Expanded *RAS*: Refining the Patient Population

Chloe E. Atreya, *Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, CA*
Ryan B. Corcoran, *Massachusetts General Hospital Cancer Center and Harvard Medical School, Boston, MA*
Scott Kopetz, *The University of Texas MD Anderson Cancer Center, Houston, TX*

The era of precision medicine has changed the treatment paradigm for some cancers, but for others, the term precision is a stretch. New targets and therapies have broadened choices for patients with lung cancer or melanoma; however, in colorectal cancer (CRC), for example, the evolution of biomarkers has been primarily to inform whom not to treat rather than to identify the patients who will benefit from treatment. The refinements in CRC have been limiting rather than expansive; by broadening the definition of *RAS* mutations, fewer patients have multiple targeted options.

The epidermal growth factor receptor (EGFR) antibody cetuximab was first approved a decade ago for use with FOLFIRI (folinic acid, fluorouracil, and irinotecan) chemotherapy in metastatic CRC (mCRC), based on the findings of the CRYSTAL (Cetuximab Combined With Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer) trial.[1] Shortly thereafter, after the results of the PRIME (Panitumumab Randomized Trial in Combination With Chemotherapy for Metastatic Colorectal Cancer to Determine Efficacy) trial, a second EGFR antibody, panitumumab, was approved for use with FOLFOX (folinic acid, fluorouracil, and oxaliplatin) in a similar patient population (2006).[2] It would be another 3 years before the US Food and Drug Administration (FDA) acknowledged emerging data suggesting that the most common activating mutations in *KRAS*, at codons 12 and 13 in exon 2, were negative predictors for treatment benefit from EGFR antibodies. In 2009, the FDA restricted the use of cetuximab and panitumumab to the roughly 60% of patients without *KRAS* exon 2 mutations. Enrichment for *KRAS* codon 12 and 13 wild-type tumors increased response rates to approximately 60%[1,2] and spurred a quest to further characterize the downstream effectors of EGFR.

After no definitive predictive roles were identified for activating *BRAF* and *PIK3CA* mutations or for loss of PTEN expression, the focus shifted back to *RAS*.[3] First, perspective narrowed as questions arose concerning whether all *KRAS* codon 12 and 13 mutations were equivalent; retrospective analyses suggested that patients with *KRAS* G13D mutations may derive a modest benefit from EGFR antibodies, intermediate between that of *KRAS* wild-type and codon 12–mutated tumors.[4-6] However, the effect was not robust enough to merit practice change.[7]

Meanwhile, interest was growing in *KRAS* exon 3 and 4 mutations, found to be oncogenic and associated with resistance to EGFR-targeted agents in preclinical models.[8,9] These findings have yielded a bounty of secondary analyses of phase II to III clinical trials—studies large enough to investigate the effect of so-called new, extended, or expanded *RAS* mutations.[10-19] In the August 1, 2014, issue of *Lancet Oncology*, Heinemann et al[20] reported on expanded *RAS* mutations in the FIRE-3 trial, corroborating the current trend.

The definition of expanded *RAS* has evolved. For the moment, the field has settled on *KRAS* and *NRAS* codons 12 and 13 (exon 2), 59 and 61 (exon 3), and 117 and 146 (exon 4). Mutations in *KRAS* and *NRAS* are typically mutually exclusive. Among five trials of first-line therapy for mCRC, including FIRE-3, the prevalence of expanded *RAS* mutations in tumor specimens, previously determined to be wild type at exon 2 of *KRAS*, ranged from 15% to 27%.[12,15,16,19,20] Studies at both extremes of mutation prevalence used BEAMing (beads, emulsions, amplification, and magnetics) technology, with a 5% mutated/wild-type allele sensitivity cutoff. A meta-analysis of nine randomized controlled trials evaluating EGFR antibodies in all lines of mCRC therapy found that approximately 20% of *KRAS* exon 2 wild-type tumors harbored an expanded *RAS* mutation.[21] By exon, the estimated prevalence of mutations was as follows: 4.3% in exon 3 and 6.7% in exon 4 of *KRAS*, and 3.8% in exon 2, 4.8% in exon 3, and 0.5% in exon 4 of *NRAS*.[21]

Meta-analysis revealed that EGFR antibody efficacy was superior for all–*RAS* wild-type tumors compared with the expanded *RAS*-mutant subgroup with respect to both progression-free survival and overall survival. Conversely, efficacy was not significantly different between the expanded *RAS*-mutant and *KRAS* exon 2–mutant subgroups.[21] In FIRE-3 specifically, median progression-free survival was inferior among patients with expanded *RAS* mutations who received cetuximab rather than bevacizumab plus FOLFIRI (hazard ratio, 2.22; 95% CI, 1.28 to 3.86; *P* = .004).[20] Although lack of benefit for EGFR inhibitor treatment in the setting of expanded *RAS* mutations was a consistent finding, statistically significant detrimental effects of EGFR antibodies were not observed reproducibly across studies. In CALGB (Cancer and Leukemia Group B)/SWOG (Southwest Oncology Group) 80405, as distinct from FIRE-3, the all–*RAS* wild-type subgroup associated with improved outcome, independent of treatment containing cetuximab or bevacizumab, suggesting that expanded *RAS* mutations may be prognostic (A.P. Venook, personal communication, October 2014). Common to all trials, CIs around hazard ratios for the expanded *RAS* subset were large, reflecting small numbers of patients.

Do these trials provide sufficient evidence to mandate routine testing for expanded *RAS* mutations in all patients with mCRC? The European Commission has updated prescribing indications for panitumumab and cetuximab, restricting use to patients with *RAS* wild-type mCRC. In the United States, package inserts state that EGFR antibodies are not indicated for treatment of *KRAS* mutation–positive mCRC, without reference to *NRAS* or specific *KRAS* codons.[22,23] The recently updated National Comprehensive Cancer Network colon cancer guidelines state that "whenever possible," non– exon 2 *KRAS*

and *NRAS* mutation status should be determined, and patients with "any known" *KRAS* or *NRAS* mutation should not receive cetuximab or panitumumab.[24] Questions remain about how to integrate the new guidelines into clinical practice.

Which RAS *Mutations Matter?*

Because of the rarity of specific *RAS* mutations evaluated, and because analyses have pooled the effects of expanded *RAS* mutations from as many as 10 individual *KRAS* and *NRAS* codons, it is difficult to determine to what degree each specific mutation confers lack of sensitivity to EGFR antibodies. No single expanded *RAS* mutation occurs at a frequency of > 6%, and some *RAS* mutations evaluated occur at frequencies of ≤ 1% (ie, mutations at codons 59 and 117 in *KRAS* and *NRAS* and at codon 146 in *NRAS*). Without the creation of a big-data warehouse from tested and treated patients, it is unlikely that the contribution of any one rare *RAS* mutation will ever be definitively determined. Indeed, there is still debate over whether *KRAS* G13D mutations (approximately 8% of CRCs) confer the same degree of resistance as codon 12 mutations. Furthermore, as whole-exome or targeted-exome sequencing replaces hot-spot mutational analysis, the identification of rare *RAS* mutations of unknown significance outside of these codons may create additional confusion.

Because not all individual *RAS* mutations are necessarily created equal, we may not be able to safely assume that any *RAS* mutation will predict lack of response to EGFR antibodies. Our confidence in the utility of any one specific *RAS* mutation as a negative predictive biomarker may have to rely in part on indirect clinical and preclinical data. For example, we can be more assured that an individual *RAS* mutation will predict lack of response to EGFR antibodies if that same mutation has been identified in patients with CRCs originally classified as *RAS* wild type who developed acquired resistance to EGFR antibodies. Mutations in codons 12, 13, 61, and 146 and *NRAS* codons 12, 13, and 61 have been observed in the setting of acquired resistance.[25-28] Preclinical data may also be helpful. For example, *KRAS* codon 117 mutations have been shown to be activating and able to confer resistance to EGFR inhibitors in preclinical models.[8,9] Conversely, there are few preclinical data to support a functional role for the rare codon 59 *RAS* mutation. Therefore, we should exercise caution when considering whether to withhold an EGFR antibody from a patient with a codon 59 mutation or another rare *RAS* mutation about which little is known.

What Are Best Practices to Test for RAS *Mutations?*

The current landscape of molecular testing has incorporated a variety of testing platforms, each with varying technical performance, cost, and feasibility.[29] Currently, the only FDA-approved test for use with cetuximab and panitumumab is a *KRAS* companion diagnostic that tests for mutations on *KRAS* codons 12 and 13 (using Scorpion Amplified Refractory Mutation System [ARMS] polymerase chain reaction [PCR] methodology, with reported sensitivity of approximately 1% to 5%). Alternate technologies, including BEAMing assays and digital PCR, have improved sensitivity to the range of 0.1% for tissue samples, representing the ability to detect one heterozygous mutant cancer cell in a background of 500 wild-type cells. Many next-generation sequencing platforms in clinical use can reliably detect allele frequencies in the range of 5% to 10%, although lower allele frequencies are commonly seen and variably reported in clinical results. Data using these high-sensitivity assays have employed various

allele frequencies for calling mutations, with many of the recent retrospective studies applying a 5% threshold. When *KRAS* mutations are present in CRC, they are typically present in the vast majority if not all of the cancer cells (mutations in *KRAS* are approximately 95% concordant in paired CRC metastases and primary tumors).[30] Nonetheless, retrospective studies using higher-sensitivity research assays such as digital PCR have suggested that tumors with rare *KRAS*-mutant clones may be present in up to 20% to 30% of patient cases determined to be *KRAS* wild type by prior clinical testing.[31-33] At least one study has suggested that these rare clones may provide a reservoir for acquired resistance to EGFR monoclonal antibodies.[26] An analysis of various thresholds for allele frequency from the CRYSTAL study demonstrated trends toward greater discrimination of benefit down to a threshold of 0.1% mutant *RAS*, although even this large study was underpowered to robustly address this question.[31] Because it remains possible that patients whose tumors harbor rare *RAS*-mutant cells might still derive meaningful initial benefit from EGFR antibodies, exceedingly sensitive detection thresholds may not be appropriate in restricting use of EGFR antibodies.

An additional barrier to the interpretation of the allele frequency results from the variable handling of tumor tissue before DNA extraction. Microdissection of the tumor tissue from the glass slide increases the purity of the cancer cell DNA by approximately two- to 10-fold, but this is labor intensive. In the current FDA-approved assay, microdissection is recommended only for patient cases where < 20% of the cells on the slide are cancerous, but the estimation of percentage of tumor cells has substantial interobserver variation.[34] In most retrospective studies, no microdissection was performed, adding to uncertainty about the true mutant allele frequency in the tumors. Additional retrospective studies will need to incorporate microdissection with quantification to appropriately address the impact of allele frequency, including correlation with clinical outcomes.

Should BRAF *Be Included in Testing Panel?*

Activating *BRAF* codon 600 (exon 15) mutations occur in 5% to 10% of CRCs and are mutually exclusive from *RAS* mutations, with
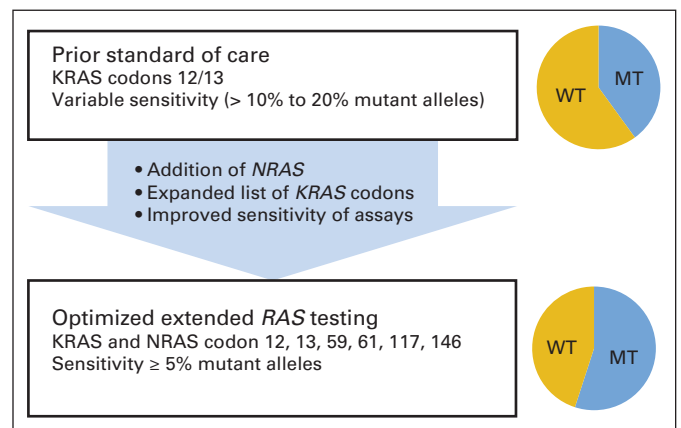

Fig 1. Advances in *RAS* testing. Optimal clinical implementation of *RAS* testing involves expansion of number of tested codons to include less common mutations and use of assays with sufficient sensitivity for *RAS*-mutant alleles. To date, preponderance of clinical data reported on expanded *RAS* mutations has used 5% threshold for detection of mutated (MT)/wild-type (WT) alleles; therefore, this represents a reasonable threshold while additional analyses are conducted.

rare exceptions.[13] Retrospective analyses of several large randomized trials have not defined a clear role for *BRAF* V600 as a negative predictive biomarker for response to EGFR antibodies.[12,13,16,35] Although limited by sample size, it is notable that no study has shown a statistically significant benefit from the addition of an EGFR antibody to chemotherapy in patients with *BRAF* V600–mutant CRC. Furthermore, these analyses have confirmed prior observations that patients with *BRAF* V600–mutant CRC have a poor prognosis and do not respond well to standard therapies. Therefore, consideration of clinical trials as an early option for patients with *BRAF* V600–mutant CRC may be of greater clinical importance than whether such patients should receive EGFR antibodies. Indeed, several recently reported clinical trials evaluating RAF inhibitor–based combinations, including with EGFR antibodies, in patients with *BRAF* V600–mutant CRC have shown promising preliminary results, with some studies achieving response rates close to 40%.[36-38]

In summary, we recommend expanded *RAS* mutation testing as part of the initial workup for mCRC, because this approach will identify an additional approximately 11% of patients with CRC who are unlikely to benefit from EGFR antibodies. We advise using a platform sensitive enough to detect mutations at an allele frequency threshold of ≤ 5% (Fig 1). Although no definitive role for *BRAF* as a negative predictive biomarker has been established, we recommend routine testing for *BRAF* V600 mutations because ongoing *BRAF*-directed clinical trials offer a promising alternative. Finally, in our pursuit of expanded *RAS* testing, we must be cautious not to expand so rapidly that we outpace the existing data by basing clinical decisions on rare *RAS* mutations, such as codon 59 or other infrequent *RAS* mutations, about which little is presently understood. Continued efforts to fine-tune our approach to the molecular classification of CRC will hone our ability to deliver precise and effective care to our patients.

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Disclosures provided by the authors are available with this article at www.jco.org.

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Chloe E. Atreya
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Ryan B. Corcoran
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Consulting or Advisory Role: Avidity Nanomedicines

Scott Kopetz
Consulting or Advisory Role: Amgen, Roche, GlaxoSmithKline, Janssen, Bristol-Myers Squibb, Agendia, Merrimack, Sysmex, Bayer
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