CRC IN FOCUS

Current Developments in the Management of Colorectal Cancer

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The Role of Microsatellite Instability Testing in Management of Colorectal Cancer

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H&O What is microsatellite instability (MSI)?

FS MSI refers to an accumulation of errors within microsatellite regions of DNA that lead to hypermutation and are caused by a deficient DNA mismatch repair system. The mismatch repair system serves an editing function—similar to a spellcheck mechanism—that identifies errors and removes them from the DNA. When this repair system is faulty, microsatellites in the DNA develop short nucleotide repeats that lead to frame-shift mutations.

H&O What causes the DNA mismatch repair system to malfunction?

FS Defective mismatch repair is most commonly caused by a methylation abnormality that inactivates the DNA mismatch repair gene *MLH1*. This is primarily a sporadic, or nonheritable, event. The other mechanism of defective mismatch repair is a germline mutation in 1 of 4 mismatch repair genes *(MLH1, MSH2, MSH6,* or *PMS2)* that produces Lynch syndrome, an autosomal dominant condition that is the most common form of hereditary colon cancer. Approximately two-thirds of MSI colorectal cancers are sporadic; the remaining one-third are caused by Lynch syndrome. Individuals with the sporadic form tend to be older and are more likely to be women, whereas patients with Lynch syndrome are significantly younger.

H&O How often does MSI occur?

FS MSI occurs in approximately 15% of all colorectal cancers. However, the frequency varies by tumor stage, with approximate frequencies of 20% in stage II disease, 11% in stage III disease, and 3.5% in stage IV (metastatic) disease.

H&O What are the characteristics of colorectal cancer tumors that have MSI?

FS Characteristic features of MSI colon cancers include proximal tumor location, poor differentiation, and abundant tumor-infiltrating lymphocytes, which are found in both sporadic and germline types. These tumors are commonly located in the cecum and ascending colon. Under the microscope, the tumor cells are usually poorly differentiated, which is not prognostic in these tumors but is associated with a worse clinical outcome in non-MSI colon cancers. The abundance of tumor-infiltrating lymphocytes in MSI tumors indicates that they are highly immunogenic.

MSI colorectal cancers are associated with a reduced rate of tumor recurrence, and these patients have more favorable survival compared with patients with non-MSI colorectal cancers, especially if the tumors are at an early stage. The survival advantage of MSI appears to attenuate with advancing tumor stage. Although the mechanism by which patients with MSI tumors have better outcomes is incompletely understood, it may be related to an enhanced antitumor immune response that is triggered by mutant proteins from hypermutation.

H&O How does MSI status affect the management of patients with colorectal cancer?

FS Detection of MSI has important implications for patient management. It is critical to diagnose Lynch

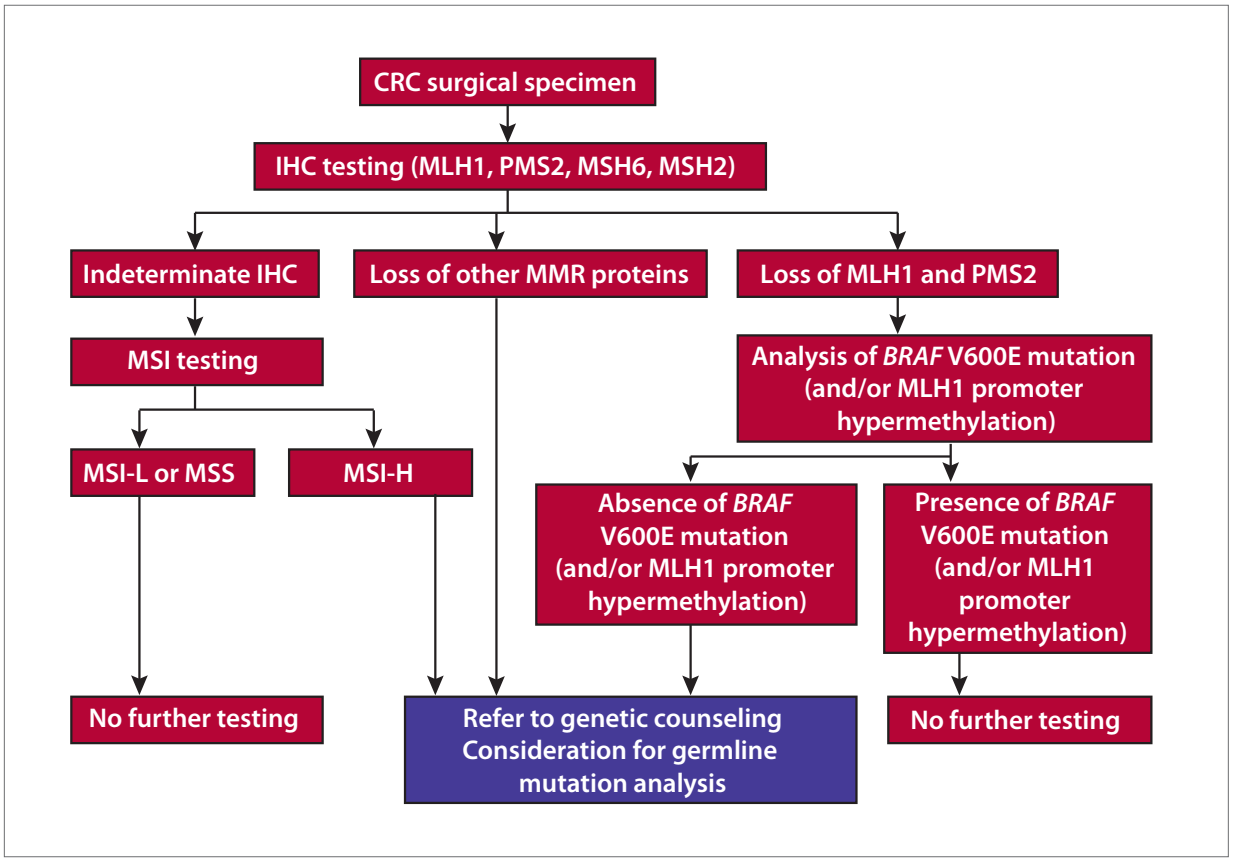

Figure. Algorithm for systematic evaluation for Lynch syndrome in patients with colorectal cancer. Republished with permission from Kawakami H, Zaanan A, Sinicrope FA. Microsatellite instability testing and its role in the management of colorectal cancer. *Curr Treat Options Oncol*. 2015;16(7):30. doi:10.1007/s11864-015-0348-2.

CRC, colorectal cancer; IHC, immunohistochemistry; MMR, mismatch repair; MSI-H, microsatellite instability-high; MSI-L, microsatellite instability-low.

syndrome, given that it is a hereditary syndrome with major implications for the patient and his or her family members. Patients with this syndrome are at the highest risk for colorectal cancers, including both synchronous and metachronous tumors. The second most common malignancy in Lynch syndrome is endometrial cancer in women. A diagnosis of colorectal cancer in Lynch syndrome influences the extent of surgical resection. Because MSI tumors tend to have a more indolent clinical course, a more aggressive surgical approach, if technically feasible, can be considered in the setting of tumor recurrence.

Regarding chemotherapy, data indicate that MSI tumors do not appear to benefit from 5-fluorouracil (5-FU), which remains the most commonly used chemotherapy drug for the treatment of colorectal cancer. The chemotherapy regimen of folinic acid, 5-FU, and oxaliplatin (FOLFOX), which is the standard adjuvant treatment regimen after surgical resection of stage III (lymph node–positive) tumors or for metastatic disease, does appear to be effective in patients with MSI cancers, who are most likely responding to the oxaliplatin.

A recent finding that has made detection of MSI so important for treatment is that metastatic colorectal cancers with MSI respond favorably to immune checkpoint inhibitors. These tumors tend to have high expression of checkpoint proteins, including programmed death 1 (PD-1) and programmed death ligand 1 (PD-L1), which interfere with the body's antitumor T-cell response. By disabling these proteins, checkpoint inhibitors enable T cells to attack and kill tumor cells—allowing the immune system to do its job more effectively. Le and colleagues reported a phase 2 study of the anti–PD-1 antibody pembrolizumab (Keytruda, Merck) in patients with MSI colorectal and extracolonic cancers associated with Lynch syndrome in the *New England Journal of Medicine* in 2015. This study, which enrolled a modest number of patients, showed that patients with colorectal cancer were substantially more likely to respond to pembrolizumab if their tumors had MSI than if they did not, and it reported

favorable response rates and prolonged progression-free survival. Multiple clinical trials are looking further at this issue (NCT01876511, NCT02563002, NCT02460198, NCT02646748).

H&O Are insurers covering the cost of checkpoint inhibitors for patients with colorectal cancer?

FS The US Food and Drug Administration (FDA) has granted "breakthrough therapy" designation to pembrolizumab for the treatment of patients with metastatic MSI colorectal cancer. Although the FDA has not yet approved the use of this drug in this patient population, it appears that some insurers are paying for its use in patients with documented metastatic MSI colorectal cancer. At this time, the best approach is for oncologists to enroll patients in a clinical trial if possible. Further study is needed to determine the optimal timing of the use of checkpoint inhibitors in the therapy of metastatic colorectal cancer.

H&O What about the use of checkpoint inhibitors in patients with nonmetastatic colorectal cancer?

FS In theory, immune checkpoint inhibition may benefit patients with earlier-stage disease, and this is an important research question at this time. I will be the principal investigator for a randomized phase 3 clinical trial in patients with MSI stage III colon cancers that will evaluate the anti–PD-L1 antibody atezolizumab (Tecentriq, Genentech) in combination with standard adjuvant FOLFOX vs FOLFOX alone. This trial, which will be conducted by the Alliance for Clinical Trials in Oncology, will evaluate the benefit of the addition of a checkpoint inhibitor to chemotherapy in earlier-stage—that is, nonmetastatic—colon cancer.

H&O What other studies have looked at MSI status and treatment?

FS In a retrospective multicenter study by Tougeron and colleagues of patients with stage II and III MSI colon cancers, adjuvant oxaliplatin-based chemotherapy was shown to significantly improve disease-free survival compared with adjuvant 5-FU alone in a multivariable analysis. In the subgroup analysis, the survival benefit of oxaliplatin-based chemotherapy was statistically significant only in stage III disease. Among patients with MSI stage II and III colon cancers treated in an adjuvant chemotherapy trial (PETACC-3, Fluorouracil and Leucovorin With or Without Irinotecan in Treating Patients Following Surgery for Stage III Colorectal Cancer) that was published by Klingbiel and colleagues, there was no benefit from the addition of irinotecan to 5-FU.

H&O Could you talk about the difference between MSI-high (MSI-H) and MSI-low (MSI-L) tumors?

FS MSI is determined using a polymerase chain reaction (PCR)-based assay that requires a molecular laboratory. To say that a tumor has MSI refers to high-frequency MSI (MSI-H). The typical MSI testing panel includes 5 mononucleotide repeat markers and 2 control markers. If instability occurs at 2 or more of the 5 markers, the patient is considered MSI-H. If instability occurs at 1 out of the 5 markers, the patient is considered MSI-L. If no instability occurs, the patient is considered microsatellite stable (MSS). MSI-L is very uncommon, and evidence suggests that these tumors behave similarly to MSS tumors. As such, they are generally grouped together.

MSI-H is a consequence of deficient DNA mismatch repair. One can detect this by performing immunohistochemistry for the DNA mismatch repair proteins MLH1, MSH2, MSH6, and PMS2. If loss of 1 or more of these proteins occurs, the tumor is considered to have deficient mismatch repair. MLH1 is commonly lost in association with PMS2, but this is not the case for MSH2 or MSH6. This approach to testing is common because immunohistochemistry is performed routinely by most hospital laboratories, and a molecular laboratory is not required. The other advantage of this type of testing is that it identifies loss of the protein product of a specific affected gene. A downside is that it involves determining whether staining is present or absent, which involves a certain degree of subjectivity. At this time, MSI testing remains the gold standard.

H&O What is the interaction between *BRAF* and MSI?

FS *BRAF* is an oncogene whose activation promotes tumor growth via the RAS/RAF/MAPK signaling pathway. Mutations in *BRAF* occur in approximately 45% of patients with sporadic colorectal cancer with MSI and essentially no patients with Lynch syndrome. In other words, the presence of a *BRAF* mutation signals that the tumor is of the sporadic type, but the absence of a *BRAF* mutation does not provide information about whether the tumor is sporadic or germline.

H&O How far away are we from universal testing for MSI in patients with colorectal cancer?

FS Guidelines from the National Comprehensive Cancer Network (NCCN) and from an upcoming multi-society consensus guideline on molecular testing in colorectal cancer recommend that MSI testing or analysis for deficient mismatch repair protein expression be done in all patients with newly diagnosed colorectal cancer. The goals are to detect patients with Lynch syndrome, and to utilize such

information for determining prognosis. Many centers around the country are preparing to do universal testing at the time that colorectal cancer is diagnosed, so testing will become increasingly prevalent. Physicians often do not obtain a detailed family history in patients with colorectal cancer that can facilitate the diagnosis of Lynch syndrome. Furthermore, a majority of sporadic tumors with MSI are not being detected. With universal testing, we can ensure that we identify these patients and provide the appropriate management and treatment going forward.

Disclosure

Dr Sinicrope previously served as an advisor to Ventana Medical Systems.

Suggested Readings

Beamer LC, Grant ML, Espenschied CR, et al. Reflex immunohistochemistry and microsatellite instability testing of colorectal tumors for Lynch syndrome among US cancer programs and follow-up of abnormal results. *J Clin Oncol*. 2012;30(10):1058-1063.

Klingbiel D, Saridaki Z, Roth AD, Bosman FT, Delorenzi M, Tejpar S. Prognosis of stage II and III colon cancer treated with adjuvant 5-fluorouracil or FOLFIRI in relation to microsatellite status: results of the PETACC-3 trial. *Ann Oncol*. 2015;26(1):126-132.

Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med*. 2015;372(26):2509-2520.

National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®). Colon Cancer. v.2.2016. https://www.nccn.org/professionals/physician_gls/f_guidelines.asp. Updated November 23, 2015. Accessed June 7, 2016.

Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol*. 2005;23(3):609-618.

Renfro LA, Shah MA, Allegra CJ, et al. Time-dependent patterns of recurrence and death in resected colon cancer (CC): pooled analysis of 12,223 patients from modern trials in the ACCENT database containing oxaliplatin [ASCO abstract 3593]. *J Clin Oncol*. 2015;33(15)(suppl).

Sargent DJ, Marsoni S, Monges G, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. *J Clin Oncol*. 2010;28(20):3219-3226.

Tougeron D, Mouillet G, Trouilloud I, et al. Efficacy of adjuvant chemotherapy in colon cancer with microsatellite instability: a large multicenter AGEO Study. *J Natl Cancer Inst*. 2016;108(7):djv438.