Biomarker testing for treatment of metastatic colorectal cancer: role of the pathologist in community practice

Rafael Rodriguez, MD

PathLogic, West Sacramento, California

The past decade has been marked by significant advancements in the treatment of patients with metastatic colorectal cancer (mCRC), including the approval of novel biologic agents such as the angiogenesis inhibitors bevacizumab and aflibercept and the epidermal growth factor receptor monoclonal antibodies (mAbs) cetuximab and panitumumab. Cetuximab was recently approved by the US Food and Drug Administration in combination with FOLFIRI (irinotecan, 5-fluorouracil, leucovorin) for the first-line treatment of patients with KRAS mutation-negative (wild-type) tumors as determined by an FDA-approved companion diagnostic. It was the first FDA approval in mCRC requiring use of a diagnostic test that is predictive of response prior to initiation of frontline therapy. The approval highlights the need for reflexive KRAS mutation testing at diagnosis to accurately determine all available treatment options. Although KRAS testing has been used by pathologists in mCRC for several years, accurate and timely reporting of test results and open communication with medical oncologists is even more essential to ensure appropriate first-line treatment selection and avoid any treatment delays. Consequently, it is critical that pathologists are highly trained and educated on KRAS testing methodology and the pros and cons of the various methods available. In this review, we discuss the development of KRAS as a biomarker in mCRC and key topics of concern to the pathologist who works with the oncologist in the community setting, including specimen preparation, KRAS testing methods, and reporting of test results. In this era of personalized medicine, pathologist education and communication with oncologists on biomarkers is paramount for optimal patient care.

Colorectal cancer (CRC) is the second most common cancer in the United States, leading to more than 50,000 estimated deaths in 2012.[1] Despite advances, most patients (50%-60%) will be diagnosed with metastatic CRC (mCRC), which has a 5-year survival rate of 12%.[2] Therefore, there is an ongoing clinical need for novel treatments and improvements in diagnostic assays.

For nearly 50 years, the only effective treatment for mCRC was the fluoropyrimidine, 5-fluorouracil (5-FU). The folic acid derivative leucovorin (LV) enhanced response rates to 5-FU.[3] However, there have been significant advancements in the treatment of mCRC over the past 15 years. Today, 5-FU–LV is combined with other agents, such as irinotecan (FOLFIRI) or oxaliplatin (FOLFOX), to form the most commonly used chemotherapy regimens for the initial treatment of mCRC. More recently, the oral 5-FU prodrug capecitabine has been used in place of 5-FU–LV in these treatment strategies (eg, capecitabine and oxaliplatin).[4] Monoclonal antibody (mAb)-based therapies are also used in combination with first-line chemotherapy regimens. These include the vascular endothelial growth factor inhibitor bevacizumab and the epidermal growth factor receptor (EGFR) inhibitor cetuximab, which are approved in combination with various chemotherapies in the first- and second-line treatment of mCRC. Bevacizumab is approved for the treatment of mCRC with intravenous 5-FU–based chemotherapy for first- or second-line treatment.[5] Notably, cetuximab is approved for treatment of patients with *KRAS* wild-type mCRC.[6] The data leading to this approval will be discussed in more detail later in this article.

Other agents that show activity beyond first-line include the angiogenesis inhibitor aflibercept, which is approved as second-line therapy in combination with FOLFIRI, and the EGFR mAb panitumumab, which is approved as single-agent therapy in the third-line setting.[7,8] The multikinase inhibitor regorafenib has also demonstrated a survival benefit compared with placebo in patients who had progressed on standard therapies and was recently approved.[9] The availability of a greater number of agents significantly improves outcomes for patients; however, it also increases the complexity of selecting the best treatment for the patient.

Accepted for publication October 14, 2013. Correspondence: Rafael Rodriguez, MD (Rafael.Rodriguez@pathlogic.com).
Disclosures: Financial support for editorial assistance was provided by Bristol-Myers Squibb Company.
Dr. Rodriguez is a pathologist at PathLogic. He has no financial disclosures. JCSO 2014;12:27-32. DOI 10.12788/jcso.0006.

Therefore, accurate determination of biomarker status at diagnosis is necessary to inform physicians of the best potential treatment course for their patients.

Diagnostic tests for CRC

As prognosis for patients with mCRC is poor, the accurate assessment of potential prognostic or predictive factors is essential prior to initiating therapy. Communication between the oncologist and pathologist on the test(s) to be ordered, the data obtained, and the potential implications are of substantial importance. Of increasing importance is mutational testing of the *KRAS* gene. Given the importance of *KRAS* mutational analyses in determining treatment options for patients with mCRC, community practices should move toward reflexive testing of *KRAS* at diagnosis. Communication between oncologists and pathologists should be open in an effort to ensure that *KRAS* testing is conducted reflexively.

For patients with a confirmed diagnosis of metastatic disease, *KRAS* mutational status is the only known definitive biomarker for predicting therapeutic response to EGFR-targeted therapy. *KRAS* mutational analysis is also the only predictive biomarker test included in the prescribing information for therapies specific to mCRC. The recently updated prescribing information for cetuximab states that cetuximab is indicated only for patients with *KRAS* mutation-negative, EGFR-expressing mCRC as determined by a test approved by the US Food and Drug Administration (FDA).[6] The prescribing information was updated in conjunction with cetuximab's approval for use as a first-line therapy in combination with FOLFIRI. The label for panitumumab was also updated to include testing.[8] Even before this update, many oncologists and pathologists ordered these tests based on data suggesting that patients with *KRAS* mutations did not benefit from EGFR mAb therapy. On the basis of the recent approval of cetuximab in the first-line setting, accurate determination of *KRAS* mutational status at the start of therapy is essential to determine all treatment options and avoid treatment delays.

Evolution of KRAS as a predictor of response to EGFR mAb therapy

Initial FDA approval of cetuximab in February 2004 was based largely on the results of the randomized Bowel Oncology With Cetuximab Antibody (BOND) trial, which demonstrated efficacy in patients with irinotecan-refractory mCRC treated with cetuximab plus irinotecan or cetuximab monotherapy.[10] In October 2007, the FDA granted another indication for single-agent cetuximab on the basis of positive overall survival (OS) results from the phase 3 CO.17 trial of cetuximab plus best supportive care (BSC) compared with BSC alone.[11] During this time, data emerged suggesting that the benefit from EGFR-targeted mAbs was limited to patients with *KRAS* wild-type mCRC.[12] Definitive support of these data was demonstrated by a retrospective analysis of the CO.17 trial, which demonstrated a significantly longer OS for patients with *KRAS* wild-type mCRC who received cetuximab–BSC compared with BSC alone (median, 9.5 vs 4.8 months, respectively; $P < .001$), whereas there was no benefit for patients with *KRAS*-mutant mCRC (median, 4.5 vs 4.6 months; $P = .89$).[13] Similar results were observed in a retrospective analysis of a phase 3 trial comparing panitumumab and BSC, which demonstrated a significant improvement in progression-free survival (PFS) among patients with *KRAS* wild-type mCRC compared with those with *KRAS*-mutant mCRC (median, 12.3 weeks vs 7.3 weeks; $P < .0001$).[14]

In April 2009, the American Society of Clinical Oncology issued a provisional clinical opinion regarding *KRAS* testing for patients with mCRC based on the results of 10 randomized and single-arm studies of cetuximab or panitumumab that retrospectively evaluated response according to *KRAS* status.[15] This opinion stated that patients with mCRC who are candidates for anti-EGFR mAbs should have their tumors tested for *KRAS* mutations by a Clinical Laboratory Improvement Amendments (CLIA)-accredited laboratory, and patients with codon 12 or 13 *KRAS* mutations should not receive anti-EGFR mAb therapy. The National Comprehensive Cancer Network (NCCN) also concurrently updated their guidelines, which quickly led to changes in clinical practice prior to any updates to prescribing information. The labels for cetuximab and panitumumab were subsequently updated in July 2009 to state that these drugs are not recommended for patients with *KRAS* codon 12 or 13 mutations. At the time of the label updates, there were several commercially available tests for determining *KRAS* mutations, and many CLIA-accredited laboratories and university hospitals were adequately prepared to conduct *KRAS* mutational analyses. However, none of these tests were FDA approved.

As data emerged on the role of *KRAS* mutations as a marker of resistance to EGFR mAb, a phase 3 trial of cetuximab–FOLFIRI compared with FOLFIRI alone for the first-line treatment of mCRC (Irinotecan in First-line Therapy for Metastatic Colorectal Cancer [CRYSTAL]) was being conducted. Although initial results of the CRYSTAL trial did not demonstrate a benefit in OS with cetuximab–FOLFIRI over FOLFIRI alone for the entire population, a benefit was observed among patients with *KRAS* wild-type mCRC (median, 24.9 vs 21.0 months; hazard ratio [HR], 0.84).[16] Only 45.1% of patients had a discernible *KRAS* status at the time of the original report. A more recent analysis with a higher ascertainment rate for *KRAS* status (89%) and longer median follow-up time (46.8 months) demonstrated a significant survival benefit for patients with *KRAS* wild-type tumors who received cetuximab.[16] These data led to the label update for cetuximab use only in patients with *KRAS* mutation-negative mCRC.[6]

TABLE 1 Technologies for *KRAS* mutation analysis

Method or technology	Kit (manufacturer)	Sensitivity MT/WT, %	Time to result	Advantages	Disadvantages
Sanger-based sequencing: dideoxynucleotide termination	BigDye Terminator Sequencing Kit (Life Technologies Corp, Grand Island, NY)[a]	15-25	≈ 1 wk	Sequencing reaction can be repeated in the same tube Can be automated in a DNA thermocycler	Low sensitivity Labor intensive
Pyrosequencing: analysis of pyrophosphate release during DNA synthesis	PyroMark KRAS v2.0 test (Qiagen, Manchester, UK)	5-10	Fast	High throughput Precise/reproducible Suitable for partially degraded samples	Expensive
Real-time PCR with melt-curve analysis for mutation detection	cobas KRAS Mutation Test (Roche Molecular Diagnostics, Pleasanton, CA)	< 5	Rapid: < 8 h	High-performance amplification and detection Automated software	Separate assay must be run for each mutation
Real-time PCR using allele-specific primers: ARMS with Scorpions technology	therascreen KRAS RGQ PCR Kit (Qiagen)	1-5	Rapid: 2 d total; 2 h to process samples	Rapid results High sensitivity Commercially available FDA approved	Detects only 7 common mutations Requires more tissue Very expensive

ARMS, amplification refractory mutation system; FDA, US Food and Drug Administration; PCR, polymerase chain reaction
[a]Test is not specific for KRAS, primers encompassing exon 2 of the KRAS gene can be used in conjunction with this test. Ross J. Arch Pathol Lab Med. 2012;136:1298-1307. Copyright College of American Pathologists. Adapted with permission.

Ideally, *KRAS* mutational status should be determined by an FDA-approved test. Currently, the only FDA-approved test for *KRAS* mutational status is the therascreen KRAS RGQ PCR Kit (Qiagen). The test is approved as a companion diagnostic to identify patients who may benefit from cetuximab. These recent approvals will undoubtedly affect treatment practices and testing patterns, particularly in the community setting.

Current KRAS mutational analysis considerations and practices

The most recent NCCN guidelines for CRC provide guidance on *KRAS* mutational analysis.[4] They suggest that *KRAS* status of tumor tissue (primary or metastatic) should be determined at the time of diagnosis of stage IV disease. Early determination of *KRAS* mutational status is important so that the physician can discuss potential treatment options with the patient. For patients with nonmetastatic disease (stages I-III), *KRAS* testing is not recommended, as the benefit of cetuximab has not been established in these settings. In certain instances, confusion may arise when a tissue is indicated as pathological stage 4 (pT4), which is indicative of a tumor that has penetrated the visceral peritoneum or invaded an adjacent structure and may not necessarily be metastatic (TNM stage IV). The NCCN recommends that testing be performed in laboratories that are CLIA 88 certified but does not currently recommend any specific methodology.[4]

Before cetuximab was approved as a first-line therapy, *KRAS* mutational analysis was often not conducted prior to the initiation of first-line therapy. Therefore, this afforded oncologists and pathologists more time to analyze patient samples. However, the use of EGFR-targeted therapy in the first-line setting will require more efficient sample analysis and will make it more important to address the limitations and issues facing community oncologists and pathologists with respect to *KRAS* testing, such as tissue collection, *KRAS* testing methods (including the FDA-approved assay), and accuracy of pathology results.

The first consideration prior to conducting a *KRAS* test is tissue collection. *KRAS* mutations occur early in the disease process, and there is significant correlation in *KRAS* mutation status between primary tumor and metastases. Therefore, sampling from either site is acceptable and recommended by the NCCN, although samples from the primary site are preferred if they are available.[4,17] Most *KRAS* assays use formalin-fixed (10% buffered formalin) paraffin-embedded tissue as the starting material, although frozen tissue may be acceptable in certain instances. The site of the tumor tissue should be adequately identified, and the pathologist should seek to identify nuclei-rich samples that have a potential for high tumor-DNA content and a low level of necrotic tissue. Pathologists generally collect the deepest invasive portion of the tumor for sampling. This practice is supported by evidence suggesting that invasive tumors are reflective of a malignant subclone that outgrew the other lesions. These tumors often contain tumorigenic mutations, such as *KRAS*. Investigation of differences in intratumoral mutations has shown that premalignant lesions demonstrate a high level of heterogeneity, while advanced stage primary tumors are generally more homogenous.[18] Awareness of the sensitivity of the *KRAS* testing method to be used will help to determine whether the tissue is adequate for analysis. In instances in which obtaining adequate tissue is a challenge should be noted so that caution can be exercised when interpreting results or additional tissue or blocks can be obtained.

Although an FDA-approved assay is now available, some hospitals and reference laboratories may use other avail-

able methods for determination of *KRAS* mutational status. Physicians recognize the level of scrutiny that is required for FDA approval; however, several factors may affect the choice of the *KRAS* test, including cost, availability of reagents and equipment, turnaround time, and experience and comfort level with a specific method and/or reference laboratory. As noted, the most recent NCCN guidelines do not provide specific guidance on assay selection. In community practice, samples are often sent out to reference laboratories. Knowledge of the methods used by these laboratories can help to inform on the implications of the results. A summary of some of the available methods is shown in Table 1.[19]

The traditional method for detection of mutations in DNA, including *KRAS* mutation, is Sanger (dideoxy) sequencing. The original methodology was developed in 1977 and is considered the gold standard in DNA sequencing.[20] For this method, DNA sequencing reactions are conducted using a self-terminating dideoxynucleotides (in place of standard deoxy). Four separate reactions are conducted for each dideoxynucleotide. The reaction products are analyzed by gel electrophoresis and analyzed visually or using a sequence analyzer.[21] Although this method has the potential of detecting all mutations within the sequence analyzed, a key advantage over other methods, drawbacks include a lack of sensitivity and a significant effort needed to complete, which leads to a slow turnaround time (Table 1). A more modern form of direct sequencing, known as pyrosequencing, is based on detection of the released pyrophosphate during the DNA synthesis reaction. This method has higher sensitivity than Sanger sequencing and is also faster and less labor intensive; however, it is much more expensive as it requires an instrument capable of detection. A kit specifically designed for detection of *KRAS* mutations is commercially available (PyroMark KRAS, Qiagen); however, it is not currently recommended for diagnostic use by the manufacturer. This kit can detect all mutations within codons 12 and 13 as well as codon 61. It should be noted that the clinical implications of codon 61 mutations remain inconclusive, and testing is not part of the current NCCN recommendations.

Newer polymerase chain reaction (PCR)–based detection methods have improved sensitivity, reproducibility, and rapid turnaround times, which can be essential when information is needed to help guide treatment decisions. These methods rely on novel real-time detection methods and are associated with a higher cost. One PCR-based assay is the cobas KRAS Mutation Test kit (Roche Molecular Diagnostics). This test uses PCR amplification of exons 2 and 3 of the *KRAS* gene and fluorescent-labeled probes that specifically identify all known mutations within codons 12, 13, and 61.[22] Following the PCR, the products are analyzed for changes in their melting temperature (melting curve analysis) with the software package, and a report containing information on the *KRAS* mutation is generated. This kit is not currently commercially available in the United States.

As discussed, the only FDA-approved test for detecting *KRAS* mutations is the therascreen KRAS RGQ PCR kit. This test detects the 7 most common mutations within codons 12 and 13: 12Ala (G12A), 12Asp (G12D), 12Arg (G12R), 12Cys (G12C), 12Ser (G12S), 12Val (G12V), and 13Asp (G13D). Mutations are identified using a technology known as amplification refractory mutation system (ARMS) and Scorpions primers (Sigma-Aldrich).[23] The ARMS component of the primer preferentially amplifies the mutant sequences, and the Scorpions portion recognizes the newly formed amplicon, leading to fluorescence, which can be detected using a real-time thermocycler. Additional advantages of this test include reproducibility, sensitivity, and a rapid time to result. The primary advantage of this test is that it has FDA approval, which likely will lead to widespread use of the test. Table 2 outlines the laboratories identified by the manufacturer that are currently using the therascreen kit.

As pathologists often use the same reference laboratories for specific tests they may not be aware of the specific methodology used by the reference laboratory or whether the laboratory is using an FDA-approved assay. Pathologists often have access to different laboratories offering the same tests, and knowing the methodologies used by each has become even more important in selecting the appropriate laboratory. Table 3 outlines the *KRAS* testing methodologies used by several reference laboratories commonly used by community physicians. Awareness of the methodology, including any limitations and potential implications depending on the results, is of interest to all health care providers and patients. Often the pathologist will act as the liaison between the oncologist and reference laboratory; therefore, it is important to maintain consistent flow of information.

A key part of maintaining consistent flow of information is standardization of reporting. Unfortunately, there is no current standard for reporting *KRAS* mutations, and this means that certain details may not be included or

TABLE 2 Laboratories that currently offer the therascreen KRAS test

Applied Diagnostics
Boyce and Bynum Pathology Laboratories
CellNetix Pathology and Laboratories
Clarient Diagnostic Services
Companion Dx Reference Lab
Dahl-Chase Diagnostic Services
Indiana University Health Pathology Laboratory
Lab21
Mayo Clinic
University of Kansas Medical Center, Department of Pathology and Laboratory Medicine

recorded consistently in pathology reports. Another challenge with the reports is that they often become cumbersome and complicated. In an attempt to address this, the International Organization for Standardization and the College of American Pathologists have provided guidance on proper reporting of molecular testing results, including *KRAS* mutation results.[24,25] Fundamental information about the patient, the specimen, the ordering physician, and the laboratory where testing was done should be included in all reports.[24] Reports should be clear and concise and should always include whether a mutation was detected, the specific mutation identified (eg, G12D), the method used and specific mutations that were tested, and the potential clinical implications. Incomplete reporting has been cited as one of the key sources of inaccuracy in *KRAS* testing.[19,25] In a study designed to examine the reliability of community-based *KRAS* testing throughout Europe, a *KRAS* external quality assessment scheme was set up to evaluate results from 59 laboratories in 8 European countries.[25] The pages of most reports were incorrectly numbered, and reports often lacked the list of mutations tested, a name and/or address of the person the report was referred to, and a referral to anti-EGFR therapy if a positive result was found; incorrect nomenclature was also used.

Now that cetuximab in combination with FOLFIRI is approved for first-line treatment of patients who have *KRAS* wild-type mCRC, there is a greater need to determine *KRAS* status at diagnosis. Although having this information at diagnosis was useful before the first-line approval of cetuximab so that physicians would be aware of all potential treatment options, it may not have been viewed as being necessary because EGFR mAbs were typically used later in the course of a patient's therapy. As *KRAS* testing at diagnosis becomes more common, it is important to be aware not only of the key issues mentioned above, but also to keep abreast of ongoing developments and emergence of other novel biomarkers.

New developments and considerations in KRAS testing and research

Data on the role of KRAS mutations and resistance to EGFR mAb therapy is fairly straightforward, indicating that patients with codon 12 and 13 KRAS mutations do not benefit; however, similar to most clinical phenomena, there are exceptions and evolving data may change our understanding of current clinical practice. One example of this is recent research on the role of the KRAS G13D mutation. This mutation is currently detected by most tests, including the therascreen kit, and cetuximab is not recommended for patients with this mutation. A pooled analysis of 579 patients was conducted to study the influence of the KRAS G13D mutation compared with other KRAS mutations in patients with chemorefractory mCRC who were treated across 7 clinical trials.26 This analysis demonstrated that patients with G13D (n = 32) demonstrated longer median OS than did patients with other KRAS mutations (7.6 months vs 5.7 months, respectively; P = .005) and longer median PFS (4.6 months vs 1.9 months; P = .004). Another study pooled data from 1,378 evaluable patients from the frontline CRYSTAL and Oxaliplatin and Cetuximab in First-Line Treatment of mCRC (OPUS) studies and found that the addition of cetuximab to first-line chemotherapy seemed to benefit patients with KRAS G13D-mutant tumors; relative treatment effects were similar to those in patients with KRAS wild-type tumors but with lower absolute values. 27 Although these data are intriguing, prospective analyses are needed to confirm these results.

TABLE 3 Large reference laboratories and CRC KRAS testing methods

Laboratory	Testing method
ARUP	PCR/pyrosequencing
Quest Diagnostics	Colorectal cancer mutation panel PCR/sequencing
Laboratory Corp of America	ARMS + Scorpions PCR/pyrosequencing

ARMS, amplification refractory mutation system; PCR, polymerase chain reaction

As noted in the methodology section, the *KRAS* therascreen kit does not detect mutations other than the common 12 and 13 mutations. Additional mutations in the *KRAS* gene, including codon 61 and 146 mutations, have been described and have been suggested to confer resistance to anti-EGFR mAbs.[28] It should be noted that these observations are limited and need to be verified in larger randomized studies. These emerging data highlight the importance of recording the specific mutation identified.

It is generally accepted that there is a high concordance rate of *KRAS* mutations between primary and metastatic tumors. Of note, a recent study from Memorial Sloan Kettering Cancer Center of 613 patients demonstrated a concordance of more than 90% for *KRAS* mutation.[17] However, in a recent study of 143 Korean patients with mCRC, the rate of concordance differed depending on the site of the initial metastatic lesion, with liver metastases demonstrating a higher concordance with the primary tumor compared with lung metastases.[29] Another study demonstrated that *KRAS* mutations can be acquired in patients treated with an EGFR mAb.[30] Together, these data highlight the importance of treatment history and the nature of the tumor sample analyzed for *KRAS* mutational analysis when using test results to influence treatment decisions.

Several additional biomarkers have been implicated in resistance to EGFR mAb therapy, including mutations of the *BRAF*, *NRAS*, and *PIK3CA* genes and loss of expression of the *PTEN* gene.[31] Of these biomarkers, *BRAF* currently has the most robust data. Although *BRAF* status was originally thought to confer resistance to cetuximab, analysis of *BRAF* status of patients treated on the CRYSTAL

trial demonstrated that all patients with *BRAF* mutations demonstrated poorer outcomes regardless of therapy.[32] Current data suggest that *BRAF* mutation is a prognostic marker but not predictive of efficacy of therapies used in the treatment of mCRC. An FDA-approved test for determining suitability to receive vemurafenib for patients with melanoma, cobas 4800 BRAF V600 Mutation Test (Roche Molecular Diagnostics), is available. Although there is no recommendation at this time for *BRAF* testing even when *KRAS* is wild-type, this information may be useful.

Conclusions

KRAS mutation remains the most robust predictive biomarker for patients with mCRC. Several testing methods are currently available, and a thorough understanding of the methodology as well as the pros and cons of each method is important for interpretation of the test results. The approval of cetuximab in combination with FOLFIRI for the first-line treatment of patients with *KRAS* wild-type mCRC as determined by an FDA-approved test increases the importance of determining *KRAS* mutation status at diagnosis of mCRC. Information about the patient, the specimen, the ordering physician, and the laboratory where testing was done will be important for making treatment decisions and for interpretation of results. This information should be included in all reports and should guide communication between pathologists and oncologists who are involved in patient care. As new clinical data and advancements in *KRAS* methodology and additional biomarkers are identified, continued collaboration between pathologists and oncologists will be important for improving patient care.

Acknowledgments

The author thanks Andrea Hammons, PhD, and William Fazzone, PhD, for their medical editorial assistance.

References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin. 2012;62:10-29.
2. American Cancer Society. Cancer Facts & Figures 2012. Atlanta, GA: American Cancer Society; 2012.
3. Erlichman C, Fine S, Wong A, Elhakim T. A randomized trial of fluorouracil and folinic acid in patients with metastatic colorectal carcinoma. J Clin Oncol. 1988;6:469-475.
4. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology: Colon cancer version 3.2012. http://www.nccn.org/professionals/physician_gls/pdf/colon.pdf. Accessed November 26, 2012.
5. Avastin (bevacizumab) [package insert]. South San Francisco, CA: Genentech Inc; 2012.
6. Erbitux (cetuximab) [package insert]. Princeton, NJ: Bristol-Myers Squibb Company; 2012.
7. Zaltrap (ziv-aflibercept) [package insert]. Tarrytown, NY, and Bridgewater, NJ: Regeneron Pharmaceuticals Inc and sanofi-aventis US LLC; 2012.
8. Vectibix (panatumumab) [package insert]. Thousand Oaks, CA: Amgen Inc; 2012.
9. Van Cutsem E, Sobrero AF, Siena S, et al. Phase III CORRECT trial of regorafenib in metastatic colorectal cancer (mCRC) [ASCO abstract 3502]. J Clin Oncol. 2012;30(suppl 15).
10. Cunningham D, Humblet Y, Siena S, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med. 2004;351:337-345.
11. Jonker DJ, O'Callaghan CJ, Karapetis CS, et al. Cetuximab for the treatment of colorectal cancer. N Engl J Med. 2007;357:2040-2048.
12. Lievre A, Bachet JB, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res. 2006;66:3992-3995.
13. Karapetis CS, Khambata-Ford S, Jonker DJ, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med. 2008;359:1757-1765.
14. Amado RG, Wolf M, Peeters M, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol. 2008;26:1626-1634.
15. Allegra CJ, Jessup JM, Somerfield MR, et al. American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. J Clin Oncol. 2009;27:2091-2096.
16. Van Cutsem E, Kohne CH, Hitre E, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med. 2009;360:1408-1417.
17. Vakiani E, Janakiraman M, Shen R, et al. Comparative genomic analysis of primary versus metastatic colorectal carcinomas. J Clin Oncol. 2012;30:2956-2962.
18. Losi L, Baisse B, Bouzourene H, Benhattar J. Evolution of intratumoral genetic heterogeneity during colorectal cancer progression. Carcinogenesis. 2005;26:916-922.
19. Ross JS. Clinical implementation of KRAS testing in metastatic colorectal carcinoma: the pathologist's perspective. Arch Pathol Lab Med. 2012;136:1298-1307.
20. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA. 1977;74:5463-5467.
21. Monzon FA, Ogino S, Hammond ME, Halling KC, Bloom KJ, Nikiforova MN. The role of KRAS mutation testing in the management of patients with metastatic colorectal cancer. Arch Pathol Lab Med. 2009;133:1600-1606.
22. Lee S, Brophy VH, Cao J, et al. Analytical performance of a PCR assay for the detection of KRAS mutations (codons 12/13 and 61) in formalin-fixed paraffin-embedded tissue samples of colorectal carcinoma. Virchows Arch. 2012;460:141-149.
23. Franklin WA, Haney J, Sugita M, Bemis L, Jimeno A, Messersmith WA. KRAS mutation: comparison of testing methods and tissue sampling techniques in colon cancer. J Mol Diagn. 2010;12:43-50.
24. Gulley ML, Braziel RM, Halling KC, et al. Clinical laboratory reports in molecular pathology. Arch Pathol Lab Med. 2007;131:852-863.
25. Bellon E, Ligtenberg MJ, Tejpar S, et al. External quality assessment for KRAS testing is needed: setup of a European program and report of the first joined regional quality assessment rounds. Oncologist. 2011;16:467-478.
26. De Roock W, Jonker DJ, Di Nicolantonio F, et al. Association of KRAS p.G13D mutation with outcome in patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab. JAMA. 2010;304:1812-1820.
27. Tejpar S, Celik I, Schlichting M, Sartorius U, Bokemeyer C, Van Cutsem E. Association of KRAS G13D tumor mutations with outcome in patients with metastatic colorectal cancer treated with first-line chemotherapy with or without cetuximab. J Clin Oncol. 2012;30:3570-3577.
28. Loupakis F, Ruzzo A, Cremolini C, et al. KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer. Br J Cancer. 2009;101:715-721.
29. Kim MJ, Lee HS, Kim JH, et al. Different metastatic pattern according to the KRAS mutational status and site-specific discordance of KRAS status in patients with colorectal cancer. BMC Cancer. 2012;12:347.
30. Otsuka K, Satoyoshi R, Nanjo H, et al. Acquired/intratumoral mutation of KRAS during metastatic progression of colorectal carcinogenesis. Oncol Lett. 2012;3:649-653.
31. Hawkes E, Cunningham D. Relationship between colorectal cancer biomarkers and response to epidermal growth factor receptor monoclonal antibodies. J Clin Oncol. 2010;28:e529-e531; author reply e532-e533.
32. Van Cutsem E, Kohne CH, Lang I, et al. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. J Clin Oncol. 2011;29:2011-2019.