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Original Article

Systematic review of pharmacogenetic testing for predicting clinical benefit to anti-EGFR therapy in metastatic colorectal cancer

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Abstract: Pharmacogenetic testing can help identify patients with metastatic colorectal cancer more likely to respond to anti-EGFR therapy. We systematically reviewed the benefits and harms of EGFR-related pharmacogenetic testing of molecular targets downstream to *KRAS* in the treatment of metastatic colorectal cancer. We searched five electronic databases from January 2000 through November 2010, and conducted separate grey literature and conference abstracts searches. Two reviewers independently assessed all articles for relevance and quality. We identified 27 studies, primarily fair- to marginal-quality, small retrospective, and single-arm cohort studies with significant overlap in patient populations. We identified seven studies that studied *BRAF* in independent patient populations, one that studied *NRAS*, four that studied *PIK3CA*, eight that studied PTEN expression, and five that studied AKT expression. The best evidence for *BRAF*, *NRAS*, and *PIK3CA* comes from the largest retrospective study (n=649) of chemorefractory patients from seven European countries. In this study, *BRAF* mutation was present in 6.5% of *KRAS* wild-type tumors. Only 8.3% of persons with *BRAF* mutations, compared to 38% of persons without *BRAF* mutations (p=0.0012), responded to chemotherapy with cetuximab. Clinical sensitivity and the false positive fraction (1- specificity) were estimated at 9.8% (95% CI 6.3, 14.5) and 1.6% (95% CI 0.2, 5.6), respectively. *BRAF* mutation was also associated with worse median progression-free survival (absolute difference 18 weeks, p<0.0001), and overall survival (absolute difference 28 weeks, p<0.0001). In the only study comparing outcomes in persons who did (n=227) and did not (n=332) receive cetuximab with combination chemotherapy, those with *BRAF* mutation had worse survival outcomes regardless of whether or not they received cetuximab. Although *NRAS* and *PIK3CA* exon 20 mutations were also associated with worse outcomes compared to persons without these mutations, evidence is based on a small number of identified mutations. Evidence for protein expression of PTEN and AKT is more sparse and limited by variable methods for assessing protein expression. Low-quality evidence addressing clinical validity of pharmacogenetic testing in metastatic colorectal cancer patients suggests that *BRAF* mutations are associated with poorer treatment response and survival outcomes, although this association may be independent of treatment with EGFR inhibitors.

Keywords: BRAF, NRAS, PTEN, AKT, metastatic colorectal cancer, anti-EGFR monoclonal antibodies, cetuximab, panitumumab, pharmacogenetic test, systematic review

Introduction

Colorectal cancer is the third leading cause of cancer-related death in the United States [1]. Despite improvements in chemotherapy for metastatic colorectal cancer, overall five-year survival remains poor at just 11% for patients with metastatic disease [1]. Currently, cetuximab (Erbitux®, ImClone Systems) and panitumumab (Vectibix®, Amgen) are approved by the U.S. Food and Drug Administration (FDA) for the treatment of metastatic colorectal cancer in the refractory disease setting [2,3]. These monoclonal antibodies bind to the EGFR, preventing binding and activation of the downstream signaling pathways, which are important for cancer cell proliferation, invasion, metastasis, and neovascularization [4]. Tumors with molecular alterations in the EGFR signaling pathway (e.g., mutations in *KRAS*, *NRAS*, *BRAF*, *PIK3CA*, loss of PTEN protein expression, or AKT over expression), however, may lead to a constitutively acti-

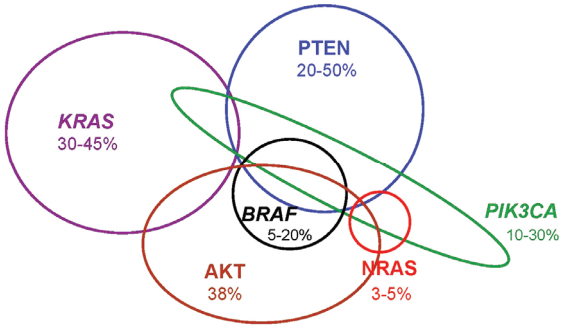

Figure 1. Mutation frequencies and loss of protein expression in the EGFR signaling pathway.

vated pathway not responsive to EGFR-targeted treatment.

Pharmacogenetic testing for *KRAS* has already entered clinical practice, such that persons with metastatic colorectal cancer whose tumors have *KRAS* mutations are not treated with EGFR monoclonal antibodies. In April 2009, ASCO issued a Provisional Clinical Opinion stating that all patients with metastatic colorectal cancer who are candidates for EGFR antibody therapy should have their tumor tested for *KRAS* mutations, and that persons with a *KRAS* mutation in codon 12 or 13 should not receive EGFR antibody as part of their treatment [5]. In July 2009, the FDA revised the label for cetuximab and panitumumab to advise against use of these agents in persons with colorectal cancer positive for *KRAS* mutations [6]. Even among patients with wild-type *KRAS*, however, the response rate to EGFR monoclonal antibodies is less than 20% [7]. Primary research suggests that molecular alterations in the EGFR signaling pathway downstream to *KRAS* may also predict non-response to EGFR monoclonal antibodies. These alterations are less frequently occurring than *KRAS* (**Figure 1**), but testing for additional molecular alterations in those without KRAS mutations has the potential to identify other patients not likely to respond to anti-EGFR therapy before treatment begins, therefore preventing unnecessary treatment and associated harms and costs [8,7].

We systematically reviewed the evidence for the clinical benefit and harms of EGFR-related pharmacogenetic testing (downstream to KRAS) in predicting non-response to treatment with anti-EGFR therapy. We asked four key questions (KQ) (**Figure 2**):

Clinical utility

KQ 1: In patients with mCRC, can other EGFR-related testing improve (or lead to non-inferior) patient outcomes or decision making compared

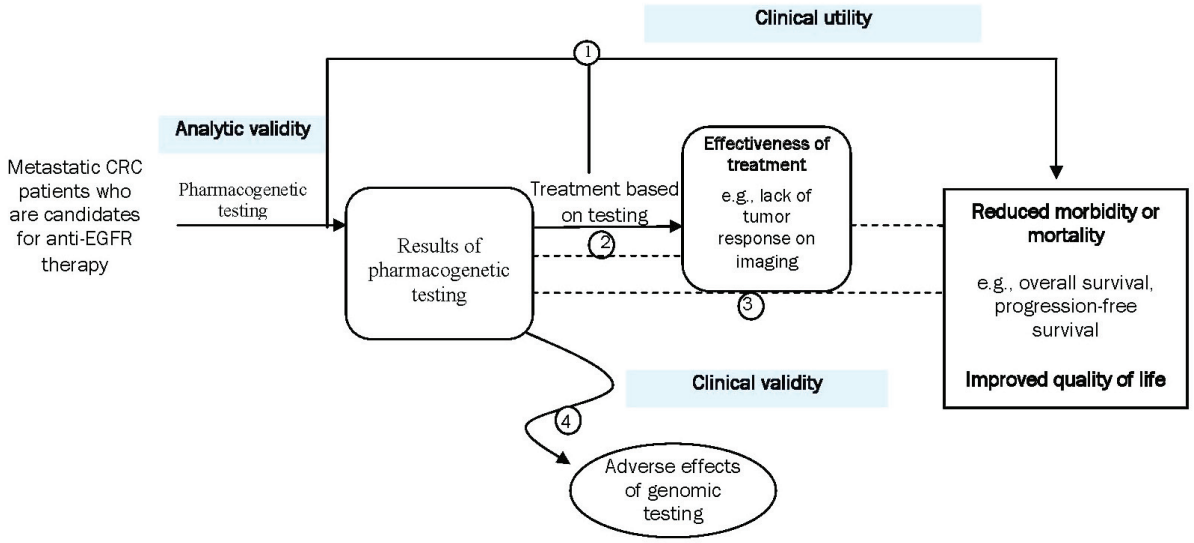

Figure 2. Analytic framework for clinical benefit and harms of EGFR-related pharmacogenetic testing of molecular targets in the treatment of metastatic colorectal cancer.

to not using additional testing?

Clinical validity

KQ 2: How well do each of these tests predict treatment effectiveness?

KQ 3: How well do each of these tests predict important health outcomes?

Harms

KQ 4: What are the potential harms to patients in using these tests to guide treatment decisions?

Methods

Studies were identified by searching electronic databases, conference abstracts, regulatory documents, and trial registries. MEDLINE was searched from January 2000 to November 2010 for English language abstracts. This search was adapted for four additional databases (Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials, Database of Abstracts of Reviews of Effects, and Health Technology Assessments Database) and limited to publications between 2000 and 2010, with no language restrictions. We also searched Conference Papers Index (via CSA) from 2009 to 2010 and hand searched selected scientific conferences from 2009 to November 2010. Relevant studies were also identified by searching clinicaltrials.gov, NIH RePORTER, Current Controlled Trials (International Standard Randomized Controlled Trial Number Register), WHO International Clinical Trials Registry Platform, and FDA regulatory documents via Drugs@FDA. The last search for all databases was performed on November 24, 2010. Search details are provided in Search Strategies document (Supplementary Table 1).

Two investigators independently reviewed 3,365 abstracts and 191 articles against *a priori* specified inclusion criteria (**Figure 3**). For all key questions, we considered studies that included persons with metastatic colorectal cancer being treated with cetuximab or panitumumab, either alone or in combination with other chemotherapeutic agents. Studies that only included patients with locally advanced disease were excluded. Testing included assays for mutations in *BRAF*, *NRAS*, *PIK3CA*, and protein expression for PTEN and AKT. We also included studies examining *PTEN* and *AKT* mutations and gene copy number. We excluded testing for EGFR protein expression or gene copy number; upstream molecular drivers (i.e., EGFR ligands epiregulin and amphiregulin); and molecular targets not directly part of the EGFR signaling cascade, but mediators in adjacent pathways. We considered any study reporting one or more of the following outcomes: overall survival, clinical response to treatment (e.g., progression free survival, time to progression), health-related quality of life, radiologic evidence of tumor progression, or potential adverse effects (e.g., incorrect genotype assignment leading to incorrect treatment assignment, delayed treatment, negative psychological effects, and ethical, legal, and social issues/concerns). We did not exclude studies based on study design or study quality. Excluded studies are listed in Supplementary Table 2.

Two investigators independently assessed the quality of each study using the quality criteria proposed by the EGAPP working group [9], supplemented by the Newcastle Ottawa Scale developed for observational studies [10], and reporting standards checklist (REMARK) developed for prognostic and predictive studies [11]. Articles were rated good-, fair-, or marginal-quality. Good-quality studies were those that met the following criteria: prospective design; large, well-defined, and representative study population; genetic testing was described well; blinded assessment of genetic testing in relation to outcome; homogeneous treatment; low rate of missing data; sufficiently long follow-up; and well-described and well-conducted analysis of outcomes. Fair-quality studies did not meet all the criteria, but did not have any fatal flaws in study design. Marginal- or poor-quality studies had significant flaws or lack of reporting that implied bias affecting interpretation of study results. Disagreements about inclusion and quality were resolved by consensus with a third reviewer.

One investigator extracted all relevant data from the studies into evidence tables that included the following study details: critical features of study design and quality, funding source, patient characteristics (e.g., age, sex, race/ethnicity, Eastern Cooperative Oncology Group [ECOG] performance status, metastatic disease), treatment regimen and setting, genetic

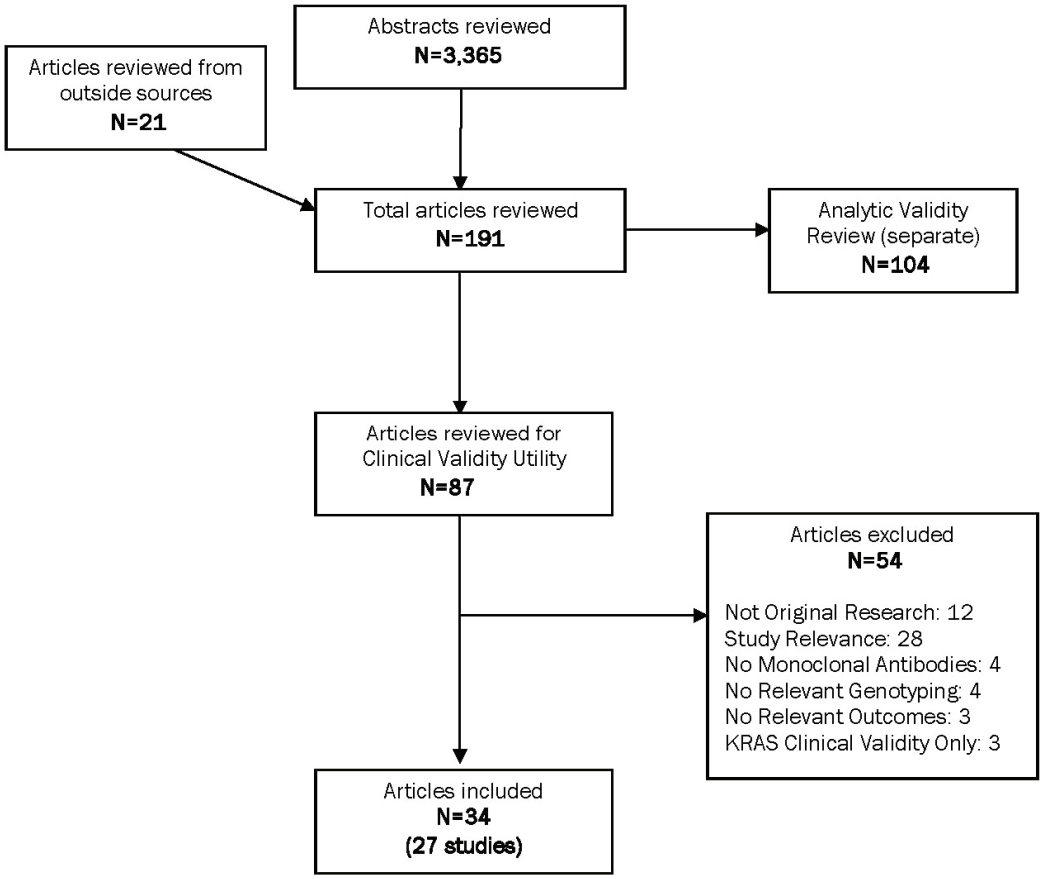

Figure 3. Search result and article flow.

testing details (e.g., gene mutation(s)/protein expression, tumor sample, assay technique, scoring method), frequency of gene mutation or protein expression, *a priori* specified study outcomes (stratified by *KRAS* wild-type if available), and any outcome representing potential adverse effects. A second reviewer verified all extracted data.

We identified 27 studies (reported in 34 articles); however there was a significant overlap of populations studied. Our evidence synthesis focuses on studies with independent patient populations and, when possible, results in persons with metastatic colorectal cancer that have *KRAS* wild-type tumors. We focus on three primary outcomes— tumor response (or disease control if tumor response is not reported) based on radiographic findings, progression-free survival (PFS), and overall survival (OS). Studies either used RECIST or World Health Organization (WHO) criteria (based on radiographic findings) to assess tumor response or disease control. Most individual studies reported tumor response rates with or without an odds ratio (OR). For tumor response, we also calculated the true positive fraction (TPF or clinical sensitivity) and false positive fraction (FPF or 1-specificity) if sufficient data were reported in persons with *KRAS* wild-type tumors [12]. For continuous outcomes (survival), in addition to reporting hazard ratios (HR), we also report absolute differences between groups in weeks or months of median progression-free or overall survival.

We summarize results qualitatively and provide these results in tables for easy comparison across studies representing unique populations. For tumor response based on imaging, we attempted quantitative synthesis (meta-analyses) for sensitivity, specificity, and odds ratios to evaluate the predictive value for each genetic

test with sufficient data. Due to overlapping populations and lack of outcome reporting for individuals with *KRAS* wild-type tumors, only 3 studies could be included in the meta-analyses. We attempted bivariate analyses for sensitivity and specificity (of *BRAF* and PTEN testing) simultaneously [13], as well as univariate meta-analyses for sensitivity, specificity, and diagnostic odds ratios, separately using random effects models [14,15]. However, the small number of studies and clinical heterogeneity among studies prohibited us from producing meaningful combined estimates. We instead focused on the best available evidence (e.g., single large, well-reported study) to provide the best estimate of clinical validity. We also considered how additional studies with independent and overlapping populations confirmed, disagreed, and/or contributed additional information to the best evidence detailed.

In addition to a summary of evidence table, we also provide a summary table focusing on the strength of the body of evidence, based on the GRADE (Grading of Recommendations Assessment, Development and Evaluations) approach [16]. The following four domains were assessed: risk of bias, consistency, directness, and precision. The overall strength of evidence was graded as high, moderate, low, or very low (insufficient).

Results

Key Question 1 to 3: Benefits of pharmacogenetic testing

We found no studies that directly assessed whether pharmacogenetic testing improves (or leads to non-inferior) important patient health outcomes (e.g., morbidity, mortality, health related quality of life) in metastatic colorectal cancer patients who received pharmacogenetic testing to guide EGFR monoclonal antibody treatment decisions, compared to those who did not receive pharmacogenetic testing (Key Question 1).

We found a total of 27 fair- to marginal-quality studies that evaluated pharmacogenetic testing for EGFR molecular targets downstream to *KRAS* and their association with tumor response (Key Question 2) or survival outcomes (Key Question 3) in patients with metastatic colorectal cancer treated with cetuximab or panitumumab [17-39]. Most trials had very limited reporting on important patient characteristics (Table 3) and were conducted in European countries. Patients in the few trials or centers that reported race/ethnicity were overwhelmingly white. Only two small studies included patients from the US [21,20], one of which included 23% non-white participants [21]. One small study was conducted in South Korea [40]. In studies that provided baseline patient characteristics, the age of patients ranged from 22 to 94 years, with the mean age ranging from 57 to 67 years. The cohorts of patients studied were 46 to 71% male.

Most studies evaluated response to cetuximab, either as monotherapy or in combination with other chemotherapy. Two studies included patients who received either cetuximab or panitumumab [27,24], and only one study included patients who exclusively received panitumumab [21]. All studies, except for one, included a majority of patients who had received prior chemotherapy, or in some cases included exclusively patients identified as chemorefractory. This study by Tol and colleagues, a retrospective evaluation of the Dutch RCT CAIRO2, evaluated the addition of cetuximab to combination chemotherapy (capecitabine, oxaliplatin, bevacizumab) as first-line treatment in patients with metastatic colorectal cancer [18]. Only eight studies reported the patients' performance score, and the majority of patients in these cohorts had no significant activity impairments (ECOG performance status of 0-1) [37,30,21,22,41, 28,40,18]. Among all patients studied, *BRAF* mutations ranged from 0 to 17%, *NRAS* 3%, *PIK3CA* 3 to 18%, loss of PTEN expression ranged from 12 to 42%, and loss of AKT expression ranged from 33 to 60%.

We found no good-quality studies. Most studies were retrospective single-arm evaluations of mutations or protein expression in cohorts of patients who received chemotherapy with cetuximab or panitumumab, comparing outcomes for persons with and without identified mutations or protein expression. Of the two studies that included both persons treated with and without cetuximab [18,20], only one reported outcomes comparing those receiving cetuximab versus those who did not [18]. Most of the studies were small, with less than 100 patients included in the analysis. Therefore, these studies may not have been adequately

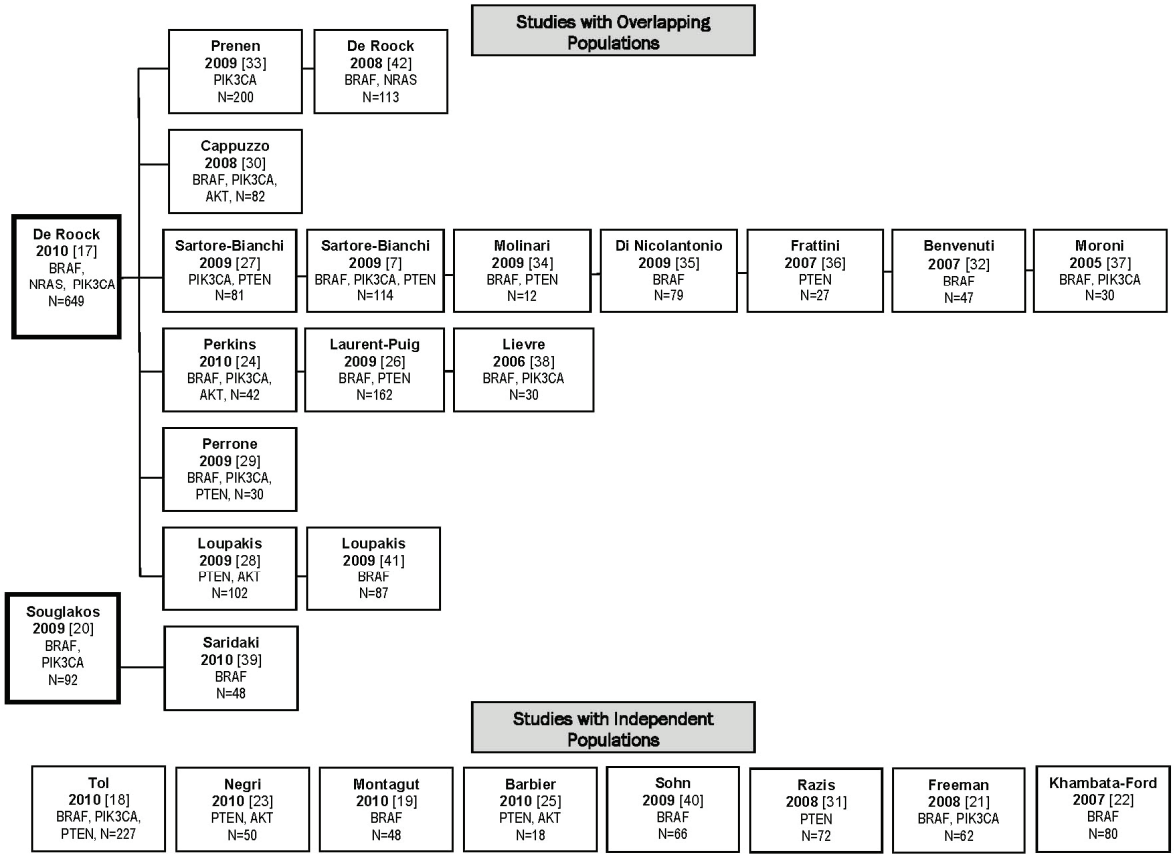

Figure 4. Included studies illustrating overlapping populations.

powered to examine the predictive ability of less frequently occurring tumor mutations. Most of the studies evaluated cetuximab in various treatment settings, and many studies combined patients receiving different combinations of chemotherapy, with differing histories of prior chemotherapy. Some studies combined persons who received either cetuximab or panitumumab. All but one study were retrospective and stated *a priori* for inclusion that "sufficient" tumor sample had to be available. Some studies specified that tumor samples had to be EGFR expression positive. Details about how patients were selected were not reported, or were very minimal, in about one-third of the studies. In the single prospective study (n=110), only 73% (80 of 110) of the tumor samples were assessed for *BRAF* [22]. No *BRAF* mutations were identified in this cohort. The level of reporting in the remaining studies was frequently inadequate to determine the proportion of missing data (from all persons eligible for the study). Information about unanalyzable or missing data (i.e., the number of and reasons for) was only reported in a few studies. Most studies did not provide sufficient data to determine if patients in the retrospectively-identified cohort were similar in terms of prognostic risk. Only a few of the studies performed analyses to determine if any important patient and treatment setting characteristics influenced the mutations' association with tumor response or survival. Studies rarely reported duration of study follow-up.

In addition to individual study quality concerns, included studies had significant overlap in populations studied. Of the 27 included studies, only seven studies evaluating *BRAF* (n=1,224) [17-22,40], one evaluating *NRAS* (n=649) [17], four evaluating *PIK3CA* (n=1,030) [17,18,20, 21], eight evaluating PTEN (n=742) [7,18,23, 25,26,28,29,31], and five evaluating AKT (n=249) [23-25,28,30] included independent patient populations (**Figure 4**). The largest

study, conducted by DeRoock and colleagues, was a retrospective analysis of chemorefractory metastatic colorectal cancer patients who received cetuximab (n=649). This study provides the best evidence for the predictive value of *BRAF*, *NRAS* and *PIK3CA* [17]. The best evidence for PTEN protein expression comes from a smaller retrospective study (n=173) [26]. No studies of AKT expression reported outcomes stratified by *KRAS* wild-type.

BRAF (codon 600, V600E)

Overall, we identified seven studies (n=1224) that reported tumor response outcomes [17-22,40], and three studies (n=968) [17,18,20] that reported survival outcomes, in independent patient populations. The best evidence for *BRAF* pharmacogenetic testing comes from one large, fair-quality retrospective analysis of a consortium of European patients with chemorefractory metastatic colorectal cancer who received cetuximab (Supplementary Tables 3 and 4) [17]. This study by DeRoock and colleagues (n=649) included chemorefractory patients with sufficient primary tumor sample available from 11 centers in seven European countries who were treated with cetuximab in combination with other chemotherapy from 2001 to 2008. This cohort was mostly men (58%) with an average age of 61 years. Mutations from primary tumor samples were assessed using MassARRAY multiplex polymerase chain reaction (PCR), with a subset of samples independently validated using direct sequencing or allele-specific PCR. Ninety-five percent (350/370) of *KRAS* wild-type samples had *BRAF* mutation status assigned and outcome data available. *BRAF* mutation (V600E) was present in 6.5% (24/350) of *KRAS* wild-type. Only 8.3% of persons with BRAF mutations responded to chemotherapy with cetuximab (p= 0.0012), compared to 38% of persons with *BRAF* and *KRAS* wild-type tumors. Calculated true positive fraction (clinical sensitivity) and false positive fraction (1 -specificity) were estimated at 9.8% (95% CI 6.3 -14.5) and 1.6% (95% CI 0.2-5.6), respectively. *BRAF* mutation was also associated with worse progression-free survival (absolute difference 18 weeks, p< 0.0001), and overall survival (absolute difference 28 weeks, p< 0.0001). Odds ratios for tumor response (Supplementary Table 4) and hazard ratios for progression-free and overall survival (Supplementary Table 5) were adjusted for age, sex, previous chemotherapy, and treatment center. Findings from other studies with [42,30,7,34,35,32,37,24,26, 38, 29,41,20,39,18] and without [22,19,40, 20,18] overlapping populations were either not informative (because of low or no mutations identified, limitations in outcome reporting) or consistent with findings from the study by DeRoock and colleagues.

We identified only one retrospective evaluation of pharmacogenetic testing in an RCT of persons with metastatic colorectal cancer receiving chemotherapy with (n=227) or without cetuximab (n=332) as first-line treatment [18]. In this study by Tol and colleagues, *BRAF* mutation was identified in 8.7% (45 of 518) of all persons. Outcomes based on imaging criteria were not reported in the subset of those with *KRAS* wild-type. For all patients, disease control was not statistically significantly different between persons with *BRAF* mutations versus *BRAF* wild-type, whether or not patients received cetuximab (Supplementary Table 4). For persons treated with cetuximab, median PFS was shorter for persons with *BRAF* mutation versus *KRAS* and *BRAF* wild-type, 6.5 months versus 11.4 months, respectively (absolute difference 4.9 months, p<0.0001). Results were similar for persons who received combination chemotherapy without cetuximab; median PFS was also shorter for persons with *BRAF* mutation versus wild-type, 5.7 months versus 10.8 months, respectively (absolute difference 5.1 months, p<0.0001). Overall survival, a secondary outcome in this study, showed a pattern consistent with that of PFS. Differences by *BRAF* mutation status for PFS and OS were essentially the same for both treatment groups (with or without cetuximab). PFS and OS were noticeably better for patients in this study (receiving first-line chemotherapy) compared with the chemorefractory patients in the two other retrospective analyses that reported survival outcomes, which suggests important clinical heterogeneity among studies [20,17].

NRAS (codons 12, 13, and 61) and PIK3CA (exons 9 and 20)

Pharmacogenetic testing for mutations other than *BRAF* have less evidence. We found only one study for *NRAS* (n=649) [17] and four studies for *PIK3CA* (n=1030) [17,18,20,21]. Again, the best evidence comes from the largest multicenter retrospective analysis (n=649) by

DeRoock and colleagues in chemorefractory metastatic colorectal cancer [17]. In this study, only 82% (302/370) of *KRAS* wild-type tumors had *NRAS* status and outcomes. Four percent (13/302) of *KRAS* wild-type tumors had *NRAS* mutations and 13% (49/370) had *PIK3CA* mutations (exon 9 and 20). Although *NRAS* and *PIK3CA* exon 20 (not exon 9) mutations were associated with poorer outcomes, this evidence is based on a very small number of mutations from one study (Supplementary Tables 3 and 4). Overall, there was no statistically significant difference in PFS or OS between persons with tumors that had *PIK3CA* mutations versus wild-type (Supplementary Table 5). Authors conducted subgroup analyses (presumed *a priori*) for mutations in exon 9 versus mutations in exon 20 because of different proposed biological effects for domains encoded by these two exons. Compared with *PIK3CA* wild-type, *PIK3CA* exon 20 mutations, but not mutations in exon 9, appeared to predict poor tumor response and survival outcomes (Tables 3 and 4). The remaining two studies with independent patient populations did not report results by *KRAS* wild-type [20,21] (Supplementary Table 4). No other studies examining *PIK3CA,* with [18,20,33,7,37,38,24,29] or without [21] overlapping populations, report results for *PIK3CA* exon 9 and 20 separately.

Protein expression of PTEN and AKT

Most studies focusing on molecular alterations in PTEN and AKT studied protein expression instead of mutations or gene copy number. Studies used immunohistochemistry (IHC) to examine protein expression of PTEN and AKT, but used different antibodies and scoring systems. We found eight studies for PTEN (n=742) [7,18,23,25,26,28,29,31], six of which (n=652) reported survival outcomes [7,18,23,26,28,29] (Supplementary Tables 3 and 4). There was some evidence to suggest that PTEN loss may be associated with non-response, though results are conflicting between studies. The best evidence comes from a retrospective cohort (n=173) by Laurent-Puig and colleagues [26]. In this study, about 20% of the *KRAS* wild-type tumors had loss of PTEN protein expression. Loss of protein expression was not associated with tumor response or progression-free survival, but was associated with slightly worse overall survival (Supplementary Tables 3 and 4). Based on a small number of fair- to marginal-quality studies with differences in assay methodologies, PTEN expression does not appear to have clinically robust ability to predict survival response to cetuximab or panitumumab. Only five small studies (n=294) studied AKT protein expression [23-25,28,30], only two of which (n=194) reported survival outcomes [23,28] (Supplementary Tables 3 and 4) and none of which reported results for AKT loss in *KRAS* wild-type. None of the five studies showed a statistically significant association between AKT expression and tumor response or survival. Based on one study, PTEN and AKT protein expression are only concordant in 60% and 68% respectively, of primary and metastatic tumors [28].

Key Question 4: Harms of pharmacogenetic testing

We did not hypothesize any clinically significant harms to testing other than incorrect genotype assignment leading to incorrect treatment assignment (i.e., leading to subsequent withholding of potentially effective therapy, or giving therapy that has significant adverse effects and cost with little to no benefit). None of the included studies reported harms of testing, and we found no studies that explicitly addressed harms or that addressed psychological, ethical, legal, or social implications of testing.

The best evidence to estimate harms associated with incorrect treatment assignment based on testing for mutations in *BRAF*, *NRAS*, and *PIK3CA* comes from the largest retrospective study of chemorefractory patients with metastatic colorectal cancer by DeRoock and colleagues [17]. Overall, the specificity of mutations in EGRF-related genes was very high, and therefore the false positive fraction (1-specificity) was low. These false positives are those few patients who would respond to treatment despite a mutation identified through genetic testing, from whom potentially effective treatment is withheld. Point estimates of false positive fractions for *BRAF*, *NRAS*, and *PIK3CA* exon 20 are 1.6%, 0.9%, and 0.0% of responders, respectively (Supplementary Table 4).

Discussion

Of the studied molecular targets downstream from *KRAS*, the evidence is most promising for *BRAF* mutation as a negative predictor of response to EGFR monoclonal antibodies, and is

most robust for persons with chemorefractory metastatic disease receiving cetuximab in combination chemotherapy (Supplementary Table 6). *BRAF* mutation is less common than *KRAS* mutations, approximately 5 to 20% versus 30 to 45%, respectively [43,44,45,46]. In the largest study, which was exclusively in chemorefractory patients, *BRAF* mutation was present in 6.5% of *KRAS* wild-type [17]. The calculated true positive fraction (possible benefit), was 9.8% (95% CI 6.3-14.5), which meant that an additional (after *KRAS* testing) 9.8% of persons who did not respond to treatment were identified with *BRAF* testing. The calculated false positive fraction (possible harm) was 1.6% (95% CI 0.2-5.6), which meant that of those who responded to treatment, the proportion with *BRAF* mutation was small. *BRAF* mutation also was associated with worse median progression-free survival (absolute difference of 18 weeks) and overall survival (absolute difference of 28 weeks) in chemorefractory persons. One retrospective evaluation of an RCT that compared persons receiving cetuximab and combination chemotherapy to those receiving combination chemotherapy without cetuximab as first-line chemotherapy showed that persons with BRAF mutations had shorter progression-free and overall survival regardless of cetuximab, suggesting prognostic ability independent of treatment with cetuximab [18]. While the overall magnitude of association (odds ratio) of *BRAF* mutation on tumor response and survival is similar to the association of *KRAS* mutation on tumor response and survival, the clinical sensitivity is much lower. In a recent good quality systematic review of *KRAS* testing in this clinical scenario, the sensitivity was 49% (95% CI 44-54) [47]. In addition, the body of evidence for *BRAF* testing is much smaller and primarily comprises single-arm retrospective studies with poorly-characterized cohorts of patients (Supplementary Table 7). Similar to *KRAS* mutations, two studies that currently represent the best evidence for *BRAF* mutations showed association is greater in chemorefractory patients than in patients receiving cetuximab in combination with other chemotherapy as first-line therapy [17,18]. However, unlike *KRAS*, *BRAF* mutation appears to have prognostic ability independent of predicting response to cetuximab. Additional retrospective evaluations of RCTs comparing persons who received chemotherapy with and without EGFR monoclonal antibodies would help clarify the extent to which *BRAF* mutation predicts poor response to anti-EGFR therapy, or predicts poor prognosis independent of treatment effect. We identified conference abstracts, without full publication of results, of retrospective analyses of RCTs evaluating the addition of cetuximab to first-line therapy that also suggest that BRAF mutation in persons with metastatic colorectal cancer was a strong negative prognostic factor (independent of treatment effect) [47,48]. When these and other studies are fully reported, it would be important to attempt to clarify the prognostic significance of *BRAF* mutations in metastatic colorectal cancer, and separate out whether there is any additional pharmacogenetic treatment selection role for *BRAF* mutation testing for anti-EGFR therapy in first-line versus second-line or higher treatment of metastatic colorectal cancer.

Important details that may the affect test accuracy and reproducibility of assays are not routinely reported in studies addressing clinical validity. The analytic validity of *BRAF* testing in colorectal cancer was not part of this systematic review. In general, we found that the published literature on analytic validity was sparse, and does not reflect the technology of the assays used in the studies. The analytic validity of *BRAF* testing in colorectal cancer is likely good, based on the one study by DeRoock and colleagues that independently validated assay results in a subset of patients using allele-specific PCR [17]. Most applicable and least biased analytic validity evidence for *BRAF* testing should be available from proficiency testing programs, although the proficiency testing data would not address important pre-analytic factors (that relate to tumor specimen and dissection of tissue) that may also influence test performance.

Evidence for EGFR-related pharmacogenetic testing, other than *KRAS* testing, in metastatic colorectal cancer to guide the use of anti-EGFR chemotherapy is still very limited (Supplementary Table 8). Evidence for these tests comes almost exclusively from fair- to marginal-quality retrospective studies without a comparison cohort who did not receive EGFR monoclonal antibodies. In general, if the gene mutation is not uncommon, prospective studies are clearly preferred. If prospective evaluation studies are not available or feasible, then better retrospective studies are needed. These studies should be based on well described cohorts, either nested in trials or clinical settings with high

quality of information (patient and outcome assessment and documentation), with good descriptions of patient and setting characteristics (in terms of prognostic factors and treatment), and good follow-up and measurement of patient outcomes.

Low GRADE retrospective observational evidence suggests that *BRAF* testing in metastatic colorectal cancer patients is a negative predictor of response and survival in those treated with cetuximab (Supplementary Table 8). However, it is unclear if the association of *BRAF* mutation with worse tumor response and survival is due to predicting response to treatment with cetuximab or prognosis independent of treatment. Evidence for *NRAS* and *PIK3CA* exon 20 is thus far based on a very limited number of tumors with identified mutations and needs to be replicated in other populations and treatment settings. Evidence for PTEN loss of expression is conflicting, and may be due to clinical heterogeneity or variation in analytic and preanalytic factors. IHC assays (the antibodies and scoring system) for protein expression need to be validated and standardized. The evidence is rapidly evolving, with numerous relevant conference abstracts presented in 2009 and 2010, without full publication of results, which most certainly will add to the knowledge base about the clinical validity of these tests (Supplementary Table 9). Improved reporting of important patient characteristics, treatment setting, and details of assays and tumor sample will help inform the applicability and implementation of clinically valid pharmacogenetic tests into practice.

Competing interests

The authors declare that they have no competing interests.

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Supplementary Table 1. Search Strategies

Published Literature		
Source	**Dates**	**Search Details**
OvidMEDLINE	OvidMedline 1996-November Week 3 2010 Medline In Process November 26, 2010 Daily Update November 26, 2010	1 Colorectal Neoplasms/ 2 Colonic Neoplasms/ 3 Sigmoid Neoplasms/ 4 Rectal Neoplasms/ 5 Anus Neoplasms/ 6 Anal Gland Neoplasms/ 7 CRC.ti,ab. 8 mCRC.ti,ab. 9 ((colon or colorectal or colonic or sigmoid or rectal or rectum) adj3 (cancer* or neoplasm* or neoplasia* or tumor* or tumour* or carcinoma* or adenocarcinoma* or metastatic or metastasis or metastases)).ti,ab. 10 or/1-9 11 "Proto-Oncogene Proteins B-raf"/ 12 "Proto-Oncogene Proteins C-raf"/ 13 BRAF protein, human.nm. 14 KRAS protein, human.nm. 15 Genes, ras/ 16 ras Proteins.sh,nm. 17 PTEN Phosphohydrolase.sh,nm. 18 PTEN protein, human.nm. 19 1-Phosphatidylinositol 3-Kinase/ 20 PIK3CA protein, human.nm. 21 p13k.ti,ab. 22 PIK3CA.ti,ab. 23 PTEN.ti,ab. 24 "neuroblastoma RAS".ti,ab. 25 (k-ras or b-raf or n-ras or h-ras).ti,ab. 26 (kras or braf or nras or hras).ti,ab. 27 "V-Ki-ras2".ti,ab. 28 "Ki-ras*".ti,ab. 29 "Kirsten rat sarcoma".ti,ab. 30 "V-raf murine sarcoma".ti,ab. 31 V600E.ti,ab. 32 ((ras or raf) adj3 (family or mutation* or gene* or pathway* or signal)).ti,ab. 33 or/11-32 34 Cetuximab.nm. 35 Panitumumab.nm. 36 Pharmacogenetics/ 37 pharmacogen*.ti,ab. 38 erbitux.ti,ab. 39 vectibix.ti,ab. 40 "abx-egf".ti,ab. 41 or/34-40 42 Antibodies, Monoclonal.sh,nm. 43 "monoclonal antibod*".ti,ab. 44 or/42-43 45 Receptor, Epidermal Growth Factor.sh,nm. 46 EGFR protein, human.nm. 47 "epidermal growth factor".ti,ab. 48 egfr.ti,ab.

Supplementary Table 1. Search Strategies (cont.)

Published Literature		
Source	**Dates**	**Search Details**
		49 or/45-48 50 44 and 49 51 33 or 41 or 50 52 10 and 51 53 limit 52 to yr="2000 -Current" 54 limit 53 to english language 55 remove duplicates from 54
CDSR, CENTRAL, DARE, HTA – searched simultaneously in Cochrane Library via Wiley Online Library	2000-2010	#1 (colon or colorectal or colonic or sigmoid or rectal or anus or anal):ti,ab,kw #2 (cancer or cancers or neoplasm or neoplasms or carcinoma or carcinomas or adenocarcinoma or adenocarcinomas or metastatic or metastasis or metastases):ti,ab,kw #3 (#1 AND #2) #4 (crc or mcrc):ti,ab,kw #5 (kras or braf or nras or pten or p13k or pik3ca):ti,ab,kw #6 (ras or raf):ti,ab,kw and (family or mutation or mutations or gene or genes or pathway or pathways or signal):ti,ab,kw #7 (k-ras or b-raf or n-ras or pten or p13k or pik3ca):ti,ab,kw #8 (egfr or "epidermal growth factor"):ti,ab,kw #9 ("monoclonal antibodies" or cetuximab or panitumumab or erbitux or vectibix or pharmacogenetics or pharmacogenomics or pharmacogenetic or pharmacogenomic or oncogene or oncogenes):ti,ab,kw #10 (#3 OR #4) #11 (#5 OR #6 OR #7 OR #8 OR #9) #12 (#10 AND #11), from 2000 to 2010
Grey Literature		
Conference Papers Index – via CSA	2009-2010	(KW=(kras or braf or nras or hras or pten or plk3ca or p13k or vraf) AND (KW=(colon or colonic or colorectal or rectal or rectum) AND (KW=(cancer or cancers or neoplasm or neoplasms or carcinoma or carcinomas or tumor or tumour or metastatic) Limited to: English Only
DRUGS@FDA	No Limits	Cetuximab OR Panitumumab No action dates or other limits imposed. Medical, Statistical, Chemistry, Pharmacology, and Clinical

Supplementary Table 1. Search Strategies (cont.)

Grey Literature		
Source	**Dates**	**Search Details**
		Pharmacology Biopharmaceutics Reviews pdfs downloaded for each drug.
FDA.gov	2008-2010	Food & Drug Administration Oncologic Drugs Advisory Committee [FDA ODAC] Meetings 2008-current, hand-searched
Clinicaltrials.gov	No Limits	(braf OR kras OR nras OR hras OR PTEN OR PIK3CA OR P13K OR vraf) AND (colon OR colonic OR colorectal) AND (cancer OR cancers OR neoplasm OR neoplasms OR carcinoma OR carcinomas OR tumor OR tumour OR tumors OR tumours OR metastatic)
NIH RePORTER	No Limits	"Term search" field used for each statement (braf AND colon) OR (kras AND colon) OR (nras AND colon) OR (hras AND colon) OR (PTEN AND colon) OR (PIK3CA AND colon) OR (P13K AND colon) OR (vraf AND colon)
Current Controlled Trials (ISRCTN)	No Limits	kras OR braf OR nras OR hras OR pten OR pik3ca OR p1k3 OR vraf
WHO ICTRP Search Portal	No Limits	All searches were limited to: Condition: colorectal cancer Recruitment status: all Title: kras OR Title: braf OR Title: nras OR Title: hras OR Title: PTEN OR Title: PIK3CA OR Title: P13K OR Title: vraf
Hand searched conference abstracts	2009 to November 2010	American Society of Clinical Oncology (ASCO) General Meeting; Gastrointestinal Cancers Symposium; American Association for Cancer Research (AACR) Annual Meeting; College of American Pathologists (CAP); American Society for Therapeutic Radiology and Oncology (ASTRO) Annual Meeting; American College of Gastroenterology (ACG) Annual Meeting; Digestive Disease Week; European Crohn's and Colitis Organization (ECCO); International Symposium on Targeted Anticancer Therapies and World Congress on Gastrointestinal Cancer

Supplementary Table 2. Studies excluded from the review

Reference	Reason for exclusion
Albitar M, Yeh C, Ma W, Albitar M, Yeh C, Ma W. K-ras mutations and cetuximab in colorectal cancer. *N Engl J Med.* 2009;360:834-836.	No relevant outcomes
Al-Kuraya KS, Al-Kuraya KS. KRAS and TP53 mutations in colorectal carcinoma. *Saudi Journal of Gastroenterology.* 2009;15:217-219.	Not original research
Bibeau F, Lopez-Crapez E, Di FF et al. Impact of Fc{gamma}RIIa-Fc{gamma}RIIIa polymorphisms and KRAS mutations on the clinical outcome of patients with metastatic colorectal cancer treated with cetuximab plus irinotecan. *J Clin Oncol.* 2009;27:1122-1129.	KRAS clinical validity only
Boccia RV, Cosgriff TM, Headley DL, Badarinath S, Dakhil SR. A phase II trial of FOLFOX6 and cetuximab in the first-line treatment of patients with metastatic colorectal cancer. *Clinical Colorectal Cancer.* 2010;9:102-107.	No relevant gene
Chung KY, Shia J, Kemeny NE et al. Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. *J Clin Oncol.* 2005;23:1803-1810.	No relevant gene
Daemen A, Gevaert O, De BT et al. Integrating microarray and proteomics data to predict the response on cetuximab in patients with rectal cancer. *Pac Symp Biocomput.* 2008;166-177.	No relevant gene
Erben P, Strobel P, Horisberger K et al. KRAS and BRAF Mutations and PTEN Expression Do Not Predict Efficacy of Cetuximab-Based Chemoradiotherapy in Locally Advanced Rectal Cancer. *Int J Radiat Oncol Biol Phys.* 2010.	Genotyping not performed in tumor samples
Gebbia V, Del PS, Borsellino N et al. Efficacy and safety of cetuximab/irinotecan in chemotherapy-refractory metastatic colorectal adenocarcinomas: a clinical practice setting, multicenter experience. *Clinical Colorectal Cancer.* 2006;5:422-428.	No relevant gene
Goncalves A, Esteyries S, Taylor-Smedra B et al. A polymorphism of EGFR extracellular domain is associated with progression free-survival in metastatic colorectal cancer patients receiving cetuximab-based treatment. *BMC Cancer.* 2008;8:169.	KRAS clinical validity only
Graziano F, Canestrari E, Loupakis F et al. Genetic modulation of the Let-7 microRNA binding to KRAS 3'-untranslated region and survival of metastatic colorectal cancer patients treated with salvage cetuximab-irinotecan. *Pharmacogenomics J.* 2010.	No relevant gene
Graziano F, Ruzzo A, Loupakis F et al. Pharmacogenetic profiling for cetuximab plus irinotecan therapy in patients with refractory advanced colorectal cancer. *J Clin Oncol.* 2008;26:1427-1434.	No relevant gene
Hebbar M, Di FF, Conroy T et al. Assessment of baseline clinical predictive factors of response to cetuximab-irinotecan in patients with irinotecan-refractory metastatic colorectal cancer. *Oncology.* 2007;73:185-191.	No relevant gene
Hebbar M, Wacrenier A, Desauw C et al. Lack of usefulness of epidermal growth factor receptor expression determination for cetuximab therapy in patients with colorectal cancer. *Anticancer Drugs.* 2006;17:855-857.	No relevant gene
Hoy SM, Wagstaff AJ, Hoy SM, Wagstaff AJ. Panitumumab in the treatment of metastatic colorectal cancer: profile report. *Biodrugs.* 2007;21:135-137.	Not original research

Supplementary Table 2. Studies excluded from the review (cont.)

Reference	Reason for exclusion
Irahara N, Baba Y, Nosho K et al. NRAS mutations are rare in colorectal cancer. *Diagn Mol Pathol.* 2010;19:157-163.	No monoclonal antibodies
Italiano A, Follana P, Caroli FX et al. Cetuximab shows activity in colorectal cancer patients with tumors for which FISH analysis does not detect an increase in EGFR gene copy number. *Ann Surg Oncol.* 2008;15:649-654.	No relevant gene
Jacobs B, De RW, Piessevaux H et al. Amphiregulin and epiregulin mRNA expression in primary tumors predicts outcome in metastatic colorectal cancer treated with cetuximab. *J Clin Oncol.* 2009;27:5068-5074.	KRAS clinical validity only
Jehan Z, Bavi P, Sultana M et al. Frequent PIK3CA gene amplification and its clinical significance in colorectal cancer. *J Pathol.* 2009;219:337-346.	No monoclonal antibodies
Jhawer M, Goel S, Wilson AJ et al. 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.	Genotyping not performed in tumor samples
Katsios C, Ziogas DE, Roukos DH. Colorectal cancer: cetuximab, KRAS, BRAF, PIK3CA mutations and beyond. *Expert review of gastroenterology & hepatology.* 2010;4:525-529.	No relevant outcomes
Laurent-Puig P, Lievre A, Blons H, Laurent-Puig P, Lievre A, Blons H. Beyond the KRAS test. *Eur J Cancer.* 2009;45 Suppl 1:398-399.	Not original research
Lelli G, Cataldo S, Carandina I et al. The role of cetuximab in pre-treated refractory patients with metastatic colorectal cancer: outcome study in clinical practice. *J Chemother.* 2008;20:374-379.	No relevant gene
Lenz HJ, Van CE, Khambata-Ford S et al. Multicenter phase II and translational study of cetuximab in metastatic colorectal carcinoma refractory to irinotecan, oxaliplatin, and fluoropyrimidines. *J Clin Oncol.* 2006;24:4914-4921.	No relevant gene
Liao W, Liao Y, Zhou JX et al. Gene Mutations in Epidermal Growth Factor Receptor Signaling Network and Their Association With Survival in Chinese Patients With Metastatic Colorectal Cancers. *Anat Rec (Hoboken).* 2010.	No monoclonal antibodies
Lopez-Crapez E, Mineur L, Emptas H et al. KRAS status analysis and anti-EGFR therapies: is comprehensiveness a biologist's fancy or a clinical necessity? *Br J Cancer.* 2010;102:1074-1075.	Not original research
Lurje G, Nagashima F, Zhang W et al. Polymorphisms in cyclooxygenase-2 and epidermal growth factor receptor are associated with progression-free survival independent of K-ras in metastatic colorectal cancer patients treated with single-agent cetuximab. *Clin Cancer Res.* 2008;14:7884-7895.	No relevant gene
Mao C, Liao RY, Chen Q, Mao C, Liao RY, Chen Q. Loss of PTEN expression predicts resistance to EGFR-targeted monoclonal antibodies in patients with metastatic colorectal cancer. *Br J Cancer.* 2010;102:940.	Not original research
Moosmann N, Fischer-von WL, Vehling KU et al. Cetuximab plus XELIRI (capecitabine + irinotecan) versus cetuximab plus XELOX (capecitabine + oxaliplatin) for first-line treatment of metastatic colorectal cancer: a randomized trial of the AIO CRC Study Group. *SO: Onkologie.* 2006;29:141.	No relevant gene
Oden-Gangloff A, Di FF, Bibeau F et al. TP53 mutations predict disease control in metastatic colorectal cancer treated with cetuximab-based chemotherapy. *Br J Cancer.* 2009;100:1330-1335.	No relevant gene

Supplementary Table 2. Studies excluded from the review (cont.)

Reference	Reason for exclusion
Pantaleo MA, Fanti S, Lollini PL et al. PET detection of epidermal growth factor receptor in colorectal cancer: a real predictor of response to cetuximab treatment? *European Journal of Nuclear Medicine & Molecular Imaging.* 2007;34:1510-1511.	Not original research
Paule B, Castagne V, Picard V et al. MDR1 polymorphism role in patients treated with cetuximab and irinotecan in irinotecan refractory colorectal cancer. *Med Oncol.* 2009.	No relevant gene
Personeni N, Fieuws S, Piessevaux H et al. Clinical usefulness of EGFR gene copy number as a predictive marker in colorectal cancer patients treated with cetuximab: a fluorescent in situ hybridization study. *Clin Cancer Res.* 2008;14:5869-5876.	No relevant gene
Personeni N, Hendlisz A, Gallez J et al. Correlation between the response to cetuximab alone or in combination with irinotecan and the activated/phosphorylated epidermal growth factor receptor in metastatic colorectal cancer. *Semin Oncol.* 2005;32:S59-S62.	No relevant gene
Reidy DL, Vakiani E, Fakih MG et al. Randomized, phase II study of the insulin-like growth factor-1 receptor inhibitor IMC-A12, with or without cetuximab, in patients with cetuximab- or panitumumab-refractory metastatic colorectal cancer. *J Clin Oncol.* 2010;28:4240-4246.	Not original research
Saltz LB. Can the addition of cetuximab to irinotecan improve outcome in colorectal cancer? *SO: Nature clinical practice Oncology.* 2005;2:20-21.	Not original research
Sartore BA, Moroni M, Veronese S et al. Epidermal growth factor receptor gene copy number and clinical outcome of metastatic colorectal cancer treated with panitumumab. *SO: Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2007;25:3238-3245.	No relevant gene
Scartozzi M, Bearzi I, Mandolesi A et al. Epidermal Growth Factor Receptor (EGFR) gene copy number (GCN) correlates with clinical activity of irinotecan-cetuximab in K-RAS wild-type colorectal cancer: a fluorescence in situ (FISH) and chromogenic in situ hybridization (CISH) analysis. *BMC Cancer.* 2009;9:303.	No relevant gene
Scartozzi M, Bearzi I, Pierantoni C et al. Nuclear factor-kB tumor expression predicts response and survival in irinotecan-refractory metastatic colorectal cancer treated with cetuximab-irinotecan therapy. *J Clin Oncol.* 2007;25:3930-3935.	No relevant gene
Scartozzi M, Mandolesi A, Giampieri R et al. Insulin-like growth factor 1 expression correlates with clinical outcome in K-RAS wild type colorectal cancer patients treated with cetuximab and irinotecan. *Int J Cancer.* 2010.	No relevant gene
Seruca R, Velho S, Oliveira C, Leite M, Matos P, Jordan P. Unmasking the role of KRAS and BRAF pathways in MSI colorectal tumors. *Expert review of gastroenterology & hepatology.* 2009;3:5-9.	Not original research
Survival Data in FDA Approval for ERBITUX((R)) (CETUXIMAB) Supports Use as a Single Agent in Patients with Advanced Colorectal Cancer. *Cancer Biol Ther.* 2007;6:1671-1673.	No relevant gene

Supplementary Table 2. Studies excluded from the review (cont.)

Reference	Reason for exclusion
Tappenden P, Jones R, Paisley S, Carroll C. Systematic review and economic evaluation of bevacizumab and cetuximab for the treatment of metastatic colorectal cancer (Provisional abstract). *SO: Health Technology Assessment.* 2007;11:1-146.	No relevant gene
Tie J, Gibbs P, Lipton L et al. Optimizing targeted therapeutic development: Analysis of a colorectal cancer patient population with the BRAFV600E mutation. *Int J Cancer.* 2010.	No monoclonal antibodies
Tol J, Koopman M, Rodenburg CJ et al. A randomised phase III study on capecitabine, oxaliplatin and bevacizumab with or without cetuximab in first-line advanced colorectal cancer, the CAIRO2 study of the Dutch Colorectal Cancer Group (DCCG). An interim analysis of toxicity. *Ann Oncol.* 2008;19:734-738.	No relevant gene
Tsuchihashi Z, Khambata-Ford S, Hanna N et al. Responsiveness to cetuximab without mutations in EGFR. *N Engl J Med.* 2005;353:208-209.	No relevant gene
Vallbohmer D, Zhang W, Gordon M et al. Molecular determinants of cetuximab efficacy. *J Clin Oncol.* 2005;23:3536-3544.	No relevant gene
Vincenzi B, Santini D, Tonini G, Vincenzi B, Santini D, Tonini G. Lack of response of cetuximab plus oxaliplatin in advanced colorectal cancer patients resistant to both oxaliplatin and cetuximab plus irinotecan. *Ann Oncol.* 2006;17:527-528.	No relevant gene
Wainberg Z, Hecht JR. A phase III randomized, open-label, controlled trial of chemotherapy and bevacizumab with or without panitumumab in the first-line treatment of patients with metastatic colorectal cancer. *Clinical Colorectal Cancer.* 2006;5:363-367.	Not original research
Winder T, Zhang W, Yang D et al. Germline Polymorphisms in Genes Involved in the IGF1 Pathway Predict Efficacy of Cetuximab in Wild-type KRAS mCRC Patients. *Clin Cancer Res.* 2010;16:5591-5602.	Genotyping not performed in tumor samples
Windsor AC, Cohen R, Jiao LR et al. Cetuximab in the first-line therapy of metastatic colorectal carcinoma: not so CRYSTAL clear. *Future Oncology.* 2008;4:741-744.	Not original research
Wong R, Cunningham D, Wong R, Cunningham D. Using predictive biomarkers to select patients with advanced colorectal cancer for treatment with epidermal growth factor receptor antibodies.[Erratum appears in J Clin Oncol. 2009 Jun 20;27(18):3070]. *J Clin Oncol.* 2008;26:5668-5670.	Not original research
Zhang W, Azuma M, Lurje G et al. Molecular predictors of combination targeted therapies (cetuximab, bevacizumab) in irinotecan-refractory colorectal cancer (BOND-2 study). *Anticancer Res.* 2010;30:4209-4217.	No relevant gene
Zhang W, Gordon M, Press OA et al. Cyclin D1 and epidermal growth factor polymorphisms associated with survival in patients with advanced colorectal cancer treated with Cetuximab. *Pharmacogenetics & Genomics.* 2006;16:475-483.	Genotyping not performed in tumor samples
Zlobec I, Molinari F, Kovac M et al. Prognostic and predictive value of TOPK stratified by KRAS and BRAF gene alterations in sporadic, hereditary and metastatic colorectal cancer patients. *Br J Cancer.* 2010;102:151-161.	No relevant outcomes

Supplementary Table 3. Overview of included studies

Author, Year	% Mutation		% Negative protein expression		N	Median Age, years	% Male	% White	Rx	Prior chemo	Perf score	Length of follow-up	Rx efficacy	Survival outcomes
DeRoock 2010[17]	*BRAF* *NRAS* *PIK3CA*	6.5 4.1 13.0	NR		649	61	58.1	NR	C-mab	Yes	NR	NR	RECIST or WHO	PFS OS
Prenen 2009[33]*	*PIK3CA*	11.6§	NR		200	61	60	NR	C-mab	Yes	NR	NR	RECIST	PFS OS
DeRoock 2008[42]*	*BRAF* *NRAS*	5.6§ 4.4§	NR		113	60	61.9	NR	C-mab	Yes	NR	NR	RECIST and WHO	NR
Cappuzzo 2008[30]*	*BRAF* *PIK3CA*	5.1§ 17.7§	AKT	NR‖	82	63	64	NR	C-mab	Yes	Yes	18 months	RECIST	OS
Sartore-Bianchi 2009[27]*	*PIK3CA* exon 9 exon 20	5.2 14.3	PTEN	8.3§	81	64	65	NR	C-mab	Yes	NR	NR	RECIST	PFS OS
Sartore-Bianchi 2009[7]*	*BRAF*	11.6	PTEN	36§	114	64	65	NR	Both	Yes	NR	NR	RECIST	NR
Molinari 2009[34]*	*BRAF*	28.5 ¶	PTEN	14.3	12	67	63	NR	Both	Yes	NR	NR	RECIST	NR
DiNicolantonio 2008[35]*	*BRAF*	13.9§	NR		79	63‡	71	NR	Both	Yes	NR	NR	RECIST	PFS OS
Frattini 2007[36]*	NR		PTEN	40.1§	27	66‡	67	NR	C-mab	Yes	NR	NR	RECIST	NR
Benvenuti 2007[32]*	*BRAF*	12.8§	NR		47	62‡	63	NR	Both	Yes	NR	60 weeks	RECIST	NR
Moroni 2005[37]*	*BRAF* *PIK3CA*	3.3§ 9.7§	NR		30	66‡	71	NR	Both	Yes	Yes	48 weeks	RECIST	NR
Perkins 2010[24]*	*BRAF* *PIK3CA*	4.3 8.7	AKT	NR	42	61.8‡	57	NR	Both	Yes	NR	10 months	RECIST	NR
Laurent-Puig 2009[26]*	*BRAF*	2.9§	PTEN	19.8§	162	NR	NR	NR	C-mab	Yes	NR	NR	RECIST	PFS OS
Lievre 2006[38]*	*BRAF* *PIK3CA*	0§ 6.7§	NR		30	62‡	63	NR	C-mab	Yes	NR	NR	RECIST	OS
Perrone 2009[29]*	*BRAF* *PIK3CA*	9.7§ 12.9§	PTEN	NR‖	30	57‡	63	NR	C-mab	Yes	NR	27 weeks	RECIST	PFS
Loupakis 2009[28]*	NR		PTEN AKT	48.9 59.8§	102	62	59	NR	C-mab	Yes	Yes	21 months	RECIST	PFS OS

Table 3. Overview of included studies (cont.)

Author, Year	% Mutation		% Negative protein expression		N	Median Age, years	% Male	% White	Rx	Prior chemo	Perf score	Length of follow-up	Rx efficacy	Survival outcomes
Loupakis 2009[41]*	*BRAF*	14.9	NR		87	66	60	NR	C-mab	Yes	Yes	NR	RECIST	PFS OS
Souglakos 2009[20]	*BRAF* *PIK3CA*	12.3 17.9	NR		92	59	52	NR	C-mab	Yes	NR	NR	NR	PFS
Saridaki 2010[39]†	*BRAF*	8.3§	NR		48	64	57	NR	C-mab	Yes	NR	NR	NR	PFS OS
Tol 2010[18]	*BRAF* *PIK3CA*	8.7 9.9§	PTEN	42§	227	62	60	NR	C-mab	No	Yes	35 months	WHO	PFS OS
Negri 2010[23]	NR		PTEN AKT	11.6§ NR	50	NR	NR	NR	C-mab	NR	NR	23 months	NR	PFS OS
Montagut 2010[19]	*BRAF*	6.3	NR		48	NR	65	NR	C-mab	Yes	NR	NR	RECIST	NR
Barbier 2010[25]	NR		PTEN AKT	40.7§ 28.6§	18	64	57	NR	C-mab	Yes	NR	NR	RECIST	NR
Sohn 2009[40]	*BRAF*	0	NR		66	58	61	NR	C-mab	Yes	Yes	NR	RECIST	NR
Razis 2008[31]	NR		PTEN	13.9§	72	60	56	NR	C-mab	Yes	NR	17 months	NR	NR
Freeman 2008[21]	*BRAF* *PIK3CA*	6.5 3.2§	NR		62	62	60	77	P-mab	Yes	Yes	NR	RECIST and WHO	NR
Khambata-Ford 2007[22]	*BRAF*	0	NR		110	61	46	NR	C-mab	Yes	Yes	NR	WHO	NR

*Overlaps with DeRoock 2010
†Overlaps with Souglakos 2009
‡ Mean age
§ In all patients, not just *KRAS* WT
|| Mutation reported, not protein expression
¶ Based on very small sample (2/7)

Chemo- chemotherapy, C-mab- cetuximab, N- sample analyzed, NR- not reported, OS- overall survival, P-mab- panitumumab, Perf score- performance score, PFS- progression free survival, RECIST- Response Evaluation Criteria In Solid Tumors, Rx- treatment, Rx efficacy- treatment efficacy based on radiographic criteria, WHO- World Health Organization

Table 4. Key Question 2 results for included studies with independent patient populations that evaluate pharmacogenetic testing to predict treatment response or non-response by radiographic criteria

A. NRAS testing (codons 12, 13, 61)

Study details		Population details		Intervention details	Outcomes: NRAS (codons 12, 13, 61) (In patients with KRAS wild-type tumor, unless otherwise specified)			
Author, year Country Quality	Design Selection criteria	N (patients) Median Age Sex	Treatment	Genetic test	Mutation frequency	Objective tumor response†	Calculated TPF (95%CI)‡ FPF (95%CI) §	Odds ratio for tumor response ‖ (95%CI)
DeRoock 2010[17] 7 European countries Fair	Retrospective cohort Chemo-therapy refractory patients from 11 centers, 2001-2008	N= 649 61 yrs [28-86] 58% men	C-mab combin-ation therapy	MassArray genotyping, subset independently validated by direct sequencing or allele-specific PCR	All patients : 2.6% (17/644) In KRAS WT : 4.1% (13/315)	Mut: 7.7% (1/13) WT: 38.1% (110/289)* p= 0.013	TPF : 0.063 (0.033, 0.107) FPF : 0.009 (0.0002, 0.49)	OR : 0.14 (0.01, 0.70) Adj OR : 0.087 (0.004, 0.511) Adjusted for age sex, number of previous chemotherapy, center OR defined with mutation predicting response

* Number is not reported consistently between text and tables
† Tumor response= partial or complete response, tumor non-response= stable disease or progressive disease
‡ True-positive fraction (TPF) or clinical sensitivity= proportion of non-responders (disease positive) who had a particular mutation (test positive)
§ False-positive fraction (FPF) or 1-specificity= proportion of responders (disease negative) who had a specific mutation (test positive)
‖ Odds ratio (OR)= unless otherwise specified, defined with presence of mutation predicting non-response; compares odds of non-response in persons with mutation to odds of non-response in persons with wild-type tumor (no mutation)

Adj- adjusted, Mut- mutation, PCR- polymerase chain reaction, WT- wild type (no mutation present), Yrs- years

B. BRAF testing (V600E)

Study details		Population details		Intervention details	Outcomes: BRAF (V600E) (In patients with KRAS wild-type tumor, unless otherwise specified)			
Author, year Country Quality	Design Selection criteria	N (patients) Median Age Sex	Treatment	Genetic test	Mutation frequency	Objective tumor response†	Calculated* TPF (sens) ‡ FPF (1-spec) §	Odds ratio for tumor response ‖ (95%CI)*
DeRoock 2010[17] 7 European countries Fair	Retrospective cohort Chemo-therapy refractory patients from 11 centers, 2001-2008	N= 649 61 yrs [28-86] 58% men	C-mab combin-ation therapy	MassArray genotyping, subset independently validated by direct sequencing or allele-specific PCR	All patients : 4.7% (36/761) In KRAS WT : 6.5% (24/350)	Mut: 8.3% (2/24) WT: 38% (124/326) p= 0.0012	TPF : 0.098 (0.062, 0.145) FPF : 0.016 (0.002, 0.056)	OR: 0.15 (0.02, 0.51) Adj OR : 0.109 (0.0165, 0.410) Adjusted for age sex, number of previous chemotherapy, center OR defined with mutation predicting response
Tol 2010[18] Tol 2009[49] Netherlands Fair	Retrospective analysis of RCT (CAIRO2) No previous chemotherapy perf score 0-1, 2005-2006	N= 559 (all) N= 227 (c-mab) 62 yrs [NR] 60% men	C-mab combin-ation therapy	Direct sequencing	All patients : 8.7% (45/518) In KRAS WT : NR	All patients (NR by KRAS WT) Disease control (tumor response NR) <u>C-mab (CBC)</u> Mut: 39% WT 48% p= 0.43 <u>No c-mab (CB)</u> Mut: 35% WT 50% p= 0.32	Cannot calculate	NR

B. BRAF testing (V600E) (cont.)

Study details		Population details		Intervention details	Outcomes: BRAF (V600E) (In patients with KRAS wild-type tumor, unless otherwise specified)			
Author, year Country Quality	Design Selection criteria	N (patients) Median Age Sex	Treatment	Genetic test	Mutation frequency	Objective tumor response†	Calculated* TPF (sens) ‡ FPF (1-spec) §	Odds ratio for tumor response ‖ (95%CI)*
Montagut 2010[19] Spain Fair	Retrospective cohort Consecutive patients from single hospital, 2004 and 2009	N= 48 Age NR 65% men	C-mab combin-ation therapy	Direct Sequencing	All patients : 6.3% (3/48) In KRAS WT : NR	All patients (NR in KRAS WT) Mut: 0% (0/3) WT: 33% (15/45) p= 0.54	Cannot calculate	NR
Souglakos 2009[20] United States and Greece Fair	Retrospective cohort Patients from two centers (Dana-Farber and University Hospital of Heraklion), 2004-2007	N= 168 (all) N= 92 (c-mab) 59 yrs [23-86] 52% men	C-mab combin-ation therapy	Mass-spectromic genotyping and Sanger sequencing	All patients : 7.7% (13/168) In KRAS WT : 12.3% (13/106)	All patients (NR in KRAS WT) Mut: 0% (0/9) WT: 16.9% (14/83) p= NR	Cannot calculate	NR
Sohn 2009[40] South Korea Fair	Retrospective cohort Consecutive irinotecan refractory patients, 2005-2008	N= 66 58 yrs [28-77] 61% men	C-mab combin-ation therapy	Direct sequencing confirmed with SNaPshot Multiplex	No mutations identified	N/A	N/A	N/A

B. BRAF testing (V600E) (cont.)

Study details		Population details		Intervention details	Outcomes: BRAF (V600E) (In patients with KRAS wild-type tumor, unless otherwise specified)			
Author, year Country Quality	Design Selection criteria	N (patients) Median Age Sex	Treatment	Genetic test	Mutation frequency	Objective tumor response†	Calculated* TPF (sens) ‡ FPF (1-spec) §	Odds ratio for tumor response ‖ (95%CI)*
Freeman 2008[21] United States Fair	Retrospective cohort Chemo-therapy refractory, perf score 0-2 from 3 phase II p-mab trials, through April 2007	N= 62 62 yrs [29-85] 60% men	P-mab mono-therapy	Direct Sequencing	All patients : 6.5% (4/62) In KRAS WT : NR	All patients (NR in KRAS WT) Mut: 25% (1/4) WT: NR p= NR	Cannot calculate	NR
Khambata-Ford 2007[22] NR Fair	Prospective cohort Chemo-therapy refractory or refused prior chemotherapy treatment, perf score 0-2, patient source NR	N= 80 61 yrs [25-89] 46% men	C-mab mono-therapy	Direct Sequencing	No mutations identified	N/A	N/A	N/A

* TPF, FPF, and OR only calculated if outcome data is reported in persons KRAS WT tumors
† Tumor response= partial or complete response, tumor non-response= stable disease or progressive disease
‡ True-positive fraction (TPF) or clinical sensitivity= proportion of non-responders (disease positive) who had a particular mutation (test positive)
§ False-positive fraction (FPF) or 1-specificity= proportion of responders (disease negative) who had a specific mutation (test positive)
‖ Odds ratio (OR)= unless otherwise specified, defined with presence of mutation predicting non-response; compares odds of non-response in persons with mutation to odds of non-response in persons with wild-type tumor (no mutation)

Adj- adjusted, CB- capecitabine-bevacizumab alone, CBC- capecitabine-bevacizumab regimen+cetuximab, C-mab- cetuximab, Mut- mutation, N/A- not applicable, NR- not reported, OS- overall survival, PCR- polymerase chain reaction, Perf score- performance score, PFS- progression free survival, P-mab- panitumumab, Pts- patients, Sens- sensitivity, Spec- specificity, US- United States, WT- wild type (no mutation present), Yrs- years

C. PIK3CA testing (exons 9, 20)

Study details		Population details		Intervention details	Outcomes: PIK3CA (exons 9, 20) (In patients with KRAS wild-type tumor, unless otherwise specified)			
Author, year Country Quality	Design Selection criteria	N (patients) Median Age Sex	Treatment	Genetic test	Mutation frequency	Objective tumor response†	Calculated* TPF (sens) ‡ FPF (1-spec) §	Odds ratio for tumor response ‖ (95%CI)*
DeRoock 2010[17] 7 European countries Fair	Retrospective cohort Chemo-therapy refractory patients from 11 centers, 2001-2008	N= 649 61 yrs [28-86] 58% men	C-mab combin-ation therapy	MassArray genotyping, subset independently validated by direct sequencing or allele-specific PCR	*exons 9/20* All patients : 14.5% (108/743) In KRAS WT : 13% (49/370)	*exons 9/20* Mut: 17.7% (6/34) WT: 37.7 (115/305) p= 0.015 *exon 9* Mut: 28.6% (6/21) WT: 36.3 (115/317) p= 0.47 *exon 20* Mut: 0% (0/9) WT: 37% (121/329) p= 0.029	*Any PIK3CA mutation* TPF: 0.128 (0.087, 0.180) FPF: 0.050 (0.018, 0.105) *exon 9* TPF: 0.069 (0.039, 0.111) FPF: 0.50 (0.018, 0.105) *exon 20* TPF: 0.041 (0.019, 0.077) FPF: 0.0 (0.0, 0.030)	*exons 9/20* OR: 0.35 (0.13, 0.83) *exon 9* OR: 0.70 (0.25, 1.78) *exon 20* OR: 0.00 (0.00, 0.89) Adjusted for age sex, number of previous chemotherapy, center OR defined with mutation predicting response
Tol 2010[18] Netherlands Fair	Retrospective analysis of RCT (CAIRO2) No previous chemotherapy perf score 0-1, 2005-2006	N= 559 (all) N= 227 (c-mab) 62 yrs [NR] 60% men	C-mab combin-ation therapy	Direct sequencing	All patients : 10.6% (43/406) In KRAS WT : NR	NR	Cannot calculate	NR

C. PIK3CA testing (exons 9, 20) (cont.)

Study details		Population details		Intervention details	Outcomes: PIK3CA (exons 9, 20) (In patients with KRAS wild-type tumor, unless otherwise specified)			
Author, year Country Quality	Design Selection criteria	N (patients) Median Age Sex	Treatment	Genetic test	Mutation frequency	Objective tumor response†	Calculated* TPF (sens) ‡ FPF (1-spec) §	Odds ratio for tumor response ‖ (95%CI)*
Souglakos 2009[20] United States and Greece Fair	Retrospective cohort Patients from two centers (Dana-Farber and University Hospital of Heraklion), 2004-2007	N= 168 (all) N= 92 (c-mab) 59 yrs [23-86] 52% men	C-mab combin-ation therapy	Mass-spectromic genotyping and Sanger sequencing	All patients : 15% (26/168) In KRAS WT : 17.9% (19/106)	All patients (NR in KRAS WT) "Presence of PIK3CA mutations showed no correlation with objective tumor responses to C-mab."	Cannot calculate	NR
Freeman 2008[21] United States Fair	Retrospective cohort Chemo-therapy refractory, perf score 0-2 from 3 phase II p-mab trials, through April 2007	N= 62 62 yrs [29-85] 60% men	P-mab mono-therapy	Direct Sequencing	All patients : 3.2% (2/62) In KRAS WT : 0% (0/38)	All patients (NR in KRAS WT) Mut: 0% (0/2) WT: NR p= NR	Cannot calculate	NR

* TPF, FPF, and OR only calculated if outcome data is reported in persons KRAS WT tumors
†Tumor response= partial or complete response, tumor non-response= stable disease or progressive disease
‡ True-positive fraction (TPF) or clinical sensitivity= proportion of non-responders (disease positive) who had a particular mutation (test positive)
§ False-positive fraction (FPF) or 1-specificity= proportion of responders (disease negative) who had a specific mutation (test positive)
‖Odds ratio (OR)= unless otherwise specified, defined with presence of mutation predicting non-response; compares odds of non-response in persons with mutation to odds of non-response in persons with wild-type tumor (no mutation)

C-mab- cetuximab, Mut- mutation, NR- not reported, PCR- polymerase chain reaction, P-mab- panitumumab, Perf score- performance score, Pts- patients, Sens- sensitivity, Spec- specificity, US- United States, WT- wild type (no mutation present), Yrs- years

D. PTEN testing for protein expression

Study details		Population details		Intervention details	Outcomes: PTEN (In patients with KRAS wild-type tumor, unless otherwise specified)			
Author, year Country Quality	Design Selection criteria	N (patients) Median Age Sex	Treatment	Genetic test	Protein expression frequency	Objective tumor response§	Calculated* TPF (sens) ‖ FPF (1-spec)¶	Odds ratio for tumor response# (95%CI)*
Tol 2010[18] Netherlands Fair	Retrospective analysis of RCT (CAIRO2) No previous chemotherapy perf score 0-1, 2005-2006	N= 559 (all) N= 227 (c-mab) 62 yrs [NR] 60% men	C-mab combin-ation therapy	IHC Percent of cells with positive staining; details or threshold for positivity not reported	All patients : Negative (loss) : 42.0% (207/493) In KRAS WT : NR	NR	Cannot calculate	NR
Negri 2010[23] Italy Marginal	Retrospective cohort Selection criteria and patient source NR	N= 50 NR NR	C-mab combin-ation therapy	IHC Negative (loss of expression): expression in less than 10% of cells	All patients : Negative (loss) : 11.6% (5/43) in primary tumors, 16.7% (4/24) in metastases In KRAS WT : NR	All patients (NR in KRAS WT) Primary tumors Negative: 20.0% (1/5) Positive: 55.3% (21/38) p= 0.19 Metastases Negative: 0% (0/4) Positive: 70.0% (14/20) p= 0.02	Cannot calculate	NR

D. PTEN testing for protein expression (cont.)

Study details		Population details		Intervention details	Outcomes: PTEN (In patients with KRAS wild-type tumor, unless otherwise specified)			
Author, year Country Quality	Design Selection criteria	N (patients) Median Age Sex	Treatment	Genetic test	Protein expression frequency	Objective tumor response§	Calculated* TPF (sens)‖ FPF (1-spec)¶	Odds ratio for tumor response# (95%CI)*
Barbier 2010[25] France Marginal	Retrospective cohort Consecutive patients, post surgical resection, single hospital	N= 18 (c-mab) N= 46 (all) 64 yrs [28-79] 57% men	C-mab combin-ation therapy	IHC Scoring based on grading of immuno-labeling using immuno-reactive score (range 0-12) Negative (loss of expression): IRS=0 Positive: IRS>0	All patients : Negative (loss) : 40.7% (11/27) in primary tumors, 37.5% (12/32) in metastases In KRAS WT : NR	Disease control (PR + SD) in subset of persons getting c-mab (NR in KRAS WT) Negative: 40% (2/5) Positive: 85% (11/13) p= 0.099	Cannot calculate	NR
Laurent-Puig 2009[26] France Fair	Retrospective cohort Consecutive patients from 6 hospitals, through 2005	N= 173 NR NR	C-mab mono-therapy and combin-ation therapy	IHC Negative (loss of expression): cytoplasmic score=0 Positive: cytoplasmic score>0	All patients : Negative (loss) : 19.1% (31/162) In KRAS WT : 19.8% (22/111)	Negative: 45.5% (10/22) Positive: 46.1% (41/89) P = 1	TPF: 0.20 (0.108, 0.323) FPF: 0.196 (0.098, 0.331)	OR: 1.025 p= 1.00

D. PTEN testing for protein expression (cont.)

Study details		Population details		Intervention details	Outcomes: PTEN (In patients with KRAS wild-type tumor, unless otherwise specified)			
Author, year Country Quality	Design Selection criteria	N (patients) Median Age Sex	Treatment	Genetic test	Protein expression frequency	Objective tumor response§	Calculated* TPF (sens)‖ FPF (1-spec)¶	Odds ratio for tumor response# (95%CI)*
Sartore-Bianchi 2009[7] Italy and Switzerland Fair	Retrospective cohort EGFR+ tumor, patients from 2 hospitals in Milan and Bellinzona	N= 132 64 yrs [26-85] 65% men	C-mab mono-therapy and combin-ation therapy, P-mab mono-therapy	IHC Negative (loss of expression): absence or reduction of staining in more than 50% of cells compared with controls	All patients : Negative (loss) : 36% (41/114) In KRAS WT : NR	NR	Cannot calculate	OR: 30.46 (3.83, 1436.461) p< 0.001 OR from multivariate analysis including BRAF and PIK3CA among KRAS WT patients
Loupakis 2009[28] Italy Fair	Retrospective cohort EGFR+ tumor, Irinotecan refractory patients from multiple centers	N= 102 62 yrs [38-78] 59% men	C-mab mono-therapy and combin-ation therapy	IHC Positive expression: ≥50% of cells were positive	All patients : Negative (loss) : 42.4% (36/85) in primary tumors, 40.0% (22/55) in metastases In KRAS WT : 48.9% (22/45) in primary tumors, 34.6% (9/26) in metastases	KRAS WT in primary tumor, PTEN expression in metastases:† Negative: 0% (0/10) Positive: 47.1% (8/17) p-value NR	TPF: 0.526 (0.289, 0.756) FPF: 0.0 (0.0, 0.369)	Cannot calculate‡

D. PTEN testing for protein expression (cont.)

Study details		Population details		Intervention details	Outcomes: PTEN (In patients with KRAS wild-type tumor, unless otherwise specified)			
Author, year Country Quality	Design Selection criteria	N (patients) Median Age Sex	Treatment	Genetic test	Protein expression frequency	Objective tumor response§	Calculated* TPF (sens)‖ FPF (1-spec)¶	Odds ratio for tumor response# (95%CI)*
Perrone 2009[29] Italy Marginal	Retrospective cohort Irinotecan-refractory patients	N = 32 67 yrs [37-78] 63% men	C-mab combin-ation therapy	No testing for protein expression; PTEN mutation and gene copy number testing only	N/A	N/A	N/A	N/A
Razis 2008[31] Greece Fair	Retrospective cohort Locally advanced or mCRC patients from Hellenic Cooperative Oncology Group, 2004-2005	N= 72 60 yrs [29-76] 56% men	C-mab mono-therapy and combin-ation therapy	IHC Negative (loss of expression): less than controls or no staining Positive expression: >10% of cells positive	All patients : Negative (loss) : 13.9% (10/72) In KRAS WT : NR	All patients (NR in KRAS WT) Negative: 50.0% (5/10) Positive: 30.6% (19/62) p= NR	Cannot calculate	NR

* TPF, FPF, and OR only calculated if outcome data is reported in persons KRAS WT tumors
† Data calculated from complete data set reported in web appendix of primary study
‡ OR cannot be calculated given limitations in data reporting
§ Tumor response= partial or complete response, tumor non-response= stable disease or progressive disease
‖ True-positive fraction (TPF) or clinical sensitivity= proportion of non-responders (disease positive) who had a particular mutation (test positive)
¶ False-positive fraction (FPF) or 1-specificity= proportion of responders (disease negative) who had a specific mutation (test positive)
# Odds ratio (OR)= unless otherwise specified, defined with presence of mutation predicting non-response; compares odds of non-response in persons with mutation to odds of non-response in persons with wild-type tumor (no mutation)

C-mab- cetuximab, EGFR- epidermal growth factor, IHC- immunohistochemistry, IRS- immunoreactive score, mCRC- metastatic colorectal cancer, Mut- mutation, N/A- Not applicable, NR- not reported, OS- overall survival, P-mab- panitumumab, PCR- polymerase chain reaction, Perf score- performance score, PFS- progression free survival, RCT- randomized controlled trial, Sens- sensitivity, Spec- specificity, WT- wild type (no mutation present), Yrs- years

E. AKT testing for protein expression

Study details		Population details		Intervention details	Outcomes: AKT (In patients with KRAS wild-type tumor, unless otherwise specified)			
Author, year Country Quality	Design Selection criteria	N (patients) Median Age Sex	Treatment	Genetic test	Mutation frequency	Objective tumor response†	Calculated* TPF (sens) ‡ FPF (1-spec) §	Odds ratio for tumor response ‖ (95%CI)*
Negri 2010[23] Italy Marginal	Retrospective cohort Selection criteria and patient source NR	N= 50 NR NR	C-mab combin-ation therapy	IHC Negative (loss of expression): expression in less than 10% of cells	All patients : Positive : NR In KRAS WT : NR	Disease control (tumor response NR) All patients (NR by KRAS WT) "pAKT was not predictive of response"	Cannot calculate	NR
Perkins 2010[24] France Fair	Retrospective cohort Consecutive patients from 6 hospitals, through 2005	N= 42 62 yrs 57% men	C-mab mono-therapy and combin-ation therapy P-mab mono-therapy	IHC Expression threshold NR	All patients : NR In KRAS WT : NR	All patients (NR by KRAS WT) "No correlation between response and protein expression"	Cannot calculate	NR
Barbier 2010[25] France Marginal	Retrospective cohort Consecutive patients, post surgical resection, single hospital	N= 18 (c-mab) N= 46 (all) 64 yrs [28-79] 57% men	C-mab combin-ation therapy	IHC Scoring based on grading of immuno-labeling using immuno-reactive score (range 0-12) Negative (loss of expression): IRS=0 Positive: IRS>0	All patients : Positive : 28.6% (8/28) in primary tumors, 31.3% (10/32) in metastases In KRAS WT : NR	Disease control (tumor response NR) All patients (NR in KRAS WT) Negative: 66.7% (4/6) Positive: 75.0% (9/12) p= 1.00	Cannot calculate	NR

E. AKT testing for protein expression (cont.)

Study details		Population details		Intervention details	Outcomes: AKT (In patients with KRAS wild-type tumor, unless otherwise specified)			
Author, year Country Quality	Design Selection criteria	N (patients) Median Age Sex	Treatment	Genetic test	Mutation frequency	Objective tumor response†	Calculated* TPF (sens) ‡ FPF (1-spec) §	Odds ratio for tumor response ‖ (95%CI)*
Loupakis 2009[28] Italy Fair	Retrospective cohort EGFR+ tumor, Irinotecan refractory patients from multiple centers	N= 102 62 yrs [38-78] 59% men	C-mab mono-therapy and combin-ation therapy	IHC Positive expression: ≥50% of cells were positive	All patients : Positive : 40.2% (35/87) in primary tumors, 41.2% (21/51) in metastases In KRAS WT : NR	All patients (NR in KRAS WT) Primary tumor: Negative: 13.5% (7/52) Positive: 28.6% (10/35) p=0.083 Metastases: Negative: 26.7% (8/30) Positive: 23.8% (5/21) p=0.820	Cannot calculate	All patients (NR in KRAS WT) Primary tumor: 2.57 (0.87, 7.59) p=0.083 Metastases: 0.86 (0.24, 3.12) p=0.82
Cappuzzo 2008[30] Italy Fair	Retrospective cohort EGFR+ tumor, patients from 5 Italian centers, 2002-2006	N= 85 63 yrs [NR] 64% men	C-mab combin-ation therapy	No testing for protein expression; AKT mutation testing only	N/A	N/A	N/A	N/A

* TPF, FPF, and OR only calculated if outcome data is reported in persons KRAS WT tumors
†Tumor response= partial or complete response, tumor non-response= stable disease or progressive disease
‡ True-positive fraction (TPF) or clinical sensitivity= proportion of non-responders (disease positive) who had a particular mutation (test positive)
§ False-positive fraction (FPF) or 1-specificity= proportion of responders (disease negative) who had a specific mutation (test positive)
‖Odds ratio (OR)= unless otherwise specified, defined with presence of mutation predicting non-response; compares odds of non-response in persons with mutation to odds of non-response in persons with wild-type tumor (no mutation)

C-mab- cetuximab, EGFR- epidermal growth factor, IHC- immunohistochemistry, IRS- immunoreactive score, Mut- mutation, NR- not reported, P-mab- panitumumab, Sens- sensitivity, Spec- specificity, WT- wild type (no mutation present), Yrs- years

Table 5. Key Question 3 results for included studies with independent patient populations that evaluate pharmacogenetic testing to predict survival (progression-free survival [PFS], overall survival [OS])

A. *NRAS* testing (codons 12, 13, 61)

Study details		Population details		Intervention details	Outcomes: NRAS (codons 12, 13, 61) (In patients with KRAS wild-type tumor, unless otherwise specified)		
Author, year Country Quality	Design Selection criteria	N (patients) Median Age Sex	Treatment	Genetic test	Mutation frequency	Median Survival (PFS, OS)	Hazard ratio (95% CI)*
DeRoock 2010[17] 7 European countries Fair	Retrospective cohort Chemo-therapy refractory patients from 11 centers, 2001-2008	N= 649 61 yrs [28-86] 58% men	C-mab combin-ation therapy	MassArray genotyping, subset independently validated by direct sequencing or allele-specific PCR	All patients : 2.6% (17/644) In *KRAS* WT : 4.1% (13/315)	*PFS (weeks)* Mut:14 WT: 26 p= .06 *OS (weeks)* Mut: 38 WT: 50 p= 0.05	*PFS* HR: 1.82 (1.04, 3.18) Adj HR: 1.81(1.00, 3.28) *OS* HR: 1.89 (1.05, 3.39) Adj HR: 1.98 (1.08, 3.62) Adjusted for age sex, number of previous chemotherapy, center

*Hazard ratio (HR) for survival (event=progression for PFS or death for OS) in persons with mutation as compared to persons with wild-type tumor (no mutation), unless otherwise specified

Adj- adjusted, C-mab- cetuximab, HR- hazard ratio, Mut- mutation, OS- overall survival, PCR- polymerase chain reaction, PFS- progression free survival, WT- wild type (no mutation present), Yrs- years

B. *BRAF* testing (V600E)

Study details		Population details		Intervention details	Outcomes: BRAF (V600E) (In patients with KRAS wild-type tumor, unless otherwise specified)		
Author, year Country Quality	Design Selection criteria	N (patients) Median Age Sex	Treatment	Genetic test	Mutation frequency	Median Survival (PFS, OS)	Hazard ratio (95% CI)*
DeRoock 2010[17] 7 European countries Fair	Retrospective cohort Chemo-therapy refractory patients from 11 centers, 2001-2008	N= 649 61 yrs [28-86] 58% men	C-mab combin-ation therapy	MassArray genotyping, subset independently validated by direct sequencing or allele-specific PCR	All patients : 4.7% (36/761) In *KRAS* WT : 6.5% (24/350)	*PFS (weeks)* Mut: 8 WT: 26 p< 0.0001 *OS (weeks)* Mut: 26 WT: 54 p< 0.0001	*PFS* HR: 3.74 (2.44, 5.75) Adj HR: 4.01 (2.46, 6.53) *OS* HR: 3.03 (1.98, 4.63) Adj HR: 3.35 (2.08, 5.39) Adjusted for age sex, number of previous chemotherapy, center
Tol 2010[18] Netherlands Fair	Retrospective analysis of RCT (CAIRO2) No previous chemotherapy perf score 0-1, 2005-2006	N= 559 (all) N= 227 (c-mab) 62 yrs [NR] 60% men	C-mab combin-ation therapy	Direct sequencing	All patients : 8.7% (45/518) In *KRAS* WT : NR	*PFS (months)* <u>c-mab (CBC)</u> Mut: 6.5 WT: 11.4 p= 0.0001 <u>No c-mab (CB)</u> Mut: 5.7 WT: 10.8 p<0.0001	<u>Patients treated with c-mab (CBC)</u> *PFS* Adj HR: 2.3 (95% CI NR) p= 0.0002 *OS* Adj HR: 3.2 (95% CI NR) p< 0.0001

B. *BRAF* testing (V600E) (cont.)

Study details		Population details		Intervention details	Outcomes: BRAF (V600E) (In patients with KRAS wild-type tumor, unless otherwise specified)		
Author, year Country Quality	Design Selection criteria	N (patients) Median Age Sex	Treatment	Genetic test	Mutation frequency	Median Survival (PFS, OS)	Hazard ratio (95% CI)*
Tol 2010[18] Cont.						*OS (months)* c-mab (CBC) Mut: 12.9 WT: 24.5 p< 0.0001 No c-mab (CB) Mut: 12.8 WT: 23.0 p= 0.0002	Adjusted for serum LDH, number of affected organs, prior adjuvant chemotherapy
Souglakos 2009[20] United States and Greece Fair	Retrospective cohort Patients from two centers (Dana-Farber and University Hospital of Heraklion), 2004-2007	N= 168 (all) N= 92 (c-mab) 59 yrs [23-86] 52% men	C-mab combin-ation therapy	Mass-spectromic genotyping and Sanger sequencing	All patients : 7.7% (13/168) In *KRAS* WT : 12.3% (13/106) All patients receiving c-mab: 9.8% (9/92)	All patients (NR by *KRAS* WT) treated with c-mab: *PFS (months)* Mut: 2.0 WT: 3.9 p= 0.0005	All patients (NR by *KRAS* WT) treated with c-mab: *PFS* HR: 3.6 (1.80, 7.40)

*Hazard ratio (HR) for survival (event=progression for PFS or death for OS) in persons with mutation as compared to persons with wild-type tumor (no mutation), unless otherwise specified

Adj- adjusted, CB- capecitabine-bevacizumab alone, CBC- capecitabine-bevacizumab regimen +cetuximab, C-mab- cetuximab, LDH- lactate dehydrogenase, Mut- mutation, NR- not reported, OS- overall survival, PCR- polymerase chain reaction, Perf score- performance score, PFS- progression free survival, RCT- randomized controlled trial, WT- wild type (no mutation present), Yrs- years

C. *PIK3CA* testing (exons 9, 20)

Study details		Population details		Intervention details	Outcomes: PIK3CA (exons 9, 20) (In patients with KRAS wild-type tumor, unless otherwise specified)		
Author, year Country Quality	Design Selection criteria	N (patients) Median Age Sex	Treatment	Genetic test	Mutation frequency	Median Survival (PFS, OS)	Hazard ratio (95% CI)*
DeRoock 2010[17] 7 European countries Fair	Retrospective cohort Chemo-therapy refractory patients from 11 centers, 2001-2008	N= 649 61 yrs [28-86] 58% men	C-mab Combin-ation therapy	MassArray genotyping, subset independently validated by direct sequencing or allele-specific PCR	*exons 9/20* All patients : 14.5% (108/743) In *KRAS* WT : 13% (49/370)	PFS (weeks) *Exons 9/20* Mut: 18 WT: 24 p= 0.17 *Exon 20* Mut: 11.5 WT: 24 p=0.013 *Exon 9* Mut: 23.5 WT: 24 p= 0.65 OS (weeks) *Exon 9/20* Mut: 39 WT: 51 p= 0.09 *Exon 20* Mut: 34 WT: 51 P= 0.01 *Exon 9* Mut: 46 WT: 51 p= 0.28	PFS (weeks) *Exons 9/20* HR: 1.30 (0.91, 1.86) *Exon 20* HR: 2.52 (1.33, 4.78) Adj HR: 2.27 (1.10, 4.66) *Exon 9* HR: 1.11 (0.72, 1.71) Adj HR: 1.28 (0.77, 2.14) OS (weeks) *Exon 9/20* HR: 1.41 (0.96, 2.06) *Exon 20* HR : 3.29 (1.60, 6.74) Adj HR: 3.30 (1.46, 7.45) *Exon 9* HR: 1.30 (0.82, 2.05) Adj HR: 1.23 (0.72, 2.11) Adjusted for age sex, number of previous chemotherapy, & center

C. *PIK3CA* testing (exons 9, 20) (cont.)

Study details		Population details		Intervention details	Outcomes: PIK3CA (exons 9, 20) (In patients with KRAS wild-type tumor, unless otherwise specified)		
Author, year Country Quality	Design Selection criteria	N (patients) Median Age Sex	Treatment	Genetic test	Mutation frequency	Median Survival (PFS, OS)	Hazard ratio (95% CI)*
Tol 2010[18] Netherlands Fair	Retrospective analysis of RCT (CAIRO2) No previous chemotherapy perf score 0-1, 2005-2006	N= 559 (all) N= 227 (c-mab) 62 yrs [NR] 60% men	C-mab combin-ation therapy	Direct sequencing	All patients : 10.6% (43/406) In *KRAS* WT: NR	*PFS (months)* c-mab (CBC) Mut: 10.3 WT: 10.4 p= 0.50 No c-mab (CB) Mut: 8.3 WT: 9.7 p= 0.88 *OS (months)* c-mab (CBC) Mut: 17.6 WT: 22.4 p= 0.22 No c-mab (CB) Mut:13.1 WT: 20.3 p= 0.47	NR
Souglakos 2009[20] United States and Greece Fair	Retrospective cohort Patients from two centers (Dana-Farber and University Hospital of Heraklion), 2004-2007	N= 168 (all) N= 92 (c-mab) 59 yrs [23-86] 52% men	C-mab Combin-ation therapy	Mass-spectromic genotyping and Sanger sequencing	All patients : 15% (26/168) In *KRAS* WT : 17.9% (19/106) In all patients receiving c-mab : 14.1% (13/92)	All patients (NR by *KRAS* WT) treated with c-mab: *PFS (months)* Mut: 2.5 WT: 3.9 p= 0.001	All patients (NR by *KRAS* WT) treated with c-mab: *PFS* HR: 2.10 (1.20, 3.90)

*Hazard ratio (HR) for survival (event=progression for PFS or death for OS) in persons with mutation as compared to persons with wild-type tumor (no mutation), unless otherwise specified. Adj- adjusted, CB- capecitabine-bevacizumab alone, CBC- capecitabine-bevacizumab regimen +cetuximab, C-mab- cetuximab, Mut- mutation, NR- not reported, OS- overall survival, PCR- polymerase chain reaction, Perf score- performance score, PFS- progression free survival, RCT- randomized controlled trial, WT- wild type (no mutation present), Yrs- years

D. PTEN testing for protein expression

Study details		Population details		Intervention details	Outcomes: PTEN (In patients with KRAS wild-type tumor, unless otherwise specified)		
Author, year Country Quality	Design Selection criteria	N (patients) Median Age Sex	Treatment	Genetic test	Protein expression frequency	Median Survival (PFS, OS)	Hazard ratio (95% CI)*
Tol 2010[18] Netherlands Fair	Retrospective analysis of RCT (CAIRO2) No previous chemotherapy perf score 0-1, 2005-2006	N= 559 (all) N= 227 (c-mab) 62 yrs [NR] 60% men	C-mab combin-ation therapy	IHC Percent of cells with positive staining; details or threshold for positivity not reported	All patients : Negative (loss) : 42.0% (207/493) In *KRAS* WT : NR	*PFS (months)* c-mab exp +: 9.4 exp -: 10.6 p= 0.25 no c-mab exp +: 8.4 exp -: 10.7 p= 0.19 *OS (months)* c-mab exp +: 21.0 exp -: 22.2 p=0.83 no c-mab exp +: 16.7 exp -: 23.8 p= 0.11	NR
Negri 2010[23] Italy Marginal	Retrospective cohort Selection criteria and patient source NR	N= 50 NR NR	C-mab Combin-ation therapy	IHC Negative (loss of expression): expression in less than 10% of cells	All patients : Negative (loss) : 11.6% (5/43) in primary tumors, 16.7% (4/24) in metastases In *KRAS* WT : NR	All patients (NR by *KRAS* WT) *PFS (months)* exp +: 8.2 exp -: 0.80 p< 0.001 *OS (months)* exp +: 14.2 exp -: 2.9 p< 0.001	NR

D. PTEN testing for protein expression (cont.)

Study details		Population details		Intervention details	Outcomes: PTEN (In patients with KRAS wild-type tumor, unless otherwise specified)		
Author, year Country Quality	Design Selection criteria	N (patients) Median Age Sex	Treatment	Genetic test	Protein expression frequency	Median Survival (PFS, OS)	Hazard ratio (95% CI)*
Laurent-Puig 2009[26] France Fair	Retrospective cohort Consecutive patients from 6 hospitals, through 2005	N= 173 NR NR	C-mab mono-therapy and combination therapy	IHC Negative (loss of expression): cytoplasmic score=0 Positive: cytoplasmic score>0	All patients : Negative (loss) : 19.1% (31/162) In *KRAS* WT : 19.8% (22/111)	*PFS (weeks)* exp+: 31.4 (26- 36) exp -: 30.0 (19.4- 43) p= 0.28 *OS (months)* exp +: 16.2 (13.9- 20.7) exp -: 11.8 (9.1- 17.9) p= 0.01	*OS in KRAS and BRAF WT* Adj HR: 1.9 (1.1, 3.2) Adjusted for sex, age, tumor location, number of previous chemotherapy
Sartore-Bianchi 2009[7] Italy and Switzerland Fair	Retrospective cohort EGFR+ tumor, patients from 2 hospitals in Milan and Bellinzona	N= 132 64 yrs [26-85] 65% men	C-mab Mono-therapy and combination therapy, p-mab mono-therapy	IHC Negative (loss of expression): absence or reduction of staining in more than 50% of cells compared with controls	All patients : Negative (loss): 36% (41/114) In *KRAS* WT : NR	PTEN loss was associated with shorter PFS (p=0.0681) and was significantly associated with worse OS (p=0.0048)	*PFS* Adj HR: 0.81 (0.47, 1.39) *OS* Adj HR: 0.43 (0.22, 0.8) From multivariate analysis including BRAF and PIK3CA, adjusted for cutaneous toxicity, previous chemotherapy HR for persons with normal expression vs. loss of expression

D. PTEN testing for protein expression (cont.)

Study details		Population details		Intervention details	Outcomes: PTEN (In patients with KRAS wild-type tumor, unless otherwise specified)		
Author, year Country Quality	Design Selection criteria	N (patients) Median Age Sex	Treatment	Genetic test	Protein expression frequency	Median Survival (PFS, OS)	Hazard ratio (95% CI)*
Loupakis 2009[28] Italy Fair	Retrospective cohort EGFR+ tumor, Irinotecan refractory patients from multiple centers	N= 102 62 yrs [38-78] 59% men	C-mab mono-therapy and combination therapy	IHC Positive expression: ≥50% of cells were positive	All patients : Negative (loss) : 42.4% (36/85) in primary tumors, 40.0% (22/55) in metastases In *KRAS* WT : 48.9% (22/45) in primary tumors, 34.6% (9/26) in metastases	Primary tumor samples: NR Metastatic tumor samples: *PFS (months)* exp +: 5.3 exp -: 3.7 p= 0.03 *OS (months)* exp +: 15.1 exp -: 13.1 p= 0.13	Primary tumor samples: NR Metastatic tumor samples: *PFS (months)* HR: 0.45 (0.12- 0.87) *OS (months)* HR: 0.50 (0.15- 1.26) HR for persons with positive expression vs. loss of expression
Perrone 2009[29] Italy Marginal	Retrospective cohort Irinotecan-refractory patients	N = 32 67 yrs [37-78] 63% men	C-mab Combin-ation therapy	No testing for protein expression; PTEN mutation and gene copy number testing only	N/A	N/A	N/A

*Hazard ratio (HR) for survival (event=progression for PFS or death for OS) in persons with mutation as compared to persons with wild-type tumor (no mutation), unless otherwise specified. Adj- adjusted, C-mab- cetuximab, EGFR- epidermal growth factor, Exp +/- positive/negative expression, IHC- immunohistochemistry, Mut- mutation, N/A- not applicable, NR- not reported, OS- overall survival, PCR- polymerase chain reaction, Perf score- performance score, PFS- progression free survival, RCT- randomized controlled trial, WT- wild type (no mutation present), Yrs- years

E. AKT testing for protein expression

Study details		Population details		Intervention details	Outcomes: AKT (In patients with KRAS wild-type tumor, unless otherwise specified)		
Author, year Country Quality	Design Selection criteria	N (patients) Median Age Sex	Treatment	Genetic test	Protein expression frequency	Median Survival (PFS, OS)	Hazard ratio (95% CI)*
Negri 2010[23] Italy Marginal	Retrospective cohort Selection criteria and patient source NR	N= 50 NR NR	C-mab Combin-ation therapy	IHC Negative (loss of expression): expression in less than 10% of cells	All patients : Positive : NR In *KRAS* WT : NR	All patients (NR by *KRAS* WT) "pAKT was not predictive of response, PFS, and OS"	NR
Perkins 2010[24] France Fair	Retrospective cohort Consecutive patients from 6 hospitals, through 2005	N= 42 62 yrs 57% men	C-mab or p-mab Mono-therapy and c-mab combin-ation therapy	IHC expression threshold NR	All patients : NR In *KRAS* WT : NR	NR	All patients (NR by *KRAS* WT) Comparison groups in survival analysis not described. Text only states: "In Cox univariate analysis, PFS was longer for patients with low expression of pAKT (HR 1.002 [1.000, 1.004])"
Cappuzzo 2008[30] Italy Fair	Retrospective cohort EGFR+ tumor, patients from 5 Italian centers, 2002-2006	N= 85 63 yrs [NR] 64% men	C-mab combin-ation therapy	No testing for protein expression; AKT mutation testing only	N/A	N/A	N/A

E. AKT testing for protein expression (cont.)

Study details		Population details		Intervention details	Outcomes: AKT (In patients with KRAS wild-type tumor, unless otherwise specified)		
Author, year Country Quality	Design Selection criteria	N (patients) Median Age Sex	Treatment	Genetic test	Protein expression frequency	Median Survival (PFS, OS)	Hazard ratio (95% CI)*
Loupakis 2009[28] Italy Fair	Retrospective cohort EGFR+ tumor, Irinotecan refractory patients from multiple centers	N= 102 62 yrs [38-78] 59% men	C-mab mono-therapy and combin-ation therapy	IHC Positive expression: ≥50% of cells were positive	All patients : Positive : 40.2% (35/87) in primary tumors, 41.2% (21/51) in metastases In *KRAS* WT : NR	All patients (NR by *KRAS* WT) <u>Primary tumor samples:</u> *PFS (months)* Exp +: 4.3 Exp -: 3.1 p= 0.37 *OS (months)* Exp +: 11.5 Exp -: 6.8 p= 0.11 <u>Metastatic samples:</u> *PFS (months)* Exp +: 3.4 Exp -: 4.4 p= 0.47 *OS (months)* Exp +: 11.1 Exp -: 13.0 p= 0.23	NR

*Hazard ratio (HR) for survival (event=progression for PFS or death for OS) in persons with mutation as compared to persons with wild-type tumor (no mutation), unless otherwise specified

Adj- adjusted, C-mab- cetuximab, EGFR- epidermal growth factor, Exp +/- positive/negative expression, IHC- immunohistochemistry, Mut- mutation, N/A- not applicable, NR- not reported, OS- overall survival, PCR- polymerase chain reaction, Perf score- performance score, PFS- progression free survival, WT- wild type (no mutation present), Yrs- years

Supplementary Table 6. Summary of evidence by key question

Number and Design of Studies	Major Limitations	Validity of Evidence	Summary of Findings
KQ1. Clinical Utility: In patients with mCRC, does EGFR-related genetic testing (downstream to KRAS) reduce morbidity and/or prolong survival compared to not testing for these markers?			
None	Not applicable	Not Applicable	Currently, there are no studies designed to assess if testing for EGFR-related markers, other than *KRAS*, in patients with metastatic colorectal cancer directly reduces morbidity and or prolongs survival, compared to patients without genetic testing.
KQ2. Clinical validity: In patients with mCRC, how well does EGFR-related genetic testing (i.e., BRAF, NRAS, PIK3CA, PTEN, AKT) predict response to anti-EGFR antibody treatment, as measured by tumor response/disease progression?			
27 studies total -- 25 retrospective single arm cohort -- 1 retrospective analysis of RCT -- 1 prospective single arm cohort Studies with unique populations: -- 7 *BRAF* (n=1,224) -- 1 *NRAS* (n=649) -- 4 *PIK3CA* (n=1,030) -- 8 PTEN (n=742) -- 5 AKT (n=294)	Overlapping populations. Retrospective and many small (n<100) studies. Limited reporting of important patient/setting characteristics. Very little evidence for panitumumab. Limited reporting of outcomes by *KRAS* wild-types (*PIK3CA,* PTEN, AKT). Only one study compared outcomes with persons who did not receive anti-EGFR therapy; this study reported outcomes by disease control (versus tumor response) and included persons eligible for combination chemotherapy as first line treatment. Limitations in reporting at the individual study level did not allow for meaningful pooled analyses.	Quality: Fair to poor Applicability: mostly European patients, poor descriptions of patient characteristics and setting of treatment; findings strongest for chemorefractory patients getting cetuximab; gene mutation testing on primary tumor sample, but may be of issue for protein expression; mutations primarily assessed or confirmed with direct sequencing	Best evidence for ***BRAF, NRAS,*** and ***PIK3CA*** comes from the largest retrospective study (n=649) of chemorefractory patients from a European consortium. In this study, ***BRAF*** mutation (V600E) was present in 6.5% of *KRAS* wild-type. Only 8.3% of persons with *BRAF* mutations, compared to 38% of persons without *BRAF* mutations responded to chemotherapy with cetuximab (p= 0.0012). Clinical sensitivity and specificity were estimated at 9.8% (95% CI 6.3-14.5) and 1.6% (95% CI 0.2-5.6), respectively. Four percent of *KRAS* wild-type tumors had ***NRAS*** mutations and 13% had ***PIK3CA*** mutations (exon 9 and 20). Although *NRAS* and *PIK3CA* exon 20 mutations are associated with poorer outcomes, confidence intervals are wide given low number of mutations. Findings from other studies with and without overlapping populations were either not informative (because low or no mutations identified, limitations in outcome reporting) or consistent with findings from the study by DeRoock and colleagues. No other studies report results for *PIK3CA* exon 9 and 20 separately. Studies evaluating protein expression of **PTEN** and **AKT** generally used IHC, but used different antibodies and scoring systems. There is some evidence to suggest that **PTEN** loss may be associated with non-response, though results are conflicting between studies. Limitations at the individual study level prevent more definitive conclusions. No studies reported results for **AKT** loss in *KRAS* wild-type. Based on one study, PTEN and AKT protein expression are only concordant in 60% and 68% respectively, of primary and metastatic tumors

Table 6. Summary of evidence by key question (cont.)

Number and Design of Studies	Major Limitations	Validity of Evidence	Summary of Findings
KQ3: Clinical validity or utility: In patients with mCRC, do treatment choices based on genetic testing (i.e., BRAF, NRAS, PIK3CA, PTEN, AKT) lead to improvement in survival (overall survival, progression-free survival), improvement in quality of life, or reduction in morbidity?			
14 studies total --13 retrospective single arm cohort -- 1 retrospective analysis of RCT Studies with unique populations: -- 3 *BRAF* (n=968) -- 1 *NRAS* (n=649) -- 3 *PIK3CA* (n=968) -- 6 PTEN (n=652) -- 2 AKT (n=194)	Overlapping populations. Retrospective and many small (n<100) studies. Limited reporting of important patient/setting characteristics. Very little evidence for panitumumab. Sparse reporting of survival outcomes. Possible differing definitions of PFS. No quality of life outcomes. Limited reporting of outcomes by *KRAS* wild-types (*PIK3CA,* PTEN, AKT). Only one study compared outcomes with persons who did not receive anti-EGFR therapy.	Quality: Fair to poor Applicability: mostly European patients, poor descriptions of patient characteristics and setting of treatment; findings strongest for chemorefractory patients getting cetuximab; gene mutation testing on primary tumor sample, but may be of issue for protein expression; mutations primarily assessed or confirmed with direct sequencing	Best evidence for ***BRAF, NRAS*, and *PIK3CA*** comes from the largest retrospective study (n=649) of a European consortium of patients. For mutation frequencies, see Key Question 2. In this study, ***BRAF*** mutation was also associated with worse progression-free survival (absolute difference 18 weeks, p< 0.0001), and overall survival (absolute difference 28 weeks, p< 0.0001). Although ***NRAS*** and ***PIK3CA*** exon 20 mutations are associated with poorer survival outcomes, confidence intervals are wide given low number of mutations. In another study comparing disease control in persons who did (n=227) and did not (n=332) receive cetuximab with combination chemotherapy, differences in PFS and OS by ***BRAF*** mutation were essentially the same for both treatment arms. Survival was noticeably better in this study, as compared to other studies, which suggests important clinical heterogeneity. Findings from other studies with and without overlapping populations were either not informative (because low or no mutations identified, limitations in outcome reporting) or consistent with findings from the study by DeRoock and colleagues. Studies used different thresholds to dichotomize protein expression positivity or loss. Based on limited evidence, **PTEN** expression does not appear to have clinically robust ability to predict survival response to cetuximab or panitumumab. No studies reported results for **AKT** loss in *KRAS* wild-type.
KQ4: Harms: What are the potential harms to patients of performing these genetic tests related to anti-EGFR antibody treatment decisions, including incorrect genotype assignment leading to incorrect treatment assignment, ELSI risks, and other risks associated with this testing?			
No additional studies identified.	No clinically significant harms of testing hypothesized (other than incorrect genotype assignment leading to incorrect treatment assignment). Limitations in same as for KQ2.	Quality: Fair to poor Applicability: applicability of test performance same as for KQ2.	Best evidence for ***BRAF, NRAS*, and *PIK3CA*** comes from the largest retrospective study (n=649) of a European consortium of patients. Calculated point estimates of false positives (from whom potentially effective treatment is withheld) for *BRAF, NRAS,* and *PIK3CA* exon 20 are estimated below 2.0%.

Table 7. Comparison of evidence for *KRAS* vs. *BRAF* pharmacogenetic testing for anti-EGFR therapy in metastatic colorectal cancer

		KRAS (codons 12, 13)*		*BRAF* (V600E)	
Purpose of study	**Mutation frequency**	~30 to 45%		~5 to 20%	
	Evidence†	24 studies, n=2242‡		7 studies, n=1224§	
	Indication	Chemorefractory persons with mCRC		Chemorefractory persons with mCRC, with *KRAS* WT tumor	
	Description of study design‖	**# Studies in ≥second-line tx**	**# Studies in first-line tx**	**# Studies in ≥second-line tx**	**# Studies in first-line tx**
Clinical utility or clinical validity	RCT of PGX testing vs. no testing strategies	None		None	
	Prospective cohort study of PGX testing vs. no testing strategies	None		None	
	Prospective evaluation of PGX test from RCT of tx vs. no tx	1	1	None	
	Retrospective evaluation of PGX test from RCT of tx vs. no tx	1	4	None	1
Clinical validity (association only)	Prospective evaluation of PGX test from cohort who received tx	1	1	1¶	None
	Retrospective evaluation of PGX test from cohort who received tx	13	1	5¶ **	None
	Well done case-control study (only if outcomes of interest are rare)	Not applicable			
Analytic validity	Not systematically reviewed	Multiple mutations in multiple codons, clinical performance may depend on analytic considerations of assay		Single mutation in single codon, assay(s) likely with good accuracy and reproducibility	

***** Evidence from Tufts' *KRAS* systematic review [47]
† Studies with non-overlapping populations
‡ Evidence for *KRAS* based on 45 studies, only 24 of which reported on independent patient populations
§ Evidence for *BRAF* based on 20 studies, only 7 of which reported on independent patient populations
‖ Quality (internal validity) of study not addressed in this table
¶ One study did not identify any *BRAF* mutations
** Only one study (n=649) reported outcomes in persons with *KRAS* wild-type tumors

- number, mCRC- metastatic colorectal cancer, PGX- pharmacogenetic, RCT- randomized controlled trial, Tx- treatment or chemotherapy, Vs.- versus, WT- wild-type

Table 8. GRADE based summary of overall quality of evidence by key question

Outcome (key question)	No. studies (n pts)	Study design	Factors that may decrease quality of evidence					Overall quality of evidence
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	
Rx efficacy: Tumor response (KQ2)	7 *BRAF* (n=1224)	Almost exclusively retrospective evaluation of single arm cohort of patients getting EGFR inhibitors	Serious	No, but mainly applicable to c-mab combo tx in chemo-refractory	No	Serious	No known publication bias	Low
	1 *NRAS* (n=649)		Serious		Only one study	Very serious		Very low
	4 *PIK3CA* (n=1030)		Serious		Serious	Very serious		
	8 PTEN (n=742)		Very serious		Very serious	Very serious		
	5 AKT (n=294)		Very serious		No	Not applicable		
Survival: PFS or OS (KQ3)	3 *BRAF* (n=968)	Almost exclusively retrospective evaluation of single arm cohort of patients getting EGFR inhibitors	Serious	No, but mainly applicable to c-mab combo tx in chemo-refractory	No	Serious	No known publication bias, possible selective reporting bias	Low
	1 *NRAS* (n=649)		Serious		Only one study	Very serious		Very low
	3 *PIK3CA* (n=968)		Serious		Serious	Very serious		
	6 PTEN (n=652)		Very serious		No	Very serious		
	2 AKT (n=194)		Very serious		No	Not applicable		
Quality of life (KQ3)	None	Not applicable						
Harms (KQ4)	No additional studies	Retrospective evaluation of cohort studies	Serious	Serious	No	Serious	Not applicable	Low to very low

Low- further research is very likely to have an important impact on confidence in the estimate of effect and is likely to change the estimate; n Pt- number of patients; No. studies- number of studies with unique populations; OS- overall survival; PFS- progression free survival; Risk of bias- limitations in quality; Rx efficacy- treatment efficacy or effectiveness; Serious- Serious limitations or inconsistency; Tumor response- based on radiographic criteria; Very low- any estimate of effect is very uncertain

Table 9. Upcoming relevant trials

Source	Study ID or First Author	Title	Details
clinicaltrials.gov	NCT01086267	Safety and Efficacy Study of BMS-908662 Alone or in Combination With Cetuximab in Subjects With K-RAS or B-RAF Mutation Positive Advanced or Metastatic Colorectal Cancer	Recruiting Completion Date: July 2012
clinicaltrials.gov	NCT00975897	Study of Tumor Tissue Testing in Selecting Treatment for Patients With Metastatic or Locally Advanced Colorectal Cancer	Recruiting Completion Date: NR
clinicaltrials.gov	NCT00655499	Panitumumab and Irinotecan as Third-Line Therapy in Treating Patients With Metastatic Colorectal Cancer	Recruiting Completion Date: NR
clinicaltrials.gov	NCT01243372	Biomarkers in Predicting Response to Cetuximab in Patients with Advanced Colorectal Cancer	Not yet recruiting Completion Date: NR
clinicaltrials.gov	NCT01126450	Lenalidomide and Cetuximab in Treating Patients with Metastatic Colorectal Cancer	Recruiting Completion Date: NR
clinicaltrials.gov	NCT00202787	Safety and Efficacy of Combination Therapy with Cetuximab and FOLFOX4 in Patients with Colorectal Cancer	Completed February 2009 No published article found
ISRCTN	ISRCTN83171665	FOCUS 3: the feasibility of molecular selection of therapy using KRAS, BRAF, and topo-1 in patients with metastatic or locally advanced colorectal cancer	Recruiting Completion Date: NR, assigned July 2009 Sponsored by the Medical Research Council (UK)
ESMO GI 2009	Saletti, P.	RAF kinase inhibitor protein (RKIP) is not a predictive factor in patients with K-RAS wild-type metastatic colorectal cancer (MCRC) treated with cetuximab or pantiumuab	Conference abstract only No published article found
ECCO15-ESMO34	Spindler, K.	Triple mutational testing for response to EGFR inhibitor treatment with cetuximab and irinotecan in metastatic colorectal cancer	Conference abstract only No published article found
ECCO15-ESMO34	van Cutsem, E.	A meta-analysis of the CRYSTAL and OPUS studies combining cetuximab with chemotherapy (CT) as 1st-line treatment for patients with metastatic colorectal cancer (mCRC): results according to KRAS and BRAF mutation status	Conference abstract only No published article found
AACR 2010	Peeters, M.	Use of massively parallel, next-generation sequencing to identify gene mutations beyond KRAS that predict response to panitumumab in a randomized, phase 3, monotherapy study of metastatic colorectal cancer (mCRC)	Conference abstract only No published article found
ASCO 2010	di Salvatore, M.	KRAS and BRAF mutational status and PTEN, cMET, and IGF1R expression as predictive markers of response to cetuximab plus chemotherapy in metastatic colorectal cancer	Conference abstract only No published article found

Table 9. Upcoming relevant trials (cont.)

Source	Study ID or First Author	Title	Details
ASCO 2010	Yokota, T.	PTEN/p-AKT expression as predictive markers for cetuximab in colorectal cancer	Conference abstract only No published article found
ASCO 2010	Sood, A.	Beyond KRAS: the quest for novel genetic markers predictive for response to anti-epidermal growth factor receptor (EGFR) therapy in patients with metastatic colorectal cancer	Conference abstract only No published article found
ASCO 2010	Weickhardt, A.	DUX study: a phase II study of evaluating dual targeting of the EGFR using the combination of cetuximab and erlotinib in patients with chemotherapy refractory metastatic colorectal cancer	Conference abstract only No published article found
ASCO 2010	Maughan, T.	Identification of potentially responsive subsets when cetuximab is added to oxaliplatin-fluoropyrimidine chemotherapy (CT) in first-line advanced colorectal cancer (aCRC): mature results of the MRC COIN trial	Conference abstract only No published article found
ASCO 2010	Schwartzberg, L.	PEAK: a randomized phase II study to compare the efficacy of panitumumab plus mFOLFOX6 in patients (pts) with previously untreated, unresectable metastatic colorectal cancer expressing wild-type KRAS	Conference abstract only No published article found
ASCO 2010	Blank, P.	Cost-effectiveness of novel predictive tests in treatment of metastatic colorectal cancer: An analysis from a Swiss perspective	Conference abstract only No published article found
ASCO 2010	Garrido-Laguna, I.	Phosphatase and tensin homologue (PTEN) loss and response to phase I trials targeting PI3K/AKT/mTOR pathway in patients with advanced cancer	Conference abstract only No published article found
ASCO 2010	Janku, F.	PIK3CA, KRAS, and BRAF mutations in patients with advanced cancers treated with PI3K/AKT/mTOR axis inhibitors	Conference abstract only No published article found
ASCO 2009	Garcia, E.	Use of combined biomarkers analysis to predict response to chemotherapy in colorectal cancer: a single-institution feasibility study	Conference abstract only No published article found
ASCO 2009	Bengala, C.	EGFR gene copy number, KRAS and BRAF status, PTEN and AKT expression analysis in patients with metastatic colon cancer treated with anti-EGFR monoclonal antibodies +/- chemotherapy	Conference abstract only No published article found
ASCO 2009	Ruzzo, A.	Association of BRAF mutations and EGFR Intron-1 L/L genotype with resistance to cetuximab plus irinotecan treatment in KRAS wild-type metastatic colorectal cancer patients	Conference abstract only Portion of results have been published: Loupakis F, et al. KRAS codon 61, 146 and BRAF Mutations…Br J Cancer. 2009. 101(4):715-21.
ASCO GI 2009	Norguet, E.	Use of nuclear PTEN expression status to predict survival of metastatic colorectal cancer patients treated with cetuximab	Conference abstract only No published article found

Table 9. Upcoming relevant trials (cont.)

Source	Study ID or First Author	Title	Details
ASCO GI 2010	Kato, K.	Efficacy of cetuximab against KRAS wild-type colorectal cancer with BRAF or PIK3CA mutations	Conference abstract only No published article found
ASCO GI 2010	Xie, L.	Practical application of using predictive biomarkers to select patients with metastatic colorectal cancer for treatment with epidermal growth factor receptor inhibitors	Conference abstract only No published article found
ASCO GI 2010	Sood, A.	Use of PTEN expression in the primary tumor as a predictive marker for radiologic response to anti-epidermal growth factor receptor (EGFR) based therapy in patients with metastatic colorectal cancer (mCRC)	Conference abstract only No published article found
ASCO GI 2010	Linot, B.	Impact of PI3K, BRAF, and KRAS mutations on efficacy intensified FOLFIRI + cetuximab regimen in advanced colorectal cancer	Conference abstract only No published article found
ASCO GI 2010	Bokemeyer, C.	Biomarkers predictive for outcome in patients with metastatic colorectal cancer (mCRC) treated with first-line FOLFOX4 plus or minus cetuximab: updated data from the OPUS study	Conference abstract only No published article found
ASCO GI 2010	Kohne, C.	Cetuximab with chemotherapy (CT) as first-line treatment for metastatic colorectal cancer: a meta-analysis for the CRYSTAL and OPUS studies according to KRAS and BRAF mutation status	Conference abstract only No published article found Results presented at ECCO15-ESMO34, ASCO 2010, and ESMO GI 2009.
ESMO 2010	Stintzing, S.	Cetuximab plus xeliri versus cetuximab plus xelox as first-line treatment for patients with metastatic colorectal cancer (mCRC): analysis of the randomized trial of the German AIO CRC study group: KRK-0204	Conference abstract only No published article found
ESMO 2010	Smith, C.G.	High throughput somatic profiling of the RAS-RAF-MAP pathways in advanced colorectal cancer and correlations with response to cetuximab	Conference abstract only No published article found
ESMO 2010	Rebersek, M.	Correlation of BRAF status with clinical response to cetuximab in KRAS wild type (WT) metastatic colorectal (mCRC) patients-single institution experience	Conference abstract only No published article found