The Role of *KRAS* Mutation Testing in the Management of Patients With Metastatic Colorectal Cancer

Federico A. Monzon, MD; Shuji Ogino, MD, PhD; M. Elizabeth H. Hammond, MD; Kevin C. Halling, MD, PhD; Kenneth J. Bloom, MD; Marina N. Nikiforova, MD

● *Context.*—*KRAS* mutations can be detected in approximately 30% to 40% of all patients with colorectal cancer. Several recent studies have shown that patients with *KRAS* mutations in codons 12 or 13 in metastatic tumors do not benefit from anti–epidermal growth factor receptor therapy with cetuximab or panitumumab.

Objective.—To review the literature on the role of *KRAS* mutation testing for management of patients with metastatic colorectal cancer and to discuss testing strategies.

Data Sources.—This review is based on published, peer-reviewed literature; available information from medical organizations (eg, National Comprehensive Cancer Network, American Society of Clinical Oncology, College of American Pathologists); and information from clinical laboratories conducting *KRAS* mutation analysis.

Conclusions.—Multiple methods for detecting *KRAS* mutations in colorectal tumors are available, and all methods in current clinical use appear to have adequate clinical sensitivity for predicting a lack of response to cetuximab and panitumumab. Pathologist expertise is essential to quality *KRAS* testing and to determining effective treatment for patients with metastatic colorectal cancer.

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In the United States, an estimated 108 070 cases of colon cancer and 40 740 cases of rectal cancer were expected to occur in 2008.[1] Colorectal cancer (CRC) is the third most-common cancer and the second most-common cause of cancer-related deaths. An estimated 49 960 deaths from colon and rectum cancer were expected to occur in 2008, accounting for almost 9% of all cancer deaths.[1] Although the 5-year survival for early, localized-stage CRC is 90%, because of low rates of screening, only 39% of cases are diagnosed at this stage. The 5-year survival drops to 68% after regional spread and to 10% for patients with distant metastases.[1]

For the past 4 decades, the treatment for stage-IV CRC (metastatic CRC) has consisted of fluoropyrimidine-based chemotherapy. Recently, the treatment for patients with metastatic CRC has changed to chemotherapeutic regimens containing irinotecan and oxaliplatin. These regimens have increased time to progression and improved overall survival.[2,3] Most recently, regimens that include 2 anti-epidermal growth factor receptor (EGFR)–targeted antibodies, cetuximab (Erbitux) and panitumumab (Vectibix), for refractory metastatic CRC have been developed.[4,5] Both of these agents have shown promising activity as first- and second-line therapy for metastatic CRC alone or in combination with oxaliplatin or irinotecan.[2,4,6]

The EGFR signaling pathway has been the focus of new drug development for colorectal cancer because it is over-expressed in approximately 80% of colorectal carcinomas.[7] When epidermal growth factor, as well as several other ligands, occupies the EGFR, it activates a signaling pathway cascade through the downstream effectors of the mitogen-activated protein kinases (MAPK) pathway. These effectors (KRAS, BRAF, ERK, and MAPK) influence cellular proliferation, adhesion, angiogenesis, migration, and survival (Figure 1, A). Other EGFR-mediated pathways include (1) the signal transducer and activator of transcription and (2) the phosphoinositide-3-kinase (P13k)/ AKT signaling pathway (STAT).[8] Blocking EGFR with cetuximab or panitumumab blocks all downstream effects of this receptor and is the basis of these therapeutic agents (Figure 1, B).

KRAS Testing in the Context of Anti-EGFR Therapy for Colon Cancer

As mentioned above, the *KRAS* gene encodes one of the proteins in the EGFR signaling pathway that is critical in the development and progression of cancer. Population-based studies have shown that approximately 30% to 40% of CRCs harbor mutations in *KRAS* that yield a constitutively active protein.[9–11] These mutations typically occur in codons 12 or 13 (exon 2) or in codon 61 (exon 3) of the *KRAS* gene.[12] In 2006, Lievre and collaborators[13] hypoth-

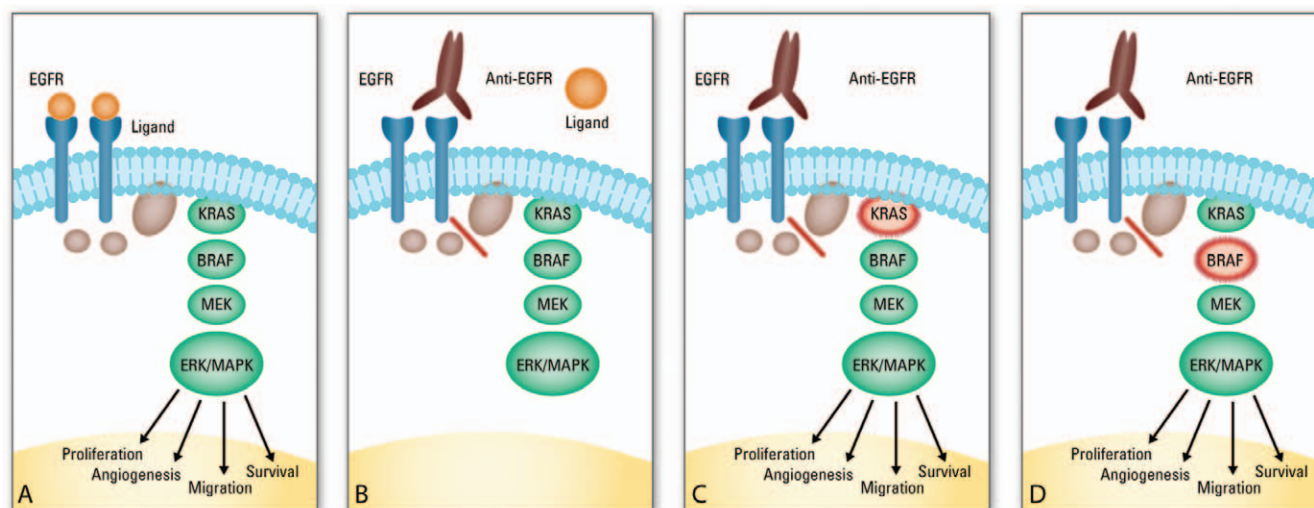KRAS *Testing in Metastatic Colorectal Cancer*—Monzon et al

Figure 1. *Epidermal growth factor receptor (EGFR) signaling pathway and the effect of anti–EGFR-targeted therapy. A, Ligand binding to EGFR results in increased cellular proliferation, angiogenesis, migration, and survival mediated by the ERK/MAPK pathway. B, Blockage of EGFR activity by directed antibodies suppresses downstream signaling. C, Mutated* KRAS *is constitutively active and bypasses EGFR blockage by therapeutic antibodies. D, Mutations in other signaling molecules could have the same effect as* KRAS *mutations.*

Table 1. Clinical Trials Showing Association of *KRAS* Mutational Status and Response to Anti–Epidermal Growth Factor (Anti-EGFR) Receptor Therapy

Source, y	Study Type, Treatment	Patients, No.	Results,[a,b] Mutant Versus Wild-Type	Level of Evidence
Lievre et al,[13] 2006	Randomized, single arm, cetuximab	30	RR, 0 versus 30%; OS, 7 versus 16 mo	II
Di Fiore et al,[21] 2007	Retrospective, cetuximab	59	RR, 5 versus 26%; PFS, 3 versus 6 mo	II
Frattini et al,[42] 2007	Retrospective, cetuximab	27	RR, 10 versus 48%	II
Khambata-Ford et al,[22] 2007	Randomized trial, cetuximab mono-tx	80	RR, 0 versus 10%	II
Amado et al,[17] 2008	Randomized trial, panitumumab mono-tx	423	RR, 0 versus 17%; PFS, 7 versus 12 wk; OS, 4.9 versus 8.1 mo	II
Cappuzzo et al,[43] 2008	Randomized, single arm, cetuximab (EGFR FISH+ patients)	85	RR, 9 versus 26%; PFS, 4 versus 5 mo; OS, 9.5 versus 10.8 mo	II
De Roock et al,[20] 2008	Randomized, single arm, cetuximab (4 trials)	113	RR, 0 versus 41%; OS, 27 versus 43 wk	II
Lievre et al,[23] 2008	Randomized, single arm, cetuximab	89	RR, 0 versus 40%; PFS, 9 versus 32 wk; OS, 10 versus 14 wk	II
Freeman et al,[24] 2008	Retrospective, panitumumab (3 trials)	62	RR, 0 versus 11%	II
Karapetis et al,[18] 2008	Randomized, cetuximab versus BSC	394	RR, 1.2 versus 13%	II
Bokemeyer et al,[14] 2008 [abstract]	Randomized, cetuximab and CT	233	RR, 33 versus 61%	II
van Cutsem et al,[16] 2008 [abstract]	Randomized, cetuximab and CT	540	RR, 36 versus 59%	II
Punt et al,[15] 2008 [abstract]	Randomized, cetuximab and CB	501	PFS, 8.6 versus 10.5 mo; OS, 19.1 versus 22.2 mo	I
Raponi et al,[44] 2008	Review	791	RR, 3 versus 35%; NPV, 97%	

Abbreviations: BSC, best supportive care; CB, capecitabine, oxaliplatin, and bevacizumab; CT, combined chemotherapy; FISH, fluorescence in situ hybridization; mono-tx, monotherapy; NPV, negative predictive value; OS, overall survival; PFS, progression-free survival; RR, response rate.

[a] Response rate in patients with *KRAS* mutation compared with those without a *KRAS* mutation.
[b] All outcomes reported here were significant with $P < .05$.

esized that mutations in the genes of downstream effectors of EGFR signaling, such as *KRAS*, could explain resistance to anti-EGFR therapy. Mutated *KRAS* is constitutively active and thus blockage of the EGFR should not affect the downstream signaling cascade of the MAPK pathway (Figure 1, C). These authors reported a retrospective study with 30 patients in which the presence of *KRAS* mutations in codons 12 or 13 was associated with a lack of response to the EGFR inhibitor cetuximab.[13]

Since then, multiple studies have corroborated these findings in tumor samples from patients with metastatic CRC in phase II and III clinical trials of cetuximab and panitumumab.[14–23] These studies have convincingly shown that only patients whose tumors carry a wild-type (normal or nonmutated) sequence of *KRAS* have a favorable response to cetuximab or panitumumab and that patients with mutations in codons 12 or 13 do not benefit from these therapies (Table 1).[24,25] These findings strongly suggest that only patients without *KRAS* mutations should receive these therapies. Given the overwhelming evidence, the National Comprehensive Cancer Network recently updated its guidelines with the recommendation that mutation analysis of the *KRAS* gene on the primary tumor or a site of metastasis should be part of the pretreatment

Table 2. Methods Used for *KRAS* Mutation Testing			
Method	Sensitivity, % of Mutant Alleles	Strengths	Weaknesses
Sanger sequencing	20	Gold standard Detects all possible mutations Inexpensive	Time consuming Open PCR system requires strict control for contamination
Pyrosequencing	5–10	Ability to sequence short PCR products (advantageous for DNA from fixed tissue) Detects all possible mutations Inexpensive Faster than Sanger sequencing	Short reading length for sequences used Open PCR system requires strict control for contamination
Allele-specific real-time PCR	1	Rapid, closed PCR system (eliminates risk of contamination with previously generated amplicons) Available as a commercial kit	Detects only the 7 most common mutations Require more tissue for analysis as compared with other methods Cost
Post-PCR fluorescent melting-curve analysis with specific probes	5–10	Rapid, closed PCR system Detects all possible mutations (heterozygous and homozygous)	Occasionally difficult to distinguish between mutation types More expensive than Sanger sequencing
PCR clamping method	1	Rapid, closed PCR system Available as a commercial kit	Does not allow to control quality of DNA and efficiency of PCR amplification

Abbreviation: PCR, polymerase chain reaction.

workup for all patients with stage-IV CRC (www.nccn. org).[26] Likewise, the American Society for Clinical Oncology recently published a provisional clinical opinion in which it is recommended that all patients with metastatic colorectal carcinoma who are candidates for anti-EGFR antibody therapy should have their tumor tested for *KRAS* mutations in a Clinical Laboratory Improvement Amendments of 1988–accredited laboratory. The American Society for Clinical Oncology also recommends that patients in whom a *KRAS* mutation in codons 12 or 13 is detected should not receive anti-EGFR antibody therapy as part of their treatment.[27] It is thus now widely accepted that patients with mutant *KRAS* are not likely to benefit from anti-EGFR therapy and are unnecessarily exposed to potential adverse events. The most common adverse reactions observed with cetuximab and panitumumab (incidence, ≥25%) are rash, headache, diarrhea, and infection. More serious adverse events, such as severe allergic reaction and myocardial infarction, have been reported.

KRAS MUTATION TESTING
Sample Considerations

For *KRAS* mutation testing, DNA needs to be extracted from a representative tumor sample, which can be obtained from fresh, frozen, or paraffin-embedded tissue. It is important that the sample is examined by a pathologist to determine the percentage of viable tumor cells in the sample. This is a visual estimation by the pathologist of the percentage of tumor cells out of all cells present in the specimen. This estimation of tumor content is important because different *KRAS* assays have different analytic sensitivities, and an attempt should be made to enrich to a level that is acceptable for the assay being used.

Testing Methods

The presence of *KRAS* mutations in colorectal tumor tissue can be detected by several different molecular methods (Table 2). Commonly used methods to evaluate samples for *KRAS* mutations are:

- **DNA Sequencing.**—*KRAS* mutations can be identified

using sequencing methods, such as Sanger sequencing and pyrosequencing. These techniques can identify all possible mutations in the analyzed *KRAS* gene segment (usually codons 12 and 13).
- **Real-Time Polymerase Chain Reaction.**—In real-time polymerase chain reaction (PCR), fluorescent probes or dyes are used to detect specific mutations in codons 12 and 13. The presence of a *KRAS* mutation is detected either by an increase of fluorescence during PCR amplification or by post-PCR fluorescent melting-curve analysis.

KRAS Sequencing

DNA sequencing is considered the gold standard for detection of mutations. This method allows determination of the order of nucleotides in a target DNA sequence. The Sanger chain-termination method is commonly used in molecular diagnostics laboratories. In this method, the incorporation of a chemically modified nucleotide (dideoxynucleotide) terminates extension of the DNA strand at the point of incorporation. This results in a mixture of DNA fragments of various lengths. Each dideoxynucleotide (A, T, C, or G) is labeled with a different fluorescent dye, allowing their individual detection. The newly synthesized and labeled DNA fragments are separated by size through capillary gel electrophoresis. The fluorescence is detected by an automated sequence analyzer (ie, ABI 3730, Applied Biosystems Inc, Carlsbad, California), and the order of nucleotides in the target DNA is depicted as a sequence electropherogram (Figure 2). A *KRAS* mutation will appear as the presence of both mutant and wild-type *KRAS* alleles (2 overlapping peaks) at the particular nucleotide that is mutated (Figure 2). Automated sequence analysis is relatively inexpensive and easy to perform, and as mentioned above, it detects all mutations present in the gene sequence. The analytic sensitivity of this method is reported to be 10% to 30% of mutant *KRAS* in a background of wild-type sequence.[28,29]

Pyrosequencing is an alternative methodology that sequences DNA as it synthesizes a short strand of DNA (sequencing by synthesis). In this method, each nucleotide
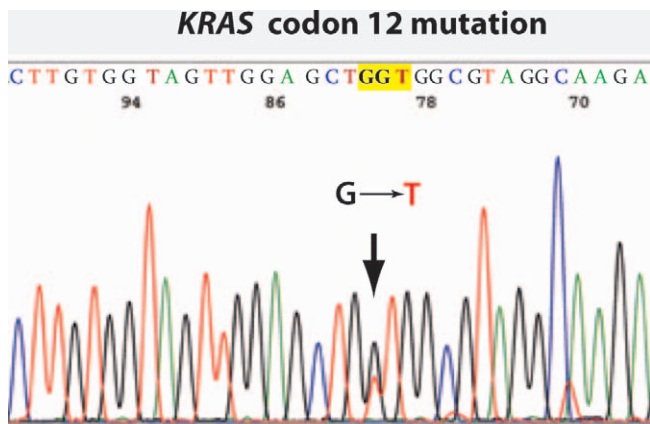
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KRAS codon 12 mutation

Figure 2. Detection of KRAS mutation in a colorectal cancer sample using DNA Sanger sequencing. The electropherogram demonstrates a G→T mutation in codon 12 (c.35G>T; GGT>GTT) corresponding to G12V. Note the presence of both a G and T peak in codon 12, indicating a heterozygous mutation because both the mutant and wild-type sequences are present.

(A, T, C, or G) is sequentially added to the reaction, and a light signal is generated when a particular nucleotide is incorporated into the DNA template by DNA polymerase. This only occurs if the added nucleotide is complementary to the one being interrogated in the template. The sequence is deduced from the order and intensity of signals (Figure 3). This technique is characterized by short read lengths but higher sensitivity than Sanger sequencing. It also has the ability to quantitate the fraction of the sample that carries the *KRAS* mutation.[30] For *KRAS* sequencing, the short reading length of the pyrosequencer instrument is not a disadvantage because *KRAS*-activating mutations are concentrated in hot spots at codons 12 and 13. A study that compared the *KRAS* pyrosequencing assay with Sanger sequencing showed that the relative sensitivity of pyrosequencing is higher than Sanger sequencing (~5%–10% mutant *KRAS* in a background of wild-type sequence).[29] The higher sensitivity is due to the higher signal-to-noise ratio of pyrosequencing compared with the Sanger se-

quencing. In addition, one can design an optimal sequencing primer and dispensation order so that most mutations can create a reading frameshift and extra peaks in pyrograms, which makes the identification of a particular mutation easier (Figure 3, B).[29] Another advantage of pyrosequencing is that PCR templates for sequencing can be designed as small as 60 to 70 bp (spanning DNA segments of only 20–30 bp among PCR primers). This improves the chances of obtaining acceptable results for fragmented DNA from paraffin-embedded tissue. Designing small PCR products is an advantage because there are more available DNA templates for amplification, less PCR bias, and more applicability to smaller amounts of DNA templates (such as DNA from a needle biopsy). In contrast, Sanger sequencing typically requires longer sequencing templates (100–150 bp).

KRAS Real-Time PCR-Based Methods

Real-time PCR-based assays are another good option for clinical testing because they are rapid, generally easy to perform, show excellent sensitivity, and are run in a closed PCR system that reduces the risk of contamination. In real-time PCR, the amplification product (amplicon) is detected as it is being produced by the release of fluorescent energy from labeled probes or DNA dyes. Two general approaches for assay design are employed for *KRAS* mutation testing: the use of allele-specific probes or the use of post-PCR melting-curve analysis.

In allele-specific real-time PCR, the assay uses multiple probes that are specific for each one of the most common 7 mutations described in *KRAS* codons 12 and 13. In the presence of one of these mutations, one of the probes will bind and will generate a fluorescent signal. Assays with this design are common for detection of genetic mutations. The reported sensitivity of *KRAS* mutation detection with this method is 1% mutant DNA in a background of wild-type DNA.[31] One of the disadvantages of this assay design is that it only detects the specific mutations for which the probes were designed but will miss other mutations present. In addition, the synthesis of multiple allele-specific probes is significantly more expensive than other assay designs. Importantly, the studies that led to approval of
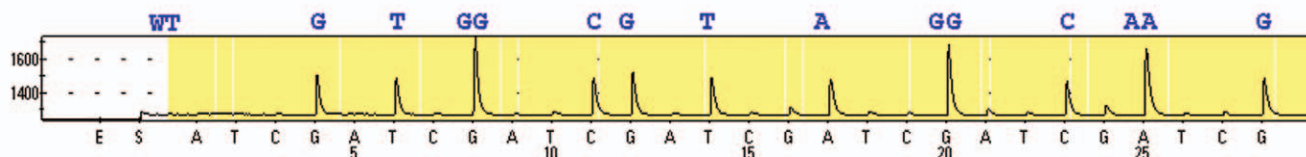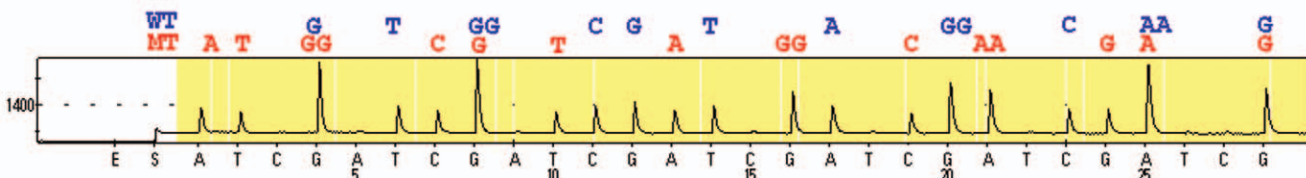

Figure 3. Detection of KRAS mutation in a colorectal cancer sample using pyrosequencing. A, Pyrogram that starts from the second base of codon 12 and shows the wild-type KRAS codons 12 and 13. B, Pyrogram shows mutant KRAS codon 12 (c.35G>A; GGT>GAT), along with the wild-type sequence. Note that the mutant allele creates additional peaks (indicated by red letters) in the pyrogram, which can confirm the presence of the mutant allele. Abbreviations: MT, mutant; WT, wild-type.

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KRAS Codon 12 Mutations

Melting Peaks

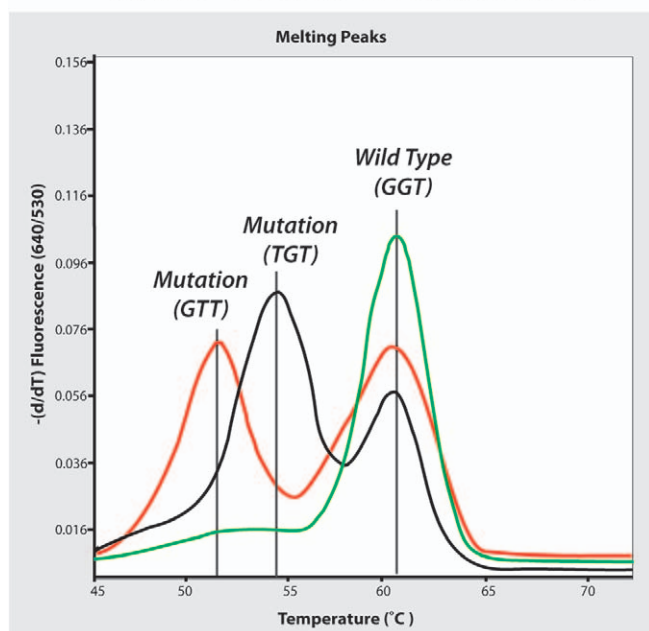*Wild Type (GGT)*

Mutation (TGT)

Mutation (GTT)

Figure 4. *Detection of KRAS mutation using real-time polymerase chain reaction (PCR) amplification and post-PCR melting-curve analysis. The picture illustrates a single melting peak (T_m of 61°C) for the normal DNA sample (green, wild type, GGT). The tumor sample with a KRAS G12C (black, c.34G>T; GGT>TGT) mutation has a mutant melting peak at 54°C, and another tumor sample carrying the KRAS G12V (red, c.35G>T; GGT>GTT) mutation shows a mutant melting peak of 52°C, whereas their wild-type sequences have melting peaks at 61°C. The mutations are heterozygous.*

panitumumab for patients with stage-IV CRC with wild-type *KRAS* by the European Committee for Medicinal Products for Human Use were performed with a commercially available assay that uses this design (Thera-Screen *KRAS* Kit, DxS Ltd, Manchester, England).

Real-time PCR with post-PCR melting-curve analysis can be performed using either fluorescently labeled probes or DNA dyes. For probe-specific real-time PCR amplification and post-PCR melting-curve analysis, 2 probes complementary to wild-type sequences are designed to span the mutation site (codons 12 and 13) of the *KRAS* gene.[32] After PCR amplification, a determination of the amplicon's melting temperature (T_m) is done (T_m is the temperature at which 50% of the amplicon separates into single stranded DNA). If no mutation is present, the probes will bind perfectly to the sample DNA and melt at a higher temperature, showing a single peak on post-PCR melting-curve analysis. In contrast, if a heterozygous mutation is present, probes will bind to the mutant amplicon imperfectly, that is, with one nucleotide mismatch, and will melt (dissociate) at a lower temperature. This will produce 2 melting peaks (a lower melting temperature peak for the mutant allele and a higher melting temperature peak for the wild-type allele; Figure 4). If a homozygous mutation is present, there will only be a single melting peak at the lower melting temperature. Each nucleotide substitution produces a melting peak at a specific T_m (Figure 4). This method showed a sensitivity for the detection of *KRAS* mutations that was similar to that of direct nucleotide sequencing as the gold standard.[32] Post-PCR melting-curve analysis can also be done with a DNA interca-

lating dye, such as SYBR Green 1, SYTO9, EvaGreen, or LC Green, instead of specific probes.[33–35] This method variation requires use of instruments with high-resolution temperature control and data acquisition and is most useful for the detection of variable single nucleotide polymorphisms and point mutations in regions without known hot spots. However, there is generally less separation observed between the melting peaks than with the probe-specific melting-curve analysis, which can make it more difficult to identify a mutation definitively.

Another variation of real-time PCR methods is the use of a PCR-clamp approach. This method uses mutation-specific hybridization probes and an additional peptide nucleic acid probe that is complementary to the wild-type sequence.[36] The peptide nucleic acid probe suppresses amplification of the wild-type *KRAS* sequence and, therefore, increases the sensitivity of detection of the mutant allele. As a result, only DNA with *KRAS* mutations will contribute to the amplification curve and specific melting curve. The reported analytic sensitivity of *KRAS* mutation detection with this method is less than 1%. A limitation of this approach is that because there is no signal for the wild-type allele, the assay does not allow for the assessment of amount or quality of the sample DNA, which makes it difficult to distinguish a wild-type result from no amplification because of an insufficient amount of DNA or to PCR inhibitors. A German-based company, TIB MolBiol (Berlin) offers a kit (KRAS LightMix) that uses this technology. At this time, the LightMix kit is not US Food and Drug Administration–approved and is sold for research use only.

Reporting Considerations

Pathologists are likely to issue *KRAS* testing result addendums to their CRC surgical pathology reports. It is important for pathologists to thoroughly review the testing results from their molecular or reference laboratory. American Society for Clinical Oncology suggests that pathologists use the terms ''*KRAS* normal (nonmutated)'' or ''*KRAS* abnormal (mutated)'' in their reports to facilitate the interpretation by the oncology community.[27] In cases in which a mutation is found, a statement indicating that ''treatment with anti-EGFR monoclonal antibody therapy is not recommended based on the *American Society for Clinical Oncology Provisional Clinical Opinion*''[27] could be included. It is also important to specify what mutation was found, the methodology employed, the sample tested (fresh tissue or paraffin block and block number), and the percentage of tumor cells present in the portion of the sample that was used for testing.

COMMENT

The importance of companion-test molecular assays for oncologic treatment decisions is on the rise. These tests are used to determine whether patients are eligible to receive a targeted therapy. In the case of *KRAS* mutation testing for CRC, the intent is to avoid unnecessary toxicity and monetary costs for patients who are not likely to respond to anti-EGFR therapies by screening them before initiating therapy. The cost of cetuximab and panitumumab therapy has been estimated to be approximately $100 000/patient/y.[37] Recently, new guidelines, which incorporate *KRAS* testing, for management of patients with CRC have been published by the National Comprehensive Cancer Network and as a provisional clinical opinion by

the American Society for Clinical Oncology.[26,27] Pathologist expertise is essential to quality *KRAS* testing and effective treatment decision making for patients with stage-IV CRC. Pathologists can assist oncologists in the appropriate use of this test and guide the interpretation of results. Pathologists who use reference laboratories for this testing should be able to carefully evaluate the *KRAS* testing technology used and quality processes employed to ensure confidence in the results.

As discussed above, there are multiple methods for determining the *KRAS* mutation status of a tumor. All of these methods appear to have adequate clinical sensitivity to detect patients unlikely to respond to cetuximab or panitumumab. In general, the advantage of sequencing-based methods is the ability to detect all possible sequence variations. The limitation of sequencing technologies is the requirement of greater than 5% to 10% mutant alleles for pyrosequencing and greater than 20% for Sanger sequencing. However, new approaches have been developed that appear to significantly increase the sensitivity of sequencing methods.[28,38,39] The advantage of real-time PCR-based methods is the ease of use, fast turnaround time, closed system to prevent contamination, and sensitivity down to 1% to 10% of mutant alleles. The limitation of the allele-specific approach is the higher cost and the potential false-negatives if mutations that the assays are not designed to detect are present in the sample. An important aspect of clinical testing is the evaluation of the method proficiency at each individual laboratory by subscribing to proficiency-testing programs or performing sample exchanges with other laboratories. Proficiency testing for *KRAS* should be available through the College of American Pathologists Molecular Oncology committee in 2009 with 2 surveys/y anticipated. These surveys may shed light on how well the different testing methodologies perform compared with one another.

It is important to mention that there is no consensus on the required sensitivity (percentage of mutant allele) for *KRAS* mutation analysis. The published studies have used a variety of methods with different sensitivities, and all of them have shown similar results, regardless of the method used. Currently, the correlation between detection of *KRAS* mutation in a small fraction of tumor cells (<1%) and treatment is unknown. *KRAS* mutations are usually acquired early in the carcinogenic process, so small subclones acquiring *KRAS* mutation at a later stage of tumor progression may not represent the genetic makeup of the main tumor mass, and it is unknown whether low percentages of mutant *KRAS* can predict the response to treatment of most tumor cells. Until clinical evidence for correlation with treatment response is established, highly sensitive detection of *KRAS* mutation may not be necessary. Furthermore, the determination of the level of sensitivity of an assay is variably approached in the literature. Most data rely on mixing studies using cell lines known to carry a *KRAS* mutation and those known to carry wild-type *KRAS* without the mutant *KRAS* allele. However, cell lines frequently exhibit copy number alterations throughout the genome, and there are often no data available on exact copy numbers of *KRAS* mutant allele and wild-type allele. Copy number changes may be further introduced during cell culture passages. With the presence of copy number alterations, mixing studies will unlikely reflect actual, relative copy number amounts of mutant and wild-type alleles. A meaningful comparison between various

methods for analytic sensitivity would be possible when the researchers compare various methods using the same DNA templates (eg, mixing DNA specimens, DNA from paraffin tissue). Such a comparison can yield data as to which method is more sensitive (ie, relative sensitivity). However, absolute sensitivity (ie, percentage of mutant allele detectable) cannot be determined unless the exact copy numbers of mutant and wild-type alleles are known before DNA mixing.

It is likely that *KRAS* testing in patients with CRC will continue to evolve. For example, in addition to hot-spot mutations in *KRAS* codons 12 and 13, mutations in codon 61 as well as other non–hot-spot mutations have been reported and may be found to have clinical significance in the future.[40] Likewise, the data supporting *KRAS* testing have brought to light other potential markers along the EGFR pathway as well as other pathways that may be important in predicting the response of a patient with CRC to other therapies. Recently, a study[41] described the lack of response to anti-EGFR therapy in patients with stage-IV CRC harboring a V600E mutation in the *BRAF* gene. As depicted in Figure 1, D, *BRAF* is another component in the EGFR signaling cascade, and its constitutive activation could have the same effects as *KRAS* activation. Although this new finding needs to be confirmed by additional independent studies, it is possible that mutations in multiple genes will be assessed before using different targeted therapeutic agents in patients with stage-IV CRC. Furthermore, because *KRAS* mutations are common in cancers other than CRC (eg, pancreatic and lung cancer), several groups are now evaluating the role of *KRAS* mutation testing in other cancers in which anti-EGFR therapies are being used.

In summary, convincing evidence about the importance of *KRAS* mutation testing for determination of eligibility to receive anti-EGFR therapy has been published. This testing is now recommended by national organizations such as National Comprehensive Cancer Network and American Society for Clinical Oncology. Multiple methods for detecting *KRAS* mutations in colorectal tumors are available, and each shows advantages and disadvantages. Importantly, all methods in current clinical use appear to have adequate clinical sensitivity for predicting lack of response to cetuximab and panitumumab. Pathologist expertise is essential to quality *KRAS* testing and to determining effective treatment for patients with metastatic CRC because pathologists play a role in the identification of suitable samples for analysis, provide consultation with surgeons and oncologists, and select reference laboratories or technologies to implement in their own laboratory.

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