

The Role of Comprehensive Molecular Profiling in Colorectal Cancer



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H&O What are the relevant mutations or other alterations that molecular profiling can pick up in colorectal cancer, and how common are they?

HH The most common mutations in colorectal cancer (CRC), specifically those in *TP53* and *PI3K*, have no clinical implications. Among the clinically relevant mutations, the most common are those in *NRAS* or *KRAS*, which occur in approximately 55% of patients with CRC. Patients with a *RAS* mutation do not benefit from treatment with an anti-epidermal growth factor receptor (EGFR) agent, either cetuximab (Erbix, Lilly) or panitumumab (Vectibix, Amgen), which means that the most common actionable mutation is a negative predictive factor.

Approximately 8% of people with CRC have a *BRAF* V600E mutation, which is a negative prognostic factor. Median survival is approximately 18 months for patients who have CRC with a *BRAF* mutation, compared with 3 years for those who have CRC without a *BRAF* mutation. Most of these *BRAF*-mutated tumors are right-sided, so the patients already have a worse prognosis than those with left-sided tumors. We have found that patients who have CRC with a *BRAF* V600E mutation tend to have a somewhat worse prognosis than those with other right-sided tumors, and overall survival is relatively short. As a result, most of them require aggressive combination chemotherapy as first-line treatment.

Human epidermal growth factor receptor 2 (*HER2*) amplification is present in approximately 4% of patients with CRC. Because *HER2* amplification occurs almost

exclusively in those without *RAS* mutations, it occurs in 8% of patients with *RAS* wild-type CRC. Patients who have *HER2* amplification have a worse prognosis than those without this amplification, and they tend to do worse than other patients with *RAS* wild-type CRC.

Another important factor in CRC is mismatch repair (MMR) enzyme deficiency, which affects approximately 4% to 5% of patients with metastatic CRC and is a useful positive marker. Defects in MMR are known to occur in families with CRC that have Lynch syndrome, and to a lesser degree in people with endometrial cancer. The rate of MMR deficiency is even higher among patients with stage 3 CRC, affecting approximately 8% of them, and among those with stage 2 disease, affecting approximately 15%.

MMR deficiency is important to identify because such patients are sensitive to immunotherapy with anti-programmed death 1 (anti-PD-1) agents. Patients who have metastatic MMR-deficient CRC also respond fairly well to chemotherapy because they lack DNA repair, so first-line treatment may involve chemotherapy, an anti-PD-1 agent, an anti-cytotoxic T-lymphocyte-associated antigen 4 agent, or a combination. How these options should be deployed in first-line therapy is still under investigation. These agents also can be used in second-line treatment and are approved by the US Food and Drug Administration (FDA) in that setting. I recommend that patients with stage 3 or 4 CRC and MMR deficiency be enrolled in a clinical trial (National Clinical Trials Network trials are available for each stage). The prognosis is good for patients with stage 2 MMR-deficient CRC,

which has a cure rate of greater than 90% with surgery. Adjuvant chemotherapy is not required for these patients because the tumors are very localized even though they are large.

The other relevant mutations are fusions in *NTRK1-3*, which affect approximately 2% to 3% of patients with CRC and can be targeted with anti-neurotrophic

Next-generation sequencing costs approximately \$3000 to \$5000 and is not always covered by insurance.

tyrosine receptor kinase (anti-NTRK) agents. Fusions in *ALK*, *ROS1*, and *RET* are also relevant because they can be targeted, but they are rare in CRC.

H&O What techniques or tests are used for molecular profiling?

HH Immunohistochemistry (IHC), polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH), and next-generation sequencing (NGS) are all used. The most important step for patients with newly diagnosed CRC is to undergo IHC testing for the presence of MMR enzymes in the biopsy specimens, to identify high microsatellite instability (MSI-high) CRC. At the same time this is being done, the pathologist can look for *HER2* amplification by IHC. If a patient has left-sided metastatic disease, we want to determine quickly whether the patient has a *RAS* mutation so we know whether to use an anti-EGFR agent or bevacizumab. In that case, PCR testing is a better choice than NGS because the results are available in 48 hours. The only role for FISH is in patients with an equivocal result, such as a 2+, on IHC staining for *HER2*.

NGS, of course, is the most comprehensive test because it detects all the relevant fusions and mutations. The results take longer, are more expensive, and are variably reimbursed, however, so NGS may not be practical before first-line therapy is started. As long as initial testing is done with PCR or IHC, NGS testing can be deferred until you see that disease is progressing on first-line treatment.

H&O How does cancer stage affect testing?

HH Patients who have early-stage tumors do not need NGS or testing for *RAS* mutations. Patients with stage 2

or 3 CRC should be evaluated for MMR deficiency. As for patients with metastatic CRC, NGS should be timed to inform second-line therapy decisions.

H&O Do any specific situations call for a different approach?

HH Some situations do call for a different approach. For example, if IHC reveals that someone has MMR deficiency, you may wish to confirm that finding with a PCR test for MSI-high CRC. NGS will not add much of value in this case because such a patient will do best with a standard chemotherapy regimen as first-line treatment and an anti-PD-1 agent as second-line treatment, according to an FDA-approved sequence. Alternatively, you could enroll this patient in a clinical trial. Again, the key tests for initiating first-line therapy in metastatic disease are MMR enzyme staining and, for left-sided tumors, *KRAS/NRAS* testing. Additional information may be obtained by NGS testing, which gives information on actionable mutations, translocations and fusions, tumor mutation burden, and MSI status. Although these are basically tissue-based tests, many of the results can be obtained by a circulating tumor DNA (ctDNA) test in the absence of tissue.

H&O How does cost factor into the decision of which type of profiling to use?

HH That depends on the physician's sensitivity to the cost of health care; not everyone has the same point of view or even the same awareness of how much these tests cost. And, of course, insurance reimbursement and out-of-pocket costs are completely opaque to the physicians. NGS costs approximately \$3000 to \$5000 and is not always covered by insurance. The other tests are significantly less expensive, with the cost of IHC testing ranging from approximately \$20 to \$50 and that of PCR testing approximately \$100. More important than cost is getting the right information at the right time, however, so it makes sense to hold off before ordering NGS testing for most patients.

H&O When should a positive test result in tumor tissue lead to referral for genetic counseling?

HH The advice regarding this is beginning to change. For example, it was recently recommended to send all patients with pancreatic cancer to genetic counseling owing to the incidence of *BRCA* mutations and the option of treating with a poly(ADP-ribose) polymerase (PARP) inhibitor. Patients who have CRC with an MMR deficiency and without a *BRAF* mutation should consult with a genetic

counselor because most of these patients have Lynch syndrome, and the family members of those who have Lynch syndrome need to be tested for this as well. Direct offspring have a 50/50 chance of inheriting Lynch syndrome from one parent, and we know that enhanced early surveillance can benefit them.

H&O What are some of the other important studies that are ongoing regarding specific mutations?

HH The most important trial in patients with MMR-deficient metastatic CRC is COMMIT (Combination Chemotherapy, Bevacizumab, and/or Atezolizumab in Treating Patients With Deficient DNA Mismatch Repair Metastatic Colorectal Cancer), from the National Cancer Institute. This open-label trial is comparing bevacizumab/chemotherapy vs atezolizumab (Tecentriq, Genentech) vs bevacizumab/chemotherapy/atezolizumab as first-line therapy.

Another important study is ATOMIC, also known as Alliance A021502 (Combination Chemotherapy With or Without Atezolizumab in Treating Patients With Stage III Colon Cancer and Deficient DNA Mismatch Repair). This is a trial for patients with stage 3 MSI-high CRC that is studying the use of leucovorin/5-fluorouracil/oxaliplatin (FOLFOX) adjuvant therapy with or without atezolizumab. For this reason, it is important to test patients with stage 3 CRC for MMR deficiency.

Trials are currently looking at combinations of anti-HER2 agents or tyrosine kinase inhibitors in patients with *HER2* amplification. In particular, an ongoing phase 2 randomized trial, S1613 (Trastuzumab and Pertuzumab or Cetuximab and Irinotecan Hydrochloride in Treating Patients With Locally Advanced or Metastatic *HER2*/Neu Amplified Colorectal Cancer That Cannot Be Removed by Surgery), is comparing trastuzumab plus pertuzumab (Perjeta, Genentech) with a standard regimen of cetuximab plus irinotecan for patients with *HER2*-overexpressing CRC. Other *HER2* inhibitor trials are also under way. No randomized trials are currently looking

at new agents in patients with *RAS* wild-type tumors, but these are much needed. One of the mechanisms of resistance to anti-EGFR antibodies is the development of a *RAS*-mutated clone. These mutations confer resistance to anti-EGFR antibodies but tend to dissipate after a few months. Therefore, re-treatment strategies for patients with *RAS* wild-type tumors are being tested with the help of ctDNA, which can show the presence or absence of the *RAS*-mutated clones.

Finally, a few registry trials are looking at the use of anti-NTRK agents in patients with *NTRK* fusions.

H&O Is there anything you would like to add?

HH A related topic is the role of ctDNA as a prognostic marker in CRC. The National Cancer Institute is planning a trial looking at stage 2 CRC with ctDNA, and a future trial will look at stage 3 CRC. These trials will track postoperative ctDNA to determine its accuracy in predicting recurrence and whether chemotherapy can “clear” the ctDNA, suggesting enhanced benefit for early treatment of those with molecular residual disease and, it is hoped, eliminating the need to treat those who are cured by surgery in the early stages of CRC.

Disclosure

Dr Hochster is a consultant to Bayer, Roche/Genentech, Merck, AstraZeneca, and Elion Labs.

Suggested Readings

ClinicalTrials.gov. Combination chemotherapy with or without atezolizumab in treating patients with stage III colon cancer and deficient DNA mismatch repair. <https://clinicaltrials.gov/ct2/show/NCT02912559>. Identifier: NCT02912559. Accessed November 4, 2019.

ClinicalTrials.gov. Combination chemotherapy, bevacizumab, and/or atezolizumab in treating patients with deficient DNA mismatch repair metastatic colorectal cancer. <https://clinicaltrials.gov/ct2/show/NCT02997228>. Identifier: NCT02997228. Accessed November 4, 2019.

ClinicalTrials.gov. Trastuzumab and pertuzumab or cetuximab and irinotecan hydrochloride in treating patients with locally advanced or metastatic *HER2*/neu amplified colorectal cancer that cannot be removed by surgery. <https://clinicaltrials.gov/ct2/show/NCT03365882>. Identifier: NCT03365882. Accessed November 4, 2019.