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The Last Word

Why a One Size Fits All Approach to *RAS* Might Not Fit Colorectal Cancer

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Since the discovery that *KRAS* exon 2 mutations predict a lack of benefit from anti–epidermal growth factor receptor (EGFR) therapy in metastatic colorectal cancer, our understanding of *RAS* mutations has expanded considerably. Guidelines now suggest extended *RAS* characterization, which includes assessment of *KRAS* and *NRAS* for mutations at exon 2 (codons 12 and 13), exon 3 (codons 59 and 61), and exon 4 (codons 117 and 146).[1] Extended *RAS* mutations appear to confer resistance to anti-EGFR therapy. With their inclusion, approximately 56% of patients will be defined as having a *RAS* mutation and will not be eligible for anti-EGFR therapy.[2,3] In addition, atypical *RAS* mutations exist that are of unclear significance and difficult to study in any prospective manner given their rarity. Even within guideline-described codons there are mutations, such as *KRAS* A59T, that are of unclear significance. Studying these mutations individually would be nearly impossible, and they highlight a pressing issue of how we deal with variants of unknown significance (VUS). The number of uncommon VUS is likely to expand exponentially as clinical sequencing moves to more comprehensive sequencing platforms, and we will need solutions to deal with these variants.

In this issue of *JNCCN*, Lou et al ("Therapeutic Response of Metastatic Colorectal Cancer Harboring a *KRAS* Missense Mutation After Combination Chemotherapy With the EGFR Inhibitor Panitumumab") present the case of a 50-year-old woman with a *KRAS* A59T–mutated metastatic colon cancer who received 4 cycles of FOLFIRI plus panitumumab and showed a radiographic partial response accompanied by a decline in carcinoembryonic antigen. The patient subsequently experienced disease control lasting 8 months while receiving FOLFIRI plus panitumumab. She was noted to have a *KRAS* A59T mutation after the treating institution changed from a Sanger sequencing platform specific to *KRAS* exon 2 to a more comprehensive amplicon-based next-generation sequencing (NGS) panel that included extended *RAS* coverage. The NGS panel was performed on DNA extracted from the same biopsy of the primary tumor as the Sanger sequencing. The authors propose that patients with *KRAS* A59T mutations may represent a population with continued sensitivity to anti-EGFR therapy.

Challenges and Opportunities in Studying Rare Biomarkers

Given the infrequency of many extended *RAS* mutations, they have not been well described individually and were considered as a group of mutations in the studies that support extended *RAS* mutation testing. Codon 59 mutations are particularly lacking in evidence to support their role in predicting resistance to anti-EGFR therapy. Only 7 patients with codon 59 mutations were described in the landmark PRIME trial analysis of extended *RAS* mutations, and these mutations were not part of the extended *RAS* mutation analysis. The evidence to support these mutations as a predictive marker was only the fact that removing these patients from the population with wild-type RAS resulted in a hazard ratio that made anti-EGFR agents appear more beneficial in wild-type patients.[3]

Clearly, further characterization of these variants is required, but how do we characterized them in a meaningful way? Using in vitro assays is one option. At our institution, the Precision Oncology Decision Support Team has a functional genomics assay using BaF3 and MCF10A cell viability studies to help characterize unknown

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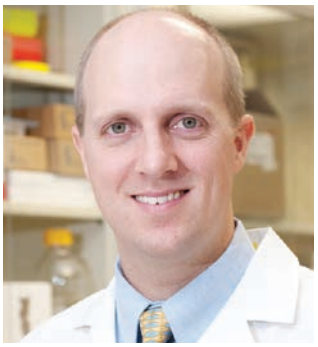

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mutations, but is this enough information to guide patient care? Another challenge with functional assays is that these techniques are not available to guide decisions outside of a specialized center, making them impractical for most clinical care.

A different approach to studying rare variants is the potential to incorporate real-world evidence. As we move into an era of electronic health records and big data, the ability to draw information from large data sets that are pooled between multiple institutions represents a unique opportunity to study uncommon situations. By harnessing the strengths of studying entire populations rather than just focusing on patients enrolled on a trial, we may be able to answer difficult questions. For example, there are 8 patients with *KRAS* A59T mutations noted in the COSMIC database; however, our own institution's database notes another 5.[4] Other institutions may also have information on a handful of these patients. If the outcomes data on all of these patients were merged, we may be able to make a meaningful statement about the particular mutation.

These efforts are complicated by privacy concerns, administrative hurdles to merging data from different health authorities, and the need for standardized reporting to facilitate pooled analyses. Despite these challenges, there is a growing realization that real-world data are useful. In fact, the FDA is in the process of drafting guidelines to suggest how premarketing and postmarketing regulatory requirements may be addressed using real-world evidence to complement traditional clinical trials.[5] Efforts such as The Cancer Genome Atlas and the American Association for Cancer Research (AACR) Project Genie are working to bring together genomic repositories and clinical annotation in platforms that can be used to ask questions regarding biomarkers. These data sets not only allow studies in large populations but also make us more efficient as a research community.

Context of a variant and biologic rationale may also help guide us in our efforts to understand the clinical significance of uncommon alterations. Many of the extended *RAS* variants in exon 3 and 4 are more commonly detected in patients who have previously been treated with anti-EGFR therapy and were originally categorized as *RAS* wild-type.[6] The fact that these alterations arose under selective pressure and coincide with clinical progression strongly argues that they predict resistance to anti-EGFR therapies and supports extended *RAS* mutations as predictive biomarkers. Unfortunately, *KRAS* codon 59 mutations have not been described as a mechanism of resistance in colorectal cancer to help guide our assessment of the current case.

Is *KRAS* A59T Truly Sensitive to Anti-EGFR Therapy?

Although reports of patients with exceptional response can provide insight into the biology underlying response, application to clinical decision-making needs to be tempered. When we see reports of a single patient with a genomic biomarker experiencing an exceptional outcome, we must always consider whether a sequencing error could be present. Tumor heterogeneity is another important consideration not mentioned by the authors that should be considered when targeted therapies result in unexpected outcomes. Concordance between primary tumor and liver metastases *RAS* status appears high in colorectal cancer, ranging from 90% to 100%; however, discordance of up to 32% has been reported between lung or lymph node metastases and primary tumors.[7,8] In the reported case, a partial response was noted in the pulmonary metastases, although whether the liver or primary tumor responded to therapy is unclear. Given that the primary tumor is where the *KRAS* mutant clone was sampled, this information is particularly important. It is possible that the partial response was noted only in *KRAS* wild-type clonal populations, and that this exceptional response is not so exceptional.

A major barrier to understanding clonal dynamics is that we cannot truly know the genotype of every cell within a patient at all times. Possibly, patients with only part of their total tumor mass containing a *RAS* mutation may still derive benefit from anti-EGFR therapy. In the CRYSTAL trial, low allele frequency *RAS* mutations identified using highly sensitive bead emulsion PCR were shown to predict a lack of benefit in overall survival from anti-EGFR therapy.[9] However, objective response rates appeared to show trends toward benefit in the low mutant allele frequency group. The strength of this trend continuously increased as the mutant allele proportion decreased. In the current report, we lack information about the mutant allele frequency and the mutation status of the metastases that responded to therapy, and this may be an important driver of response.

Conclusions

In the right context, *KRAS* A59T mutations may be sensitive to anti-EGFR agents. Does this case report prove it? No. It raises an important question about whether all *RAS* mutations are created equally and highlights the difficulties in characterizing uncommon variants. Although in vitro assays and real-world evidence may help us understand the significance of these variants, they are not definitive. A recent review of the literature and internal databases at our institution noted 98 unique *RAS* variants, many of which lacked characterization. We will never be able to fully define the significance of all of these mutations with prospectively collected data, and some degree of uncertainty will always remain. However, guidelines are built on best available evidence, and it will require more than case reports to justify a change.

References

1. Sepulveda AR, Hamilton SR, Allegra CJ, et al. Molecular biomarkers for the evaluation of colorectal cancer: guideline from the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and the American Society of Clinical Oncology [published online ahead of print February 6, 2017]. J Clin Oncol, doi: 10.1200/JCO.2016.71.9807.
2. Peeters M, Kafatos G, Taylor A, et al. Prevalence of RAS mutations and individual variation patterns among patients with metastatic colorectal cancer: a pooled analysis of randomised controlled trials. Eur J Cancer 2015;51:1704–1713.
3. Douillard JY, Oliner KS, Siena S, et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. N Engl J Med 2013;369:1023–1034.
4. Forbes SA, Beare D, Gunasekaran P, et al. COSMIC: exploring the world's knowledge of somatic mutations in human cancer. Nucleic Acids Res 2015;43:D805–811.
5. Sherman RE, Andersen SA, Dal Pan GJ, et al. Real-world evidence—what is it and what can it tell us? N Engl J Med 2016;375:2293–2297.
6. Morelli MP, Overman MJ, Dasari A, et al. Characterizing the patterns of clonal selection in circulating tumor DNA from patients with colorectal cancer refractory to anti-EGFR treatment. Ann Oncol 2015;26:731–736.
7. Han CB, Li F, Ma JT, Zou HW. Concordant KRAS mutations in primary and metastatic colorectal cancer tissue specimens: a meta-analysis and systematic review. Cancer Invest 2012;30:741–747.
8. Baas JM, Krens LL, Guchelaar HJ, et al. Concordance of predictive markers for EGFR inhibitors in primary tumors and metastases in colorectal cancer: a review. Oncologist 2011;16:1239–1249.
9. Van Cutsem E, Lenz HJ, Kohne CH, et al. Fluorouracil, leucovorin, and irinotecan plus cetuximab treatment and RAS mutations in colorectal cancer. J Clin Oncol 2015;33:692–700.