Genetics in Medicine

Recommendations from the EGAPP Working Group: Can testing of tumor tissue for mutations in EGFR pathway downstream effector genes in patients with metastatic colorectal cancer improve health outcomes by guiding decisions regarding anti-EGFR therapy?

Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group*

This recommendation statement is a product of the independent EGAPP Working Group. Although the Centers for Disease Control and Prevention (CDC) provides support to the EGAPP Working Group, including staff support in the preparation of this document, recommendations made by the EGAPP Working Group should not be construed as official positions of the CDC or the US Department of Health and Human Services.

Summary of recommendations: The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group (EWG) found that, for patients with metastatic colorectal cancer (mCRC) who are being considered for treatment with cetuximab or panitumumab, there is convincing evidence to recommend clinical use of *KRAS* mutation analysis to determine which patients are *KRAS* mutation positive and therefore unlikely to benefit from these agents before initiation of therapy. The level of certainty of the evidence was deemed high, and the magnitude of net health benefit from avoiding potentially ineffective and harmful treatment, along with promoting more immediate access to what could be the next most effective treatment, is at least moderate.

The EWG found insufficient evidence to recommend for or against *BRAF* V600E testing for the same clinical scenario. The level of certainty for *BRAF* V600E testing to guide antiepidermal growth factor receptor (EGFR) therapy was deemed low. The EWG encourages further studies of the potential value of testing in patients with mCRC who were found to have tumors that are wild type (mutation negative) for *KRAS* to predict responsiveness to therapy.

The EWG found insufficient evidence to recommend for or against testing for mutations in *NRAS*, or *PIK3CA*, and/or loss of expression of PTEN or AKT proteins. The level of certainty for this evidence was low. In the absence of supporting evidence, and with consideration of other contextual issues, the EWG discourages the use of these tests in guiding decisions on initiating anti-EGFR therapy with cetuximab or panitumumab unless further evidence supports improved clinical outcomes.

Rationale: It has been suggested that patients with mCRC whose tumors harbor certain mutations affecting EGFR pathway signaling are typically unresponsive to therapy with anti-EGFR antibodies (cetuximab and panitumumab). The EWG identified recent evidence reviews that have addressed this topic, and this recommendation statement is based on results of these reviews. In developing these recommendations the EWG considered evidence in the areas described below.

Analytic validity: Although no research syntheses that have formally evaluated analytic validity of these tests were found, the EWG was able to draw the following conclusions from assessments included in the evidence reviews under consideration. There is adequate evidence that *KRAS* mutation analysis reliably and accurately detects common mutations (codons 12 and 13), whereas evidence was inadequate for less frequent *KRAS* mutations (e.g., codon 61). There is also adequate evidence that testing for *BRAF* V600E accurately and reliably detects the mutation. For common mutations in *NRAS*, *PIK3CA*, and expression of PTEN AKT, there is adequate evidence of accurate and reliable detection. However, much less data exist in support. Furthermore, in the specific context of mCRC, no evidence was found on the analytic validity of immunohistochemistry (IHC) assays for PTEN or AKT expression.

Clinical validity: For *KRAS* mutation analysis, the EWG found convincing evidence for association with treatment response to anti-EGFR therapy, independent of prognostic association. For *BRAF* V600E mutation testing, the EWG found insufficient evidence for association with treatment response to anti-EGFR therapy independent of prognostic association. The EWG found insufficient evidence for association of results of testing for mutations in *NRAS* or *PIK3CA*, and loss of expression of PTEN or ATK proteins, with treatment response to anti-EGFR therapy.

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Clinical utility: For *KRAS* mutation analysis, the EWG found adequate evidence that improved health outcomes are achieved by avoiding ineffective chemotherapy and potential side effects and expediting access to the next most effective treatment. Inadequate evidence was found regarding association of *BRAF* V600E mutation testing or loss of PTEN expression with improved health outcomes among patients with mCRC undergoing anti-EGFR therapy as compared with patients with tumors bearing wild-type *BRAF* sequence and PTEN expression levels, respectively. No evidence was found to support improved health outcomes associated with testing results for *NRAS* or *PIK3CA* variants, or AKT protein expression levels in this clinical scenario.

Contextual issues: CRC is an important and highly prevalent health problem. Improvements in mCRC outcomes associated with pharmacogenetic testing could have important clinical, and potentially public health, impacts. Adverse events related to cancer chemotherapy can be common and severe. Therefore, successfully optimizing treatment to maximize efficacy and minimize side effects is important for reducing mCRC-related morbidity and mortality.

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CLINICAL CONSIDERATIONS

Definitions used by EGAPP

1. Analytic validity refers to a test's ability to accurately and reliably measure the genotype or analyte of interest, in this case: codon 12 and 13 mutations in *KRAS* exon 2, the *BRAF* V600E mutation, variants in *NRAS* (codons 12, 13, and 61), and in *PIK3CA* (exon 9 and exon 20), and loss of PTEN and AKT protein expression.
2. Clinical validity defines the ability of the test to accurately and reliably identify or predict the intermediate or final outcomes of interest, in this case: tumor response or overall response rate to cetuximab or panitumumab, progression-free survival, and overall survival. This is usually reported in terms of clinical sensitivity and specificity.
3. Clinical utility defines the balance of benefits and harms associated with the use of the test in practice, including improvement in measurable clinical outcomes and usefulness/added value in clinical management and decision making compared with not using the test. In this case, clinical outcomes and usefulness/value added includes avoidance of treatment-related adverse events and/or ineffective therapy.

Patient population under consideration

These recommendations apply to all patients with mCRC who are being considered for treatment with cetuximab or panitumumab. This statement is neither intended for, nor applicable to, patients in adjuvant or neo-adjuvant settings.

Considerations for practice

1. *KRAS* wild-type status has been considered necessary but not sufficient to achieve a clinical response from anti-EGFR therapy. Approximately 40–60% of patients with wild-type *KRAS* also fail to achieve a response with anti-EGFR treatment.
2. The EWG recommends that testing for *KRAS* (exon 2; codons 12 and 13) should be provided to patients with mCRC who are candidates for anti-EGFR antibody therapy using either cetuximab or panitumumab.
3. Several sources suggest that patients with metastatic CRC who test positive for *KRAS* (exon 2; codons 12 and 13) mutations should not be treated with cetuximab or panitumumab.[1–4]
4. Oncogenic mutations in *KRAS* may be present in ~30–50% of CRC tumors.[5]

BACKGROUND AND CLINICAL CONTEXT FOR THE RECOMMENDATION

Over 140,000 new cases of CRC (~102,900 colon and 39,670 rectal cancer) and more than 50,000 deaths from CRC were expected in 2010.[6] Metastatic disease may account for ~20% of new CRC diagnoses.[7] Median overall survival following mCRC diagnosis is only about 2 years,[8] whereas for those with distant disease, overall survival at 5 years is 11%.[9] Molecular markers are increasingly used for predictive and prognostic applications in CRC, and tumors can be assigned to non–mutually exclusive molecular subtypes of CpG island methylator phenotype, chromosomal instability, and microsatellite instability.[10] Among several chemotherapeutic options available for the treatment of mCRC, two agents consisting of monoclonal antibodies directed against the EGFR are currently approved by the US Food and Drug Administration for use in the refractory setting: cetuximab and panitumumab.[11] Cetuximab has been shown to improve overall survival, progression-free survival, and quality-of-life measures, following failure of other treatment, in patients with CRC.[12] Panitumumab has been shown to improve progression-free survival[13,14] and quality of life,[15] beyond best supportive care alone, in CRC patients with tumors that are wild type for *KRAS*.

EGFR expression is present in ~70–75% of CRC.[16] However, response rates for cetuximab and panitumumab monotherapy are only ~8–12%.[9] Although approval of these agents is limited to use with tumors expressing EGFR, as detected by IHC, there are data demonstrating that EGFR-negative cases can respond to cetuximab-based regimens, which could be due either to limitations in EGFR IHC methodologies or possibly to induction of antibody-dependent cell-mediated cytotoxicity by cetuximab.[16] In 2008, a key study concluded that "patients with a colorectal tumor bearing mutated *K-ras* did not benefit from cetuximab, whereas patients with a tumor bearing wild-type *K-ras* did…" and "the mutation status of the *K-ras* gene had no influence on survival among patients treated with best supportive care alone."[17] Another group reported that panitumumab monotherapy is effective only in patients in whom tumors are wild-type for *KRAS*, and that consideration of *KRAS* genotype should be applied in selection of patients with mCRC for this treatment.[5] *KRAS* mutations are increasingly acknowledged as predictive for resistance to anti-EGFR antibody therapy, and several studies involving first- and subsequent-line treatments suggest that patients with tumors that have *KRAS* mutations are

unresponsive to panitumumab or cetuximab, with no beneficial effects on survival attributable to these treatments.[18] However, even among patients with wild-type *KRAS*, response rates for anti-EGFR therapy remain <20%.[9,19] Proteins found downstream of KRAS in the mitogen-activated protein kinase signaling pathway (BRAF, PIK3CA, NRAS, PTEN, and AKT) are also purported to affect response to anti-EGFR therapy.

KRAS mutation analysis, in both mCRC and lung cancer, is increasingly being utilized in clinical practice;[20] in 2009, the yearly market was estimated at ~$1.2 billion for cetuximab and $300 million for panitumumab.[21] Although there is no obvious consensus on testing methods or which exons and/or codons should be analyzed, several guidelines for clinical practice now include *KRAS* testing.[20] In January 2009, the Blue Cross and Blue Shield Association's Technology Evaluation Center (BCBS TEC) released an assessment supporting the validity and utility of *KRAS* mutation analysis toward informing anti-EGFR therapy in mCRC.[11] The American Society of Clinical Oncology released a provisional clinical opinion in April 2009, advocating *KRAS* testing for all patients with mCRC who are candidates for therapy with anti-EGFR antibodies, and specifically advising to refrain from such therapy in patients found to have *KRAS* codon 12 or 13 mutations.[3] In July 2009, the US Food and Drug Administration enacted class labeling changes for anti-EGFR antibodies (cetuximab and panitumumab), stating that they "…are not effective for the treatment of patients with mCRC containing *KRAS* mutations," and added to the cetuximab label that it is not recommended for treatment of CRC in which *KRAS* codon 12 or 13 mutations are present.[22] In June 2010, a technology assessment on three pharmacogenetic tests for treatment of cancer, requested from the Agency for Healthcare Research and Quality by the Coverage and Analysis Group at the Centers for Medicare and Medicaid Services, was released by the Tufts Evidence-Based Practice Center.[23] A summary of the section of this assessment that addressed *KRAS* testing for anti-EGFR therapy in CRC was published by Evidence-Based Practice Center investigators in January 2011.[24] In December 2010, a technology assessment[25] on *KRAS* testing in advanced CRC was released by the Ontario Medical Advisory Secretariat, Ministry of Health and Long-Term Care, which was used by the Ontario Health Technology Assessment Advisory Committee to issue a concurrent recommendation.[26] The Kaiser Permanente Center for Health Research has conducted a systematic review of pharmacogenetic testing to predict the value of testing for markers downstream from *KRAS* (*BRAF*, *NRAS*, and *PIK3CA* mutations, and loss of PTEN and AKT protein expression) toward informing anti-EGFR antibody therapy in mCRC.[9] A summary of that review was published in 2011,[9] and the full report[19] will be made available on www.egappreviews.org.

In light of the wealth of existing and ongoing systematic reviews and technology assessments, for the first time, the EWG was able to issue this recommendation statement with no corresponding EGAPP-commissioned review on the topic. Among these works, the EWG sought out evidence-based reviews and technology assessments that addressed an overarching question regarding the following specific clinical scenario: what is the direct evidence that pharmacogenomic testing of tumor tissue in patients with mCRC aimed at predicting response to anti-EGFR antibody therapy leads to increased overall or progression-free survival, and/or decreased treatment-associated adverse events?

REVIEW OF SCIENTIFIC EVIDENCE

This statement summarizes the supporting scientific evidence used by the EWG to make recommendations regarding the use of selected pharmacogenomic tests (for codon 12 and 13 mutations in *KRAS*, the *BRAF* V600E mutation, variants in *NRAS* and in *PIK3CA*, and loss of PTEN and AKT protein expression) in patients with mCRC who are being considered for treatment with cetuximab or panitumumab.

Methods

EGAPP is a project developed by the Office of Public Health Genomics at the Centers for Disease Control and Prevention to support a rigorous, evidence-based process for evaluating genetic tests and other genomic applications that are in transition from research to clinical and public health practice in the United States.[27] Key goals of the EWG are to develop conclusions and recommendations regarding clinical genomic applications and to establish clear linkage to the supporting scientific evidence. The EWG members are nonfederal multidisciplinary experts convened to establish methods and processes, set priorities for review topics, participate in technical expert panels for commissioned evidence reviews, and develop and publish recommendations.

The EWG identified existing and ongoing systematic reviews and technology assessments that addressed the topic of pharmacogenomic testing to inform anti-EGFR antibody therapy in patients with mCRC.[9,11,19,23–25] Although none of the reviews on which this recommendation statement is based was commissioned by EGAPP, three EWG members provided expert guidance during the course of one of these systematic reviews[9] as members of a technical expert panel.

EWG members reviewed the existing evidence reports, key primary publications, and other sources of information. The process also included assessment of key gaps in knowledge and relevant contextual factors (e.g., availability of diagnostic or therapeutic alternatives, feasibility and practicality of implementation, cost effectiveness). The final EWG recommendation statement was formulated on the basis of magnitude of effect, certainty of evidence, and consideration of contextual factors.[27,28]

Technology description

Direct sequencing and real-time polymerase chain reaction (real-time PCR) are commonly used techniques for *KRAS*[21] *BRAF*, *NRAS*, and *PIK3CA* mutation analysis. Detection of PTEN and AKT protein expression, or lack thereof, can be accomplished through IHC assays.

Analytic validity

Analytic validity is defined as a test's ability to accurately and reliably measure the analytes (in this case, protein levels of PTEN and/or AKT) or the genotypes (*KRAS*, *BRAF*, *NRAS*, and *PIK3CA* variants) of interest. This includes measures of analytic sensitivity and specificity, as well as assay reproducibility, robustness (e.g., resistance to small changes in preanalytic or analytic variables), and quality control.

KRAS. Techniques for *KRAS* codon 12 and 13 assessment have been compared in a multinational study involving series of 74 formalin-fixed paraffin-embedded and 80 frozen CRC samples that were sent for analysis at seven testing centers. Each center utilized a different testing methodology: single-strand conformation polymorphism analysis, dideoxy sequencing, pyrosequencing, high-resolution melting analysis, TIB Molbiol (hybridization probe genotyping) kit, or DxS Diagnostic Innovations (allele-specific, real-time PCR) kit. Concordance was high among results from all methods except for the TIB Molbiol kit. Among the top five performing assays, concordance was 96% from formalin-fixed paraffin-embedded samples (63% with all assays included) and 83% with the frozen samples. These results suggest that reasonably good comparability exists between the different methods employed in this study.[29]

In a study funded by Amgen, *KRAS* mutation analysis results from five commercial laboratories, based on analysis of 40 formalin-fixed paraffin-embedded CRC samples, were compared with Amgen's direct-sequencing results. Moreover, the capacity of the selected laboratories to provide results that are comparable with those obtained through direct sequencing, using a variety of methods, was demonstrated (**Table 1**). In the only case in which there was poor agreement with sequencing results in this study, the corresponding assay has ceased to be available for commercial use.[30]

In a recent study comparing SNaPshot and KRAS StripAssay with direct sequencing in detecting *KRAS* exon 2 mutations from 296 serially diluted samples, detection limits were 10% tumor cells with SNaPshot, 1% with the StripAssay, and 20% with direct sequencing.[30] Another recent study compared a Sequenom mass spectrometry genotyping assay with locked nucleic acid PCR sequencing, for *KRAS* and *BRAF* mutations, using 308 CRC samples that had originally been tested through standard sequencing. Locked nucleic acid PCR detected 45%, and mass spectrometry 41%, more *KRAS* mutant samples than standard sequencing alone.[31] Although direct sequencing is considered the gold standard for *KRAS* mutation analysis,[30,32] it is less analytically sensitive (detects fewer mutated alleles in a heterogeneous mixture of mutated and unmutated cells) as compared with some real-time PCR–based *KRAS* assays. However, according to a 2009 College of American Pathologists (CAP) Perspectives on Emerging Technology report "the clinical relevance of a small percentage of cells with mutant *KRAS* has not been established."[21] Accordingly, we do not yet know exactly what level of sensitivity is necessary to allow a *KRAS* assay to provide clinically useful information to practitioners in this clinical scenario.[33]

Proficiency testing is a form of quality assurance that involves interlaboratory comparison of test results for specific analytes performed on samples distributed from a central source. Proficiency testing presents unique specimen-related challenges. For example, solid tumors have specific technical challenges that are different from techniques of similar assays in blood. Beginning in 2009, the CAP began offering proficiency testing for *KRAS* testing. The program has experienced rapid growth, with 48 participants in 2009 to 174 participating laboratories in the first half of 2011. Performance data have reflected a high degree of concordance among laboratories and with the expected results. Of the four separate challenges during this period that included three positive and one negative specimens, 16 errors out of 407 results were reported, for an accuracy of >96% (three indeterminate results were not included in this calculation because results were not reported).

The preceding analysis excludes one proficiency challenge that was characterized by substantial discordance between the results obtained from individual laboratories. Following identity testing of proficiency testing materials and reanalysis of a subset of these materials by referee laboratories using several methods of varying lower limits of detection, the inconsistent results were attributed to tumor heterogeneity as discussed below.

Table 1 Comparison with direct sequencing of *KRAS* mutation analysis between five laboratories[68]

Laboratory	Test/method	Reported	Results (κ[a], 95% confidence interval)
Amgen	Direct sequencing	Exon 2	—
Agencourt Bioscience	Picotiter plate pyrosequencing	Exon 2	0.94, 0.52–0.98
Gentris Clinical Genetics	Enriched samples for tumor tissue, then real-time polymerase chain reaction (PCR) and direct sequencing	Codons 12 and 13	0.75, 0.52–0.98
Genzyme	Allele-specific primer extension	Codons 12 and 13	0.94, 0.85–1.00
HistoGeneX	DxS K-RAS mutation test kit/allele-specific real-time PCR	Codons 12 and 13: G12A, G12D, G12R, G12V, G12C, G12S, G13D	0.95, 0.83–1.00
Invitek[b]	Invisorb spin tissue mini kit; Invigene K-ras genotyping kit/allele-specific oligonucleotide hybridization	Codons 12 and 13: six mutations	0.13, 0.15–0.42

[a]κ Statistic pertains to measure of agreement with observations from Amgen direct sequencing. [b]Assay employed is no longer available for commercial use.

Sequencing methods were most commonly used for mutation detection. The largest number of laboratories used Sanger sequencing, a smaller number used Pyrosequencing, and the remaining few reported using allele-specific PCR.

Downstream markers. Although the systematic review addressing *BRAF*, *NRAS,* and *PIK3CA* genotyping, along with PTEN/AKT protein expression, did not directly assess test accuracy or reproducibility,[9] analytic validity for *BRAF* testing was described as likely good, based on real-time PCR result validation in one study.[34] Furthermore, because *BRAF* mutation testing is intended to identify a single variant (V600E), analytic validity is likely high, similar to that found in CAP proficiency testing for other single-mutation tests; furthermore, high clinical validity for *BRAF* testing provides indirect evidence of high analytic validity.[35]

The CAP initiated *BRAF* proficiency testing in 2010 and program growth has also been rapid. Participants initially included 57 laboratories in 2010. This number expanded to 111 participants in 2011. Performance on the first three challenges, which included one mutated and two nonmutated samples, has demonstrated a high degree of concordance among laboratories and with the expected results. Of 233 reported results, only one error, a false-negative result, was reported, for a total accuracy of 99.6%.

As with *KRAS*, sequencing methods were most commonly used, with the largest number of laboratories using Sanger sequencing, followed by Pyrosequencing, and the remainder reported using allele-specific PCR.

No systematic analyses of accuracy and reproducibility of *NRAS* or *PIK3CA* testing were identified. IHC assays for PTEN and AKT are not currently subjected to CAP external proficiency testing. However, IHC testing for other proteins is offered as part of the CAP IHC Survey.[35] We found no indication that any validated, standardized scoring schema for PTEN or AKT IHC testing exists.[36] Inconsistency in clinical validity for PTEN and AKT may suggest issues related to analytic validity and/or tumor source.[19]

Enrichment and tumor heterogeneity

As previously stated, the lower limit of mutation detection by allele proportion varies by method of analysis, and little is known about the relative importance of the mutant allele proportion on clinical response for *KRAS* and *BRAF* mutations. In practice, tumor enrichment through macrodissection or microdissection is recommended in order to increase the proportion of neoplastic cells from which DNA is extracted, and the proportion of neoplastic cells in relation to the lower limit of detection of the particular method used is an important consideration in the ability of the assay to correctly identify the presence of a *KRAS* mutation. CAP checklists currently require estimation of the proportion of tumor in the material from which DNA is extracted; however, no further guidance on this issue is provided.

Adding further complexity, the proportions of mutant and wild-type alleles within the extracted DNA analyzed depend on both the neoplastic percentage of the tissue section from which DNA is taken and the percentage of malignant cells that contain a *KRAS* or *BRAF* mutation. The implications of these two factors (the percentage of neoplastic cells that are in the extracted DNA versus the percentage of neoplastic cells that actually contain mutations) for clinical response to anti-EGFR therapy likely differ. Finally, tumor heterogeneity, in which the presence of mutations or their relative proportions vary within different regions of the tumor, is a described but incompletely understood phenomenon that can confound the results of *KRAS* and *BRAF* mutation testing, as well as IHC results.[37–44] In cases in which tumor heterogeneity is present, false-negative results can occur due to sampling error.

Analytic validity conclusions

- Regarding the more commonly tested *KRAS* mutations (codons 12 and 13), the EWG agrees with conclusions of the 2009 BCBS TEC assessment, which rest on the fact that "the analytic validity of *KRAS* mutation testing by PCR methods is established as a commercially available laboratory test in CLIA-licensed laboratories."[11]
- The EWG has reasoned previously that analytic validity for *BRAF* V600E testing was adequate in a 2009 recommendation on Lynch syndrome,[35] and we have found no emergent evidence to refute this assessment.
- Although few data are available regarding analytic validity of *NRAS* and *PIK3CA* genotyping, we found no reason to expect the accuracy and reproducibility of these tests to be lower than for genotyping assays targeting other DNA polymorphisms.

Few data are available to support analytic validity of PTEN and AKT expression analysis by IHC. If inconsistencies in clinical validity for PTEN and AKT expression analysis were to be observed, however, they could be investigated as potential indicators of issues related to analytic validity.

Clinical validity

Clinical validity is defined as a test's ability to accurately and reliably identify or predict the disorder or phenotype of interest. In this case the relevant phenotype is nonresponsiveness to anti-EGFR therapy, which can be demonstrated through measures of progression-free survival or overall survival in patients who have been treated with cetuximab or panitumumab.

KRAS. In the Brown 2009 BCBS TEC assessment,[11] data from five randomized controlled trials (RCTs; quality ratings ranging from fair to good) and five single-arm retrospective studies, all published either as abstracts or full reports between 2007 and 2008, were examined. The assessment evaluated and summarized the evidence for the use of *KRAS* mutation testing in tumor tissue to predict lack of treatment response to cetuximab or panitumumab. Among the findings of this report was that existing evidence was "…sufficient to conclude that patients with mutated *KRAS* tumors in the setting of metastatic

colorectal cancer do not respond to anti-EGFR monoclonal antibody therapy, do not derive overall survival benefit, and may experience decreased progression-free survival."[11]

The summary report from Dahabreh et al.[24] details more recent "evidence that KRAS [*sic*] mutations are predictive of survival, disease progression, and treatment failure in patients with advanced colorectal cancer treated with anti-EGFR antibodies."

In the report, progression-free survival, a secondary study outcome, underwent meta-analysis. The analysis included 741 patients with *KRAS* mutations, among a total of 1,945 independent patients from 16 studies. The authors considered all study designs (prospective and retrospective), treatment settings (first-line and second-line or higher), and treatment strategies (monotherapy or combination chemotherapy). *KRAS* mutations were significantly associated with decreased median progression-free survival, with a corresponding hazard ratio (HR) of 2.11 (95% confidence interval (CI) 1.74–2.55). However, there was considerable between-study heterogeneity ($P = 0.003$ and $I^2 = 57\%$).[24]

The Medical Advisory Secretariat 2010 technology assessment[25] conducted a pooled analysis on five of seven studies reporting mean overall survival and progression-free survival among patients undergoing combination therapy with cetuximab and irinotecan. The authors found improved outcomes for patients with wild-type versus mutant *KRAS* tumors for both overall survival (mean difference of 4.11 months (95% CI 2.62–5.60)) and progression-free survival (mean difference of 3.32 months (95% CI 1.78–4.86)). There was significant heterogeneity for both overall survival ($P < 0.00001$ and $I^2 = 95\%$) and progression-free survival ($P < 0.00001$ and $I^2 = 99\%$). GRADE quality of evidence was generally deemed low.[25]

Although the Medical Advisory Secretariat investigators found that it was not possible to conduct pooled analyses for cetuximab or panitumumab used as monotherapy, they presented evidence from seven studies (four cetuximab and three panitumumab) suggesting increased overall survival and progression-free survival among patients with *KRAS* wild-type tumors in both scenarios. GRADE quality of evidence was deemed moderate for analysis of both cetuximab and panitumumab. However, quality ratings were mainly based on results of a retrospective analysis, with stratification by *KRAS* status, of one RCT for each drug.[25]

An updated survival analysis of the CRYSTAL study[45] has recently been published by Van Cutsem et al.[46] The original study showed that among 1,098 mCRC patients, adding cetuximab to the first-line regimen of fluorouracil, leucovorin, and irinotecan (FOLFIRI) reduced risk of progression, (HR = 0.85, $P = 0.48$) and increased treatment response (odds ratio (OR) = 1.40, $P = 0.004$), along with R0 resection rate of metastases with curative intent. Retrospective analysis revealed that the beneficial effects of cetuximab were limited to those patients in whose tumors *KRAS* mutations were not detected in codons 12 and 13. *KRAS* wild-type patients showed improved treatment response (OR = 1.91 (95% CI 1.24–2.93), reduced risk of disease progression (HR = 0.68, $P = 0.02$), and enhanced overall survival (HR = 0.84).[45,46] In the updated analysis, a substantially greater proportion of subjects were analyzed for *KRAS* mutation status (45 to 89%). Patients with *KRAS* wild-type tumors, demonstrated an increased cetuximab response rate as compared with FOLFIRI alone (57.3 vs. 39.7%; OR = 2.069, $P < 0.001$). The addition of cetuximab resulted in improved overall survival (23.5 vs. 20.0 months; 0.796, $P = 0.093$) and median progression-free survival (HR = 0.696, $P = 0.012$).[46]

Downstream markers. Lin et al.[9] identified seven studies that attempted to assess tumor response in the presence of the *BRAF* V600E mutations, three of which assessed survival. Of these studies, the investigators determined that the best evidence came from a retrospective analysis of a multicenter study[34] involving a total of 649 patients who underwent combination chemotherapy with cetuximab. Twenty-four patients had *KRAS* wild-type tumors that contained a *BRAF* V600E mutation. Clinical sensitivity was 9.8% (95% CI 6.3–14.5) and 1—specificity was 1.6% (95% CI 0.2–5.6). Patients in whom *BRAF* mutations were not detected demonstrated improved tumor response (OR = 0.15; 95% CI 0.02–0.51) and progression-free survival (HR = 1.82; 95% CI 1.04–3.18).[9] Lin et al.[9] also found that this study provided the best evidence of three identified studies that considered overall survival in relation to *BRAF* V600E mutation status. Overall survival in patients in whom *BRAF* V600E mutations were not detected was increased by 28 weeks (HR = 1.89; 95% CI 1.05–3.39).

A single retrospective study of testing in an RCT[47] was identified by Lin et al.[9] The study included 518 patients who underwent combination chemotherapy +/− cetuximab, 45 of whom had tumors with a *BRAF* V600E mutation. Although significantly worse survival outcomes (progression-free survival and overall survival) were observed with *BRAF* V600E versus *BRAF* and *KRAS* wild-type tumors, results were similar whether or not treatment included cetuximab. Control of mCRC was not found to be significantly affected by *BRAF* genotype, irrespective of whether cetuximab was included in the treatment regimen.[9] Consistent with these findings, results from a study nested in the CRYSTAL trial that included 599 patients randomized to FOLFIRI treatment alone and 599 who received FOLFIRI with cetuximab demonstrated decreased tumor response, progression-free survival, and overall survival in patients whose tumors contained *BRAF* V600E mutations, independent of the inclusion of cetuximab in the treatment regimen.[19,46]

Lin et al.[9] determined that the best evidence considering *NRAS* and *PIK3CA* testing to predict nonresponsiveness to anti-EGFR therapy came from the multicenter retrospective analysis discussed in the first paragraph of this Downstream Markers section with respect to *BRAF* testing.[34] Among the patients in this study with *KRAS* wild-type tumors, 13 with *NRAS*-mutated tumors and 49 with *PIK3CA*-mutated tumors were identified. Statistically significant relationships between progression-free survival and overall survival and *NRAS* or *PIK3CA* mutation status could not be established.[9] The best evidence considering PTEN protein expression testing came

from a retrospective cohort study[48] of 173 patients; in this study, no association with progression-free survival or tumor response was demonstrated among the 20% of patients with *KRAS* wild-type tumors who had lost PTEN expression, although an association with overall survival was found (HR = 1.9; 95% CI 1.1–3.2; in *KRAS*/*BRAF* wild-type background, HR adjusted for sex, age, location of tumor, and amount of previous chemotherapy).[9]

No studies identified by Lin et al.[9] demonstrated statistically significant associations between tumor response or survival and AKT expression.

Clinical validity conclusions

- There is adequate evidence for an association of *KRAS* genotype at codons 12 and 13 with diminished treatment response to anti-EGFR therapy in the treatment of mCRC.
- For *BRAF* V600E mutation testing, there is insufficient evidence for association of genotype with reduced treatment response to anti-EGFR therapy. Evidence pertaining to survival outcomes was derived from studies deemed to be of fair quality.[19] It is unclear whether adverse outcomes associated with the *BRAF* V600E mutation represent a prognostic relationship, or are reflective of decreased responsiveness to anti-EGFR therapy.
- There is insufficient evidence for an association of mutations in *NRAS* or *PIK3CA*, or loss of PTEN or AKT expression with no or reduced response to anti-EGFR therapy. Evidence pertaining to survival outcomes was derived from studies deemed to be of fair quality for *NRAS*, and *PIK3CA*, and fair to marginal quality for PTEN and AKT.[19]

Clinical utility

In the context of this recommendation, clinical utility is the likelihood that the benefits of pharmacogenomic testing of tumor tissue to predict potential response to anti-EGFR therapy in patients with mCRC exceed the possible harms of testing. These putative benefits derive from withholding administration of an ineffective treatment and include a subsequent reduction in treatment-related adverse events as well as an opportunity to pursue other therapies to which a patient's disease may be more likely to respond. Although most people will not experience life-threatening side effects, adverse events such as nausea and diarrhea can still occur, and in some cases can be as severe as anaphylaxis or cardiotoxicity. Potential harms of testing include the possibility of inappropriately withholding an effective treatment or erroneously administering suboptimal therapy based on misleading test results.

KRAS. As mentioned in the Clinical Validity section, the 2009 BCBS TEC assessment found that by identifying patients with mCRC who will not respond to (and should not receive) anti-EGFR therapy, *KRAS* testing can prevent both ineffective treatment and treatment-associated toxicity and minimize delay in identifying and administering potentially effective alternative therapies.[11] More recently, in the summary report from Dahabreh et al.,[24] a bivariate meta-analysis was conducted for *KRAS* mutation–based prediction of lack of treatment response (a secondary outcome) with cetuximab or panitumumab, as judged by imaging. The analysis included 841 patients with *KRAS* mutations, among a total of 2,242 independent patients across 22 studies. Summary sensitivity was determined to be 0.49 (95% CI 0.43–0.55), with a specificity of 0.93 (0.87–0.97). Positive and negative likelihood ratios were 7.35 (95% CI 3.72–14.50) and 0.55 (0.49–0.61), respectively. Overall survival (as a primary outcome) was investigated by meta-analysis of 13 studies involving 1,695 independent subjects (651 with *KRAS* mutant tumors). Results showed an association between *KRAS* mutations and reduced overall survival (HR = 1.79; 95% CI 1.48–2.17, $P < 0.001$). However, as with progression-free survival, some potential for heterogeneity was evident ($P = 0.007$ and $I^2 = 56\%$).[24]

Downstream markers. Lin et al.[19] stated, "none of the included studies reported harms, and we found no studies that explicitly addressed harms or that addressed psychological, ethical, legal, or social implications of testing." Nevertheless, a risk of potential harms from *BRAF*, *NRAS*, *PIK3CA*, PTEN, and AKT testing can be inferred because of the possibility that testing could result in withholding anti-EGFR treatment from patients who would benefit from it. If testing of some or all of these potential biomarkers were in fact predictive of nonresponsiveness to anti-EGFR therapy, analytic issues could still produce harms from erroneous therapeutic decisions.[19]

Clinical utility conclusions

- There is adequate evidence that improved health outcomes are achieved through *KRAS* mutation analysis, by avoiding ineffective chemotherapy and potential side effects.
- Evidence is inadequate to demonstrate an association of *BRAF* V600E mutations or loss of PTEN expression with nonresponsiveness to anti-EGFR therapy and therapy-related poorer health outcomes among patients with mCRC.
- No evidence was found to support improved health outcomes associated with testing results for *NRAS* or *PIK3CA* variants or AKT protein expression levels in this clinical scenario.

CLINICAL TRIALS: PLANNED, ONGOING, AND RECENTLY COMPLETED STUDIES

In an effort to determine whether any upcoming clinical trials may provide data in the near future that could change the recommendations provided here, advanced searches were done on the website ClinicalTrials.gov on 24 June 2011, using the search terms "KRAS," "BRAF," "V600E," "NRAS," "PIK3CA," "PTEN," and "AKT," and targeting the search by intervention category with the term "genetic." Of 15 studies returned for *KRAS*, results from the following four studies may prove relevant to this clinical scenario:

1. Phase II/III randomized study of molecular selection of chemotherapy using *K-ras*, *BRAF* mutation analysis, and topoisomerase-1 expression analysis in patients with metastatic or locally advanced colorectal cancer (FOCUS-3): Primary objectives of the definitive portion of the study include comparing response rate in patients with *KRAS* wild-type tumors treated with cetuximab or bevacizumab and irinotecan plus modified de Gramont against irinotecan plus modified de Gramont alone.[49]
2. Phase II study of panitumumab and irinotecan hydrochloride as third-line therapy in patients with previously treated mCRC without *KRAS* mutation: Tertiary objectives of the definitive portion of the study include correlating the treatment regimen with EGFR expression, proteomics, and epigenetics.[50]
3. Combination chemotherapy and cetuximab or bevacizumab in treating patients with mCRC: The purpose of this trial will be to assess tumor testing for mutations in *KRAS*, *BRAF*, and other genes for informing selection of chemotherapies.[51]
4. Presence of circulating tumor DNA in colorectal cancer (ALGECOLS): The purpose of this trial involves determining feasibility of isolating tumor mutations (e.g., *KRAS, NRAS*, and *BRAF*) from plasma.[52]

Using "*BRAF*" as a search term, no additional relevant studies were found. However, using the term "V600E," the following study was identified:

1. Study of *BRAF* mutations as predictors of efficacy in patients with advanced colorectal cancer treated with cetuximab: The primary objective of this study will be to determine, if the treatment effect, with cetuximab or bevacizumab, on progression-free survival is dependent on *BRAF* V600E tumor mutation.[53]

No additional relevant studies were identified using "*NRAS*," "*PIK3CA*," "PTEN," or "AKT" as search terms.

Lin et al.[9] noted that two recent abstracts, both describing retrospective analyses of RCTs investigating addition of cetuximab to first-line treatment, may support an association between *BRAF* mutation and poorer prognosis, independent of treatment, in patients with mCRC. In an updated search conducted in June 2011, Lin et al.[19] were able to describe these in more detail as a study nested in the OPUS trial that included only 11 patients with *BRAF* mutations, and a study nested in the CRYSTAL trial, with patients randomized to receive FOLFIRI ($n = 599$) or FOLFIRI along with cetuximab ($n = 599$). Results of the latter study demonstrated that *BRAF* mutations were associated with worse tumor response, progression-free survival, and overall survival as compared with patients with wild-type *BRAF* irrespective of whether or not cetuximab was included in the treatment. Although three potentially relevant studies involving *PIK3CA* were identified, none focused on differences between exons 9 and 20. Two of the four studies identified, which evaluated PTEN expression also, stratified patients by *KRAS* mutation status, and these demonstrated association of PTEN expression with tumor response, but not with overall survival. No relevant studies involving *NRAS* mutation analysis or AKT expression were identified. Moreover, the studies identified in the updated search did not alter the investigators' original conclusions.[9,19]

CONTEXTUAL ISSUES IMPORTANT TO THE RECOMMENDATION

- CRC is an important and highly prevalent health problem; improvements in outcomes associated with pharmacogenetic testing could have important health impacts.
- Adverse events related to cancer chemotherapy can be common and severe; successfully optimizing treatment for maximal efficacy and minimal side effects is important for reducing mCRC-related morbidity and mortality.
- Some side effects may be important prognostic indicators. A retrospective analysis of a randomized trial has shown that cetuximab-induced skin toxicity is correlated with improved tumor response and survival. In particular, significant correlation between better overall and progression-free survival with cetuximab-induced skin toxicity was evident in patients with *KRAS* codon 12 mutations.[54] There is potential for harm in assigning patients with mCRC to an ineffective therapy.

Cost effectiveness

- A recent Markov modeling analysis demonstrated that use of *KRAS* testing to select patients with mCRC for anti-EGFR treatment (with cetuximab or panitumumab) could save between $7,500 and $12,400 (US) per patient under most of the scenarios evaluated.[55]
- The Medical Advisory Secretariat 2010 technology assessment[25] included an economic analysis set in Ontario, Canada. Among the conclusions were that "while *KRAS* testing is cost effective for all strategies considered, it is not equally cost effective for all treatment options."[25]
- A cost-effectiveness analysis of *KRAS* testing with the use of cetuximab (versus best supportive care) in last-line therapy for mCRC was published by the Japanese investigators Shiroiwa et al.[56] in 2010. Although the authors recommended *KRAS* testing in this scenario, they concluded that "the ICER (incremental cost-effectiveness ratio) of cetuximab treatment (with or without KRAS testing) is too high, even if treatment is limited to patients with wild-type KRAS."
- A recent Swiss-based cost-effectiveness analysis concluded that although substantial cost is associated with testing, identification of wild-type *KRAS* and *BRAF* would be economically advantageous.[57]
- Finally, researchers in Leeds, UK, have recently published a cost-effectiveness analysis on testing of multiple formalin-fixed, paraffin-embedded sample blocks for *KRAS* and *BRAF* mutations, as a means of addressing intratumor

heterogeneity. Although tumor heterogeneity is not common, the investigators found that among 68 patients sampled in two trials (PICCOLO and FOCUS), testing of a single sample would mistakenly classify 10% of patients as having a wild-type genotype. The authors concluded that the minimal added cost of testing two or more samples would support improved detection of mutations.[58]

RESEARCH GAPS

The EWG found the research literature to be insufficient but encouraging in many respects, and recommends further studies that could address important gaps in knowledge:

- Published information on analytic validity of tests covered in this recommendation was both sparse and heterogeneous in reporting. More standardized reporting of assay performance characteristics would be helpful in allowing more meaningful evidence syntheses to be done.
- More high-quality prospective studies, as well as analyses involving RCT data (whenever possible) are needed to increase the certainty of evidence for each of the markers considered in this recommendation.
- Evidence pertaining to *BRAF* V600E, *NRAS*, *PIK3CA*, PTEN, and AKT was derived almost entirely from studies deemed to be of fair to marginal quality.[19] More studies of at least good quality will be necessary before definitive recommendations for or against use of these tests in this clinical scenario can be made.
- It remains unclear whether there is additional benefit of pharmacogenetic testing for *BRAF* with respect to ability to predict poor prognosis independent of treatment effects.
- Economic analyses on biomarkers other than *KRAS* and *BRAF* that are pertinent to this clinical scenario and focused on the US health-care system are currently lacking.

There has been increasing research focused on whether mutations in *KRAS* codon 13 have similar implications as those in codon 12 with respect to response to anti-EGFR therapy. A recent retrospective analysis of pooled data, including 579 patients involved in one of five different clinical studies, found the most frequent *KRAS* codon 13 (p.G13D) mutation in CRC to be associated with longer overall survival and progression-free survival with cetuximab treatment as compared with other *KRAS* mutations in an mCRC chemotherapy refractory setting.[59] In the first-line chemotherapy setting, a pooled analysis involving 1,378 patients derived from the CRYSTAL and OPUS studies revealed significantly improved progression-free survival and tumor response, although not overall survival, among those with tumor p.G13D mutations.[60] Two recent meta-analyses have been published on *KRAS* p.G13D and response to anti-EGFR therapy in mCRC. The first, which included 1,487 patients from 10 studies, reported significantly improved tumor response, progression-free, and overall survival with cetuximab among patients with p.G13D versus *KRAS* codon 12 mutations. Patients with p.G13D tumors had nonsignificantly shorter progression-free overall survival than those with wild-type *KRAS*, and significantly worse objective response rate.[61] The other meta-analysis involved 2,802 patients from seven studies, and reported significantly improved overall response rate for codon 13 versus other *KRAS* mutations, and significantly worse response than wild-type *KRAS*. For secondary outcomes of progression-free and overall survival, codon 13 was likewise higher than for other *KRAS* mutations, but not higher than wild-type.[62] Moreover, this area of research remains in a hypothesis-generating stage, and prospective studies in this area are needed.[63]

RECOMMENDATIONS OF OTHER GROUPS

Several recommendations and guidelines on colorectal cancer management have incorporated *KRAS* genotyping to predict nonresponsiveness to anti-EGFR therapy. Less information was found in such guidelines on *BRAF*, *NRAS*, *PIK3CA*, PTEN, or AKT. Some major examples identified from current guidelines are summarized below.

KRAS

- The American Society of Clinical Oncology released a provisional clinical opinion in April 2009, which stated 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 CLIA-accredited laboratory. If KRAS mutation in codon 12 or 13 is detected, then patients with metastatic colorectal carcinoma should not receive anti-EGFR antibody therapy as part of their treatment."[3]
- National Comprehensive Cancer Network colon and rectal cancer guidelines include a strong recommendation for *KRAS* "…genotyping of tumor tissue (either primary tumor or metastasis) in all patients with metastatic colorectal cancer at the time of diagnosis of stage IV disease. The recommendation for *KRAS* testing at this point is not meant to indicate a preference regarding regimen selection in the first-line setting, but rather, this early establishment of *KRAS* status is appropriate in order to plan for the treatment continuum, so that the information may be obtained in a non-time-sensitive manner, and the patient and provider can discuss the implications of a *KRAS* mutation, if present, while other treatment options still exist."[4,64]
- American College of Radiology Appropriateness Criteria: Rectal Cancer—Metastatic Disease at Presentation includes a discussion of several recent studies, which "collectively suggest that EGFR inhibitors should be considered in treating KRAS wild-type tumors, but should not be offered in KRAS mutant patients."[65,66]

BRAF

- An acknowledged limitation of the 2009 American Society of Clinical Oncology provisional clinical opinion statement is that it does not cover "assays for other alterations that have been reported to affect response to anti-EGFR

[monoclonal antibodies] (e.g., mutation in *BRAF*, *PI3K*, or *PTEN* genes and loss of expression of *PTEN* [*sic*] that may indicate resistance; amplification of EGFR, lack of amplification of PTEN, and expression of epiregulin, or amphiregulin that may indicate response)."[3]

- National Comprehensive Cancer Network colon cancer guidelines state that "for patients with *KRAS* wild-type tumors, the panel includes the option of *BRAF* genotyping of tumor tissue (either primary tumor or metastasis) at the time of diagnosis of *KRAS* wild-type stage IV disease."[4]

NRAS, PIK3CA, PTEN, AND AKT

- No guidelines or recommendations from United States–based groups were identified that specifically covered the use of *BRAF*, *NRAS*, *PIK3CA*, PTEN, or AKT to inform anti-EGFR antibody therapy for mCRC.
- Canadian Expert Group consensus recommendations on *KRAS* testing in CRC mention that "…data are currently insufficient to recommend testing for additional potential biomarkers such as *PTEN*…and *PIK3CA* mutations (level 2A)."[67]

DISCLOSURE

The authors declare no conflict of interest.

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