



Canadian Expert Group consensus recommendations: *KRAS* testing in colorectal cancer

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ABSTRACT

Monoclonal antibodies against the epidermal growth factor receptor (anti-EGFR) when used in the treatment of metastatic colorectal cancer are associated with improved survival. Patients whose tumours harbor a *KRAS* mutation in codon 12 or 13 have been shown not to benefit from anti-EGFR antibodies. The importance of *KRAS* mutation status in the management of patients with metastatic colorectal cancer has led to the elaboration of Canadian consensus recommendations on *KRAS* testing, with the aim of standardizing practice across Canada and reconciling testing access with the clinical demand for testing. The present guidelines were developed at a Canadian consensus meeting held in Montreal in April 2010. The best available evidence and expertise were used to develop recommendations for various aspects of *KRAS* testing, including indications and timing for testing, sample requirements, recommendations for reporting requirements, and acceptable turnaround times.

KEY WORDS

KRAS mutations testing, *BRAF* mutations testing, metastatic colorectal cancer, guidelines, consensus recommendations, cetuximab, panitumumab, anti-EGFR therapy

1. INTRODUCTION

Cetuximab and panitumumab, the monoclonal antibodies against the epidermal growth factor receptor (anti-EGFR), are currently used in the treatment of patients with pretreated metastatic colorectal cancer (mCRC) and are associated with improved progression-free survival and overall survival^{1,2}. However, not all patients benefit from anti-EGFR antibodies. Patients whose tumours harbor a *KRAS* mutation in codon 12 or 13 have consistently been demonstrated not to respond to anti-EGFR monoclonal antibody therapy. The

improvement in survival is limited to *KRAS* wild-type tumours³⁻⁷. Moreover, some studies suggest that the addition of cetuximab or panitumumab to first-line FOLFOX chemotherapy (leucovorin, 5-fluorouracil, oxaliplatin) is potentially detrimental in patients with *KRAS* mutant tumours^{5,8}.

Reports on the prognostic value of *KRAS* mutation status in mCRC are conflicting. However, in the analysis of the phase III CO.17 trial, no significant difference in survival was found by *KRAS* mutation status in patients assigned to best supportive care, thereby excluding a treatment-independent effect of *KRAS* mutation status on outcome⁶.

Funding and use of anti-EGFR therapy, and *KRAS* testing practices, are variable from province to province in Canada. That variability was evident in a survey conducted by the authors in October 2009 on the use of anti-EGFR therapy for mCRC. According to the survey, which was completed by 57 medical oncologists across Canada, 81% of respondents have funded access to cetuximab or panitumumab as therapy for mCRC, and most (96%) use it in third-line therapy. There is no single preferred anti-EGFR strategy in the third-line setting (37% prefer panitumumab monotherapy; 28%, cetuximab monotherapy; 16%, cetuximab plus irinotecan combination therapy; and 30% have no preference). Use of an anti-EGFR antibody would be considered by 14% of respondents when the *KRAS* status is unknown, and none would consider using such an antibody after failure of another anti-EGFR antibody.

Of the survey respondents, 63% have routine access at their institution to testing for *KRAS* gene mutation. Most *KRAS* mutation testing is requested during second-line (43%) or before third-line therapy (50%). Only 9% request the test during first-line therapy, and 2%, at diagnosis. With regard to biomarkers beyond *KRAS*, 88% do not currently have routine access at their institution to testing for *BRAF* mutation.

Guidelines from Cancer Care Ontario⁹ and the BC Cancer Agency¹⁰ recommend EGFR inhibitors

after failure of standard chemotherapy in patients with advanced CRC whose tumours have tested negative for *KRAS* gene mutations in codons 12 and 13. The importance of *KRAS* gene mutations in the management of mCRC patients has led to this current effort to develop Canadian consensus recommendations on *KRAS* testing, with the aim of standardizing clinical testing practices and access across Canada. The current Canadian guidelines are based on a consensus meeting held in Montreal in April 2010 and on the most current literature.

2. CONSENSUS PROCESS

Panel members included gastrointestinal medical oncologists, molecular geneticists, and pathologists from across Canada. The recommendations reflect evidence from the published literature and the collective experience of the authors. This set of consensus recommendations complements the recent review on *KRAS* mutation testing techniques in the treatment of mCRC published by Drs. Soulières, Kamel–Reid, and colleagues in *Current Oncology* in 2010¹¹. Table I describes the criteria used to rate the level of consensus. Table II summarizes the recommendations that follow.

3. RECOMMENDATIONS

3.1 Indications for and Funding of *KRAS* Testing

The *KRAS* gene is a predictive biomarker for anti-EGFR therapy, and tumour *KRAS* status should be determined whenever anti-EGFR therapy is being considered in the treatment of mCRC (level 1). In this era of personalized medicine and targeted therapies, it is imperative that funding of the drug be linked to funding of the requisite predictive test (level 2A).

3.2 Timing of *KRAS* Testing

The cost of *KRAS* testing is highly dependent on the method used for the detection of *KRAS* status, with test kits costing up to \$450 per sample tested. Generally, costs range from \$300 to \$450 depending on the method used; pre- or post-test costs associated with getting the tissue to the lab and assessing its tumour cellularity are not included.

Given the high prevalence of CRC in the Canadian population and the relatively high cost of the test, the expert group agreed that it is important to restrict *KRAS* testing to the metastatic setting, in which the results directly affect clinical management (level 2A).

The issue of testing at diagnosis was also addressed. Because *KRAS* status has not been demonstrated to be a strong prognostic biomarker, it was recommended that routine *KRAS* testing at diagnosis, regardless of stage, cannot be recommended at the present time (level 2A).

To offer maximal lead time to identify patients who would be suitable for third-line anti-EGFR therapy, it is recommended that *KRAS* testing be requested in mCRC patients when those patients are starting second-line therapy (level 2B). The high-volume testing laboratories in Canada (Princess Margaret Hospital, Mount Sinai Hospital, and the BC Cancer Agency) currently report a testing turnaround time of 10–14 working days, and so tissue transfer from the pathology department of the originating hospital to the testing laboratory can be subject to significant time delays. Should the surgical specimen submitted for analysis be deemed insufficient, a second submission or a re-biopsy may be necessary, thereby increasing the time required to obtain an actionable result (further elaborated in the next subsection, “Sample Requirements”). Hence, the practice of waiting until second-line progression for *KRAS* testing is discouraged, because delays in testing may potentially result in patients progressing to the point where they may no longer be fit for third-line anti-EGFR therapy.

It has previously been suggested that earlier testing may result in significant excess testing, because a significant proportion of patients may not proceed further than first-line therapy. In the study by Tournigand and colleagues¹², the rate of drop-off from first- to second-line chemotherapy approached 35%. Waiting until after first-line therapy to test accords with the earlier recommendation that testing be done in candidate patients when they start second-line therapy.

Indications for anti-EGFR therapy may evolve as new evidence emerges. For example, the use of anti-EGFR inhibitors in combination with first-line chemotherapy has been suggested as an option for conversion therapy in patients with liver-limited

TABLE I Categories of consensus

Level	Definition
1	Uniform consensus based on high-level evidence that the recommendation is appropriate.
2A	Uniform consensus based on lower-level evidence, including clinical experience, that the recommendation is appropriate.
2B	Non-uniform consensus, but no major disagreement, based on lower-level evidence, including clinical experience, that the recommendation is appropriate.
3	Major disagreement that the recommendation is appropriate.

TABLE II Summary of recommendations

	Recommendation	Level of consensus
1.	<p>Indications and funding for <i>KRAS</i> testing</p> <p><i>KRAS</i> is a predictive biomarker for anti-epidermal growth factor receptor (EGFR) therapy and tumour <i>KRAS</i> status should be determined whenever anti-EGFR therapy is considered in the treatment of metastatic colorectal cancer (mCRC). Funding of the drug should be linked to funding of the requisite predictive test.</p>	1 2A
2.	<p>Timing of <i>KRAS</i> testing</p> <p>When possible, it is recommended that <i>KRAS</i> testing be requested when mCRC patients start second-line therapy.</p>	2B
3.	<p>Sample requirements</p> <p>Formalin-fixed paraffin-embedded tissue blocks are typically available for mutational analysis. Tumour cell enrichment by micro- or macro-dissection or selective sampling of the paraffin block by needle core should be used to increase the sensitivity of tumour testing.</p> <p>Mutational analysis should use the primary resection specimen, if available.</p> <p>An endoscopic core biopsy of the primary tumour is preferred over a core biopsy of a distant metastasis. If core biopsy of the primary is not possible, core biopsy of a distant metastasis should be obtained. Fine-needle aspiration of a metastasis should be avoided.</p> <p>If every reasonable effort has been made to ascertain <i>KRAS</i> status, but that status remains unknown, it would be reasonable to speak with the patient and to offer the benefit of consideration for anti-EGFR therapy.</p>	2A
4.	<p>Optimal test for <i>KRAS</i> mutational analysis</p> <p><i>KRAS</i> testing strategies are deemed acceptable if they satisfy these minimal requirements: a mutation-detection sensitivity between 95% and 99%, and a specificity of 100%.</p> <p><i>KRAS</i> testing must be reproducible and should be performed by an accredited laboratory that conforms to quality guidelines for <i>KRAS</i> testing and routinely participates in proficiency testing such as that offered by the College of American Pathologists, with external validation.</p>	2A
5.	<p>Testing beyond <i>KRAS</i>: <i>BRAF</i> status</p> <p><i>BRAF</i> is a negative prognostic factor, but does not appear to be predictive. Routine testing of <i>BRAF</i> mutation status before anti-EGFR therapy is not currently recommended.</p> <p>Similarly, data are currently insufficient to recommend testing of other potential biomarkers (<i>PTEN</i>, amphiregulin, epiregulin, and <i>PIK3CA</i> mutations, among others).</p>	2A
6.	<p>Test reporting requirements</p> <p>The absence or presence of <i>KRAS</i> mutation must be reported.</p> <p>At a minimum, if a mutation is identified, the affected codon should be specified, and if available, the specific change should be indicated.</p> <p>Appropriate nomenclature should be used in reporting results.</p> <p>Report should specify the assay used.</p> <p>Reports should conform to existing reporting guidelines (American College of Medical Genetics, College of American Pathologists, Canadian College of Medical Geneticists).</p>	2A
7.	<p>Acceptable turnaround times</p> <p>Once the specimen is received by the testing laboratory, 10 working days is an acceptable turnaround time for reporting the result to the ordering physician.</p>	2A

disease who may be downstaged to resectability. The U.K. National Institute for Health and Clinical Excellence currently endorses the use of cetuximab in combination with FOLFOX (5-fluorouracil, leucovorin, oxaliplatin)—or with FOLFIRI (leucovorin, 5-fluorouracil, irinotecan) if FOLFOX is contraindicated—for the first-line treatment of *KRAS* wild-type

metastatic colorectal cancer if metastatic disease is confined to the liver and is potentially resectable¹³. This strategy is currently not routinely used in the Canadian practice setting, but as data continue to emerge, the indications for use of anti-EGFR therapy, and consequently for *KRAS* testing, may transition to earlier lines of therapy in the near future.

3.3 Sample Requirements

Formalin-fixed paraffin-embedded tissue blocks are typically available for mutational analysis. Until recently, formalin-fixed tissues were considered to be of low quality for DNA testing, but recent technique refinements have enhanced sensitivity if sufficient DNA is available¹⁴. Tumour cell enrichment by micro- or macro-dissection, or selective sampling of the paraffin block by needle core, should be used to increase the sensitivity of tumour testing (level 2A).

Mutational analysis should use the primary resection specimen, if available (level 2A). However, an estimated 20% of patients will present with metastatic disease at diagnosis¹⁵. If the primary resection specimen is not available, biopsy material is required for analysis. When the primary is left *in situ*, an endoscopic core biopsy may offer a more representative sample of the tumour and is preferred over a core biopsy of a distant metastasis in which tumour cells might represent little of the sample. If an endoscopic core biopsy of the primary is not possible, core biopsy of a distant metastasis should be obtained (level 2A). A recent Italian study¹⁶ showed a high level of concordance for *KRAS* mutational status between primary tumours and metastases. Fine-needle aspiration of a metastasis should be avoided, because the sample obtained is often insufficient to proceed with a mutational analysis. The pathologist should be responsible for choosing the most appropriate tissue block to be tested.

The probability of wild-type *KRAS* status is 60%. If every reasonable effort has been made to ascertain *KRAS* status, but that status remains unknown, it would be reasonable to speak with the patient and to offer the benefit of consideration for anti-EGFR therapy (level 2A).

3.4 Is There an Optimal Test for *KRAS* Mutational Analysis?

Most of the clinically implicated *KRAS* mutations will be identified in codons 12 and 13¹⁷. Several mutation detection procedures have been described, all of which are based on polymerase chain reaction (see the *KRAS* mutation testing review by Soulières *et al.*¹¹). Various testing strategies are used across the country: TheraScreen K-RAS test kit (Qiagen, Toronto, ON) alone or in combination with direct sequencing or restriction fragmentation length polymorphism (or both), and restriction fragmentation length polymorphism plus sequencing.

Any validated test strategy is deemed acceptable provided that it satisfies these minimal requirements (level 2A):

- A mutation-detection sensitivity between 95% and 99%
- A specificity of 100%

Because harbouring a *KRAS* mutation is a negative predictor, any assay must have a high specificity (that is, a low rate of false positives, which may deny anti-EGFR therapy to an otherwise eligible patient), but it is reasonable to accept less than 100% sensitivity. Testing for *KRAS* must be reproducible and should be performed by an accredited laboratory that conforms to quality guidelines for such testing and that routinely participates in proficiency testing such as that provided by the College of American Pathologists, with external validation (level 2A).

3.5 Testing Beyond *KRAS*: *BRAF* Status

The *BRAF* gene is a promising potential prognostic and predictive biomarker. However, its predictive value, which was observed mainly in small retrospective studies, shows some inconsistency^{18,19}. In fact, in recent analyses of the CRYSTAL trial, the pooled CRYSTAL/OPUS and CAIRO2 trials, *BRAF* was demonstrated to be a strong negative prognostic factor, but not to be predictive of lack of benefit with cetuximab^{20–22}. Hence, *BRAF* status does not appear to be consistently predictive in all trials of anti-EGFR therapy and would therefore not alter current treatment decision-making. Further supportive, preferably prospective, confirmation of the role of *BRAF* as a predictive biomarker for anti-EGFR therapy would be necessary before such use in routine clinical practice can be considered.

Similarly, the data are currently insufficient to recommend testing for additional potential biomarkers such as *PTEN*, amphiregulin, epiregulin, and *PIK3CA* mutations (level 2A).

3.6 Test Reporting Requirements

The absence or presence of *KRAS* mutation must be reported in writing. If a mutation is identified, the affected codon and, if available, the specific change should be indicated. Ambiguous terms for results such as “positive” or “negative” should be avoided, because they could lead to confusion. Appropriate nomenclature should be used to report results. The report should also specify the assay that was performed. As always, reports should conform to existing reporting guidelines from organizations such as the American College of Medical Genetics, the College of American Pathologists, and the Canadian College of Medical Geneticists (level 2A).

3.7 Acceptable Turnaround Times

Given that anti-EGFR treatment is an accepted standard of care for patients with advanced refractory CRC and wild-type *KRAS* tumours, the need for timely release of tissue by host laboratories is paramount. Once the specimen is received by the testing laboratory, 10 working days is an acceptable turnaround time for reporting the *KRAS* mutational analysis result to the ordering physician (level 2A).

4. CONFLICT OF INTEREST DISCLOSURES

None of the authors received financial compensation to participate in the consensus conference or to write the paper.

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