ADVANCES IN ONCOLOGY

Current Developments in the Management of Solid Tumor Malignancies

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Colorectal Cancer in Focus

The *BRAF* Mutation in *MLH1*-Deficient Colorectal Cancer

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H&O How common is the mismatch repair protein deficiency (MMR-d) in colorectal cancer (CRC)?

SM Approximately 15% of CRC cases are attributable to MMR-d.[1] The deficient proteins are MLH1, MSH2, MSH6, and PMS2. The most common cause of MMR-d is hypermethylation of *MLH1*.[2] Hence, the etiology of *MLH1*-deficient CRC is more often sporadic than genetic.

H&O Does MMR-d affect prognosis or treatment?

SM Patients with MMR-d CRC exhibit better prognosis than those with MMR-proficient (MMR-p) tumors.[3] In patients with MMR-d tumors, single-agent fluoropyrimidine (5-FU)-based therapy is not beneficial and may even be detrimental.[4] In stage II colon cancer, testing for MMR-d is one of the risk assessment modalities used to make decisions regarding adjuvant use of single-agent, 5-FU–based treatment.[5]

H&O What is the current status of *BRAF* mutation in CRC?

SM *BRAF* is a member of the Raf kinase family of serine/threonine-specific protein kinases. These proteins play a role in regulating the signaling pathway of mitogen-activated protein (MAP) kinases/extracellular signal-regulated kinases (ERKs), which affects cell division, differentiation, and secretion. The *BRAF* V600E mutation is found in 5–10% of patients with metastatic colon cancer and is an adverse prognostic factor, with a median survival of 9–14 months.[6,7] In early-stage CRC, the situation is less clear. Hutchins and colleagues evaluated *BRAF* status in 1,584 stage II CRC patients from the QUASAR (Quick and Simple and Reliable) trial and found a *BRAF* V600E mutation rate of 8%.[8] Risk of recurrence did not differ between *BRAF*-mutated and wild-type tumors (relative risk [RR], 0.84). However, 53% of *BRAF*-mutated tumors were MMR-d, and when the confounding effect of MMR-d was eliminated, the trend was reversed (RR, 1.32). A combined translational analysis of the PETACC (Pan-European Trials in Alimentary Tract Cancer) 3, EORTC (European Organisation for Research and Treatment) 40993, and SAKK (Swiss Group for Clinical Cancer Research) 60-00 trials showed that the *BRAF* mutation was present in 8% of patients and was negatively prognostic for overall survival in only the microsatellite-low (MSI-L) and microsatellite-stable (MSI-S) tumors. MSI-L and MSI-S are synonymous with MMR-p tumors.[9]

H&O What was the design of your study of the *BRAF* V600E mutation in colorectal cancer patients with MMR-d due to loss of *MLH1*?

SM We sought to investigate the contribution of the *BRAF* V600E mutation in *MLH1*-deficient CRC to identify any specific genotype/phenotype relationship.[10]

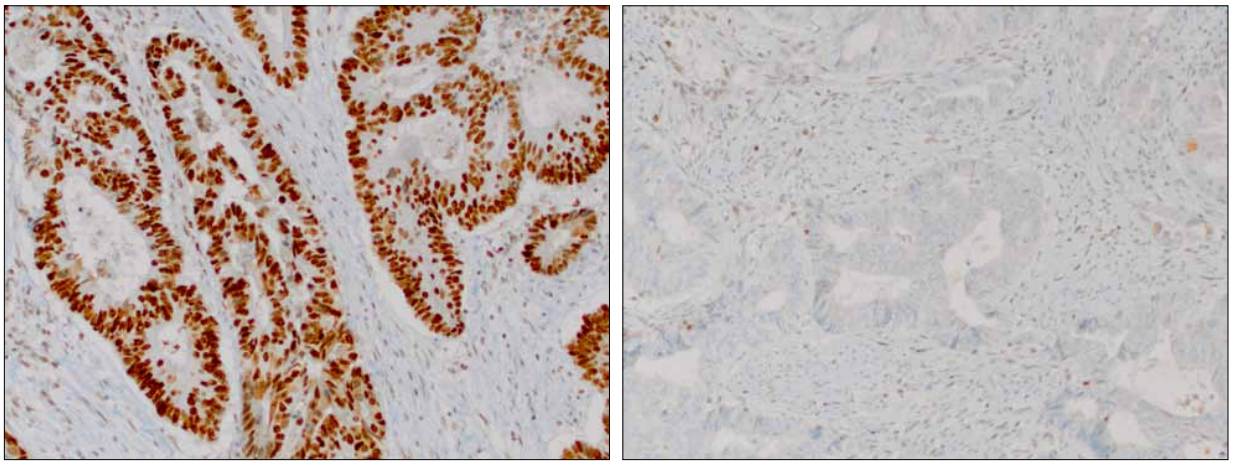

Figure 1. Immunohistochemistry staining shows present (left) and absent (right) MLH1.

Table 1. Baseline Patient Characteristics

Number of Patients	128
Age (years)	
Median	71
Range	26–91
Sex, n (%)	
Male	66 (52)
Female	62 (48)
Race, n (%)	
White	109 (85)
African American	19 (15)
Stage, n (%)	
0	5 (4)
1	28 (22)
2	40 (31)
3	38 (30)
4	17 (13)
Site, n (%)	
Right	62 (48)
Transverse	6 (5)
Left	43 (33)
Rectum	17 (14)
MMR Status, n (%)	
Deficient	18 (14)
Proficient	110 (86)

MMR=mismatch repair.

Table 2. Study Results

MMR Deficient, n	18	
MLH1 absent	16*	
MSH2 absent	1	
MSH2 and MSH6 absent	1	
MLH1 Absent, n (%)	16 (100)	
BRAF V600E mutant	13 (81)	
BRAF wild-type	3 (19)	
Characteristics of Patients With *MLH1*-Deficient CRC	*BRAF* V600E Mutant	*BRAF* Wild-Type
Median age (years)	80	67
Sex		
Female, n	12	1
Male, n	1	2
Location		
Right, n	12	2
Left, n	1	1

*MLH1 and PMS2 absent=15, MLH1 absent=1.

The study population included 128 patients newly diagnosed with CRC at Akron City Hospital from March 2010 to February 2011. The patients underwent mismatch repair (MMR) protein testing by immunohistochemistry (IHC). Their baseline characteristics are listed in Table 1. Deficiency of *MLH1* prompted *BRAF* V600E testing. If the *BRAF* V600E mutation was detected, further testing was stopped since it was unlikely that the cancer was due to Lynch syndrome. If *BRAF* was wild in *MLH1*-deficient patients, sequencing of *MLH1* was performed. If *PMS2* deficiency coexisted with *MLH1*, then sequencing of *PMS2* was performed as well if *MLH1* sequencing was normal.

IHC for the MMR proteins (MLH1, PMS2, MSH2, and MSH6) was performed on 4 µm–sections of formalin-fixed tissue. Staining protocols from the manufacturer (Cell Marque) were followed. Adequately stained positive and negative controls were included in each patient sample. The slides were interpreted by a single pathologist. Nuclear staining in greater than 50% of tumor nuclei was considered positive (ie, an indication that the protein was not deficient). Normal and abnormal *MLH1* stains are shown in Figure 1.

BRAF V600E mutation testing was performed by Clarient. Briefly, the procedure included the selection

and microdissection of the tumor with lysis and extraction of DNA. Single-primer, real-time polymerase chain reaction was used to amplify the region containing the *BRAF* mutation. Two fluorogenic probes detected *BRAF* wild-type and V600E-mutant sequences.

H&O What were the study findings?

SM Fourteen percent of the patients (n=18) were MMR-d. Among these 18 patients, 16 were *MLH1* deficient (15 were *MLH1*/*PMS2* deficient, and 1 was solely *MLH1* deficient). The *BRAF* V600E mutation was found in 81% of patients with *MLH1* deficiency (13 of 16 patients). The remaining 3 patients were sequenced for *MLH1* and *PMS2*, and no mutations in either gene were found.

The median age of patients with *MLH1*-deficient/*BRAF* V600E–mutated CRC was 80 years, which was significantly older than the overall population (70 years) as well as the population that was *MLH1*-deficient/*BRAF* V600E wild-type (67 years). Of note, among the 13 patients with *MLH1*-deficient/*BRAF* V600E–mutated CRC, 12 were women with right-sided tumors who had deficient *MLH1* and *PMS2*, and 1 was a man with a left-sided tumor who had only *MLH1* deficiency. Three had stage I disease, 5 had stage II, 4 had stage III, and 1 had stage IV. The patient with stage IV was the man with left-sided cancer. These results are summarized in Table 2. Among the 13 patients with *MLH1*-deficient/*BRAF* V600E–mutated tumors, 3 had mucinous and/or signet cell features.

H&O Does your study have implications for the management of CRC patients?

SM We identified a specific genotype/phenotype in CRC: *MLH1*/*PMS2*-deficient *BRAF*-mutated right-sided CRC in elderly women. The numbers in this study are too small to provide meaningful data on prognosis, and follow-up is not mature. However, it does seem that even within MMR-d *BRAF* tumors, there might be different subgroups based on the type of MMR-deficient protein, which could have prognostic implications. This suggestion must be confirmed in larger trials. MMR testing is relatively inexpensive, and *BRAF* mutation analysis has been performed in various large, adjuvant study samples.

Based on the reported data, it appears that early-stage CRC patients with *BRAF* mutations have 2 different prognostic subsets: MMR-p and MMR-d. The MMR-p subsets do worse when compared to their MMR-d counterparts as well as their *BRAF* wild-type counterparts. In metastatic CRC, MMR-p *BRAF*-mutated tumors might account for a higher percentage than MMR-d, leading to worse outcomes. Thus, it would be important to know the MMR status from the CRYSTAL (Cetuximab Combined With Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer) and AGITG MAX (Australasian Gastro-Intestinal Trials Group Mitomycin C, Avastin and Xeloda) trials.[4,5]

It seems like we have reached a ceiling in the one-size-fits-all approach to the adjuvant treatment of colon cancer, and it is necessary to identify poor prognostic factors that require further efforts to improve outcomes. Patients with MMR-p/*BRAF* V600E are one such subset, and might benefit from intensification of chemotherapy efforts and use of targeted agents.

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