ADVANCES IN ONCOLOGY

Current Developments in the Management of Solid Tumor Malignancies

Section Editor: Clifford A. Hudis, MD

Colorectal Cancer In Focus

KRAS: Guiding Treatment of Patients With Metastatic Colorectal Cancer

Wells Messersmith, MD, FACP
Associate Professor
Director, GI Medical Oncology
Program co-Leader, Developmental Therapeutics
University of Colorado Anschutz Medical Campus
Denver, Colorado

H&O How is KRAS mutational status guiding treatment, and how has this concept evolved?

WM KRAS testing has been around for approximately 3 years, and it is a good example of how quickly there is uptake of new information into the field of oncology. Most oncologists were incorporating KRAS testing even before the US Food and Drug Administration (FDA) changed the labels on cetuximab (Erbitux, Eli Lilly/Bristol-Myers Squibb) and panitumumab (Vectibix, Amgen). These days, it is rare to see a colorectal cancer patient whose KRAS mutation status is not known, especially one who has advanced colorectal cancer. In the adjuvant setting, we usually do not collect information on KRAS mutational status because monoclonal antibodies targeting the epidermal growth factor receptor (EGFR) are not effective in this situation. However, in advanced disease, KRAS testing has become the standard of care, determining eligibility for cetuximab and panitumumab. It is important to remember that KRAS testing is a negative predictive test, meaning that if a patient is KRAS wild-type, it does not necessarily mean that he or she will benefit from EGFR-targeting agents, and if the patient is KRAS mutant—at least codon 12 KRAS-mutant—the patient will not derive benefit from and should not receive EGFR-targeting agents.

Ironically, when cetuximab was initially approved in 2004, it was required to send EGFR staining results to many of the insurance companies in order to have the testing covered, even though EGFR immunohistochemical (IHC) staining was not predictive. At that time, I had many patients who were unable to receive cetuximab because their tumors did not stain for EGFR. This is a good example of how predictive testing can go wrong in the process of drug development, where assumptions were made about the value of IHC testing.

The concept of KRAS mutational testing is an evolving one. One of the main issues with which we are currently dealing is whether we should be "lumpers" or "splitters"; in other words, should we lump KRAS mutations together regardless of the codon or the type of mutation, or should we be splitting out these various mutations? There have been reports about different predictive effects of codon 13 mutations and specific codon 12 mutations. Other mutations in the KRAS gene, such as codons 61 and 164, have been reported, and tumors with mutations in NRAS seem to behave similarly to KRAS-mutated tumors. Hence, there are many important ongoing debates in the field.

H&O At what point in the disease course should KRAS mutation testing be discussed?

WM In advanced, metastatic disease, it is the standard of care to order KRAS testing. In the postoperative setting, it is not standard; however, there are several situations in postoperative colorectal cancer care in which I do order

KRAS testing. If I believe that the patient is at extremely high risk of recurrence and I want to quickly ascertain the patient's KRAS mutation status, I would order the test. The other situation in which I would request KRAS testing is when it is difficult to obtain tissue. For example, there are some hospitals from which we have trouble getting samples, and there are patients from rural areas for whom it may take a long time to obtain the KRAS test in the future. In these situations, I order KRAS testing a bit earlier in the disease process. When my patients relocate, I make sure they have a copy of the results.

H&O The cobas KRAS Mutation Test has recently been approved as a companion diagnostic. What are the implications of this approval?

WM The implications of this approval on day-to-day management are not very high. It is reassuring that there is a test that has been reviewed and approved by the FDA, but, in terms of affecting our practice, we are not going to do anything different from what we were doing in the past in regard to ordering the test. Whether or not the cobas KRAS Mutation Test will start to take precedence over other mutational tests is unclear, and the question of whether or not insurers will require an FDA-approved test has yet to be answered. At the University of Colorado, we have a clinical laboratory improvement amendments–certified laboratory staffed by molecular pathologists, so we interact regularly with the physician-scientists performing the tests, and they communicate results in real-time. I suspect for most hospitals, it is a send-out test, and oncologists need to be familiar with what type of test is being done.

H&O Is there a need for positive predictive factors for EGFR-targeting monoclonal antibodies?

WM We definitely need positive predictive factors. Despite the use of KRAS mutational testing, only a subset of patients who are KRAS wild-type actually achieve benefit. However, the advantage of negative predictive factors is that they are very clean, meaning that a patient who has at least some chance of benefit is not denied access to the drug. Ideally, we would combine both negative and positive predictive factors to determine treatment. Positive predictive factors, such as amphiregulin and epiregulin, are seen across study populations, but it can be very difficult to tease out whether these should be used clinically (ie, will what is seen on a population basis apply to an individual patient). Oftentimes, the positive predictive tests do not meet that criterion. Incidentally, we also need positive and negative predictive factors for bevacizumab, and cytotoxic chemotherapy such as 5-FU, oxaliplatin, and irinotecan. There are many "positive" small studies with predictive factors, which fail miserably in large prospective trials.

H&O Do different mutations have different predictive values?

WM The issue of the codon 13 mutation is controversial. In my practice, if I have codon 13–mutated patients, I discuss with them the results of randomized trials and attempt to make decisions based on their preferences and their individual clinical situations. I think that at this point it is too early to make blanket statements about codon 13, and I am waiting for more confirmatory data from the ongoing studies before I make definitive decisions. Unfortunately, we are in a very difficult spot with codon 13 because the package inserts of panitumumab and cetuximab do not recommend treating these patients, particularly because these are expensive therapies. However, there are now data to indicate that maybe we should be treating these patients like KRAS wild-type patients, and this puts oncologists in a difficult situation.

We have retrospective data mainly from one European group, and we are waiting for other groups to confirm or refute the findings. It is important to remember that we need data from prospective clinical trials; we can get very interesting signals from retrospective cases and then test them in prospective clinical trials. However, before we change practice, we need to look at prospective trials, in which patients have been randomized, to dictate clinical practice.

H&O What role does BRAF play in KRAS testing?

WM Retrospective data from longitudinal databases of patients who received standard of care (ie, gathering of patient samples annotated clinically) show that BRAF patients have no chance of responding to EGFR-targeting monoclonal antibodies. However, the CRYSTAL (Cetuximab Combined with Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer) trial, from which updated results were published in the *Journal of Clinical Oncology* in 2011, showed that although BRAF has a very powerful negative prognostic value, the magnitude of benefit for BRAF patients was similar for BRAF mutated versus BRAF wild-type groups. These patients lived about half as long, but they had the same magnitude of benefit from cetuximab in the CRYSTAL trial. Very small numbers of BRAF-mutated patients limit interpretation of the data. The treatment decisions in cases when BRAF testing is negative are difficult. In my practice, I get BRAF testing on all of my patients who are KRAS wild-type. In general, KRAS mutations and BRAF mutations do not coexist, so

only wild-type KRAS patients would need to get tested. I think in the near future we will have trials that select BRAF-mutated patients, and, in fact, we are seeing some of these trials now. For example, there is growing interest in MEK inhibitors and MEK combined with BRAF inhibitors for this subset of patients. Some of my BRAF mutated patients have done extremely well on these types of trials. Because these trials are becoming more the rule rather than the exception, I think that it will be advantageous for patients to know their BRAF mutational status not only for the ability to participate in clinical trials, but also to be aware of their eligibility for treatment with new targeted agents that may become available.

H&O What are some of the testing methods utilized in patients with colorectal cancer?

AB. There are 5 different methods that can be used to test KRAS. One is direct sequencing, which has low sensitivity, as it requires 20–50% of the cells to be cancerous. There is also amplification refractory mutation systems (ARMS); these are mutation-specific polymerase chain reactions (PCR) that are often accompanied by suppression of normal signals. ARMS testing has a lot of sensitivity. Another method is high resolution melting analysis, in which sequences of the mutations will hybridize at different temperatures. This test is usually confirmed by direct sequencing, and it is thought to have a sensitivity of approximately 10%. Allele-specific probes is another KRAS testing method that is PCR based. Finally, there is restriction fragment length polymorphism, which is also often confirmed by direct sequencing. This testing method is the most sensitive of all the tests.

At our institution, we have conducted a study looking at the different assays; we tested all the same samples using various techniques. We found that what worked best for us was to use cores from blocks instead of putting specimens on a slide and then picking them off the slide. With core biopsies, one can see where the tumor is located on the block, insert a needle in that location, and isolate the DNA right from the paraffin without putting it on a slide. Our study did not contain a large sample set, but nonetheless, it is indicative of the kinds of analyses that can be done. It also shows that tissue processing matters.

The widespread use of KRAS testing has led to issues of quality control, particularly because these tests are determining what treatments patients will be receiving. Cetuximab and panitumumab are not miracle drugs: progression-free survival and response rates are not that great in wild-type patients, and there are even examples where KRAS wild-type patients did not benefit from EGFR antibodies in large studies (COIN [Combination Chemotherapy With or Without Cetuximab as First-Line Therapy in Treating Patients With Metastatic Colorectal Cancer] and NORDIC VII [FLOX in Combination With Cetuximab in First-line Treatment of Colorectal Cancer] trials). However, as more of our treatments become more like crizotinib (Xalkori, Pfizer) in lung cancer—with response rates of 70–90% in specific patient populations—the need to emphasize quality control of biomarkers becomes greater.

It is important for oncologists to know what KRAS test they are ordering and to be aware of some of the performance issues that may arise. If direct sequencing is being ordered and a mutation is picked up, one does not need to worry that the signal is suppressed, because a mutation was picked up. However, if the direct sequencing test showed a patient as wild-type, there might be some concern that there was not enough tumor on the slide. Oncologists should also be aware of what codons the laboratory is testing. For example, the cobas Mutation Test looks at not only codons 12 and 13, but also at codon 61. In the future, other codons may be of clinical importance. This leads to the question of what is the appropriate protocol in patients who test positive for extremely rare mutations. For instance, the incidence of BRAF is probably less than 10%. Even in a large trial like CRYSTAL, which had over 1,000 patients, there were only 33 patients that actually had a BRAF mutation. Because of the rarity, it is much more difficult to draw definitive conclusions in such rare subsets. One of the challenges in the field is in standardizing KRAS testing and determining which results are clinically actionable.

Suggested Readings

André T, Boni C, Navarro M, et al. Improved overall survival with oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment in stage II or III colon cancer in the MOSAIC trial. *J Clin Oncol*. 2009;27:3109-3116.

Van Cutsem E, Köhne CH, Láng I, et al. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. *J Clin Oncol*. 2011;29:2011-2019.

Franklin WZ, Haney J, Sugita M, Bemis L, Jimeno A, Messersmith WA. KRAS mutation: comparison of testing methods and tissue sampling techniques in colon cancer. *J Mol Diagn*. 2010;12:43-50.