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DNA Testing and Molecular Screening for Colon Cancer

John M. Carethers

Division of Gastroenterology, Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan

Abstract

Colon cancer develops and progresses as a consequence of abnormal cellular molecular changes, many of which result in mutant DNA. Modern molecular techniques allow examination of individual patient genetic data that ascribe risk, predict outcome, and/or modify an approach to therapy. DNA testing and molecular screening are in use today and are becoming a critical and necessary part of routine patient care. Assessing at-risk patients for hereditary colon cancer is predicted to move from individual gene testing that is commonly performed today to whole exome or whole genome sequencing, providing additional vast information of the patient's genome that might not be related to the colon cancer syndrome. Detecting mutant DNA from shed tumor cells in fecal material for colon cancer screening will increase in diagnostic accuracy over time, with improvements in the panel of mutant DNA being examined and through clinical testing. DNA mutations and other molecular changes detected directly from within the colon cancer help to inform and guide the physician for the best approach for optimal patient care and outcome. The use of epidermal growth factor receptor–targeted therapy in advanced colon cancer patients requires knowledge of the mutation status for *KRAS* and *BRAF* genes, and knowing the mutational status of *PIK3CA* may predict how patients respond to aspirin to prevent colon cancer recurrence. Biologically driven decision-making, or precision medicine, is becoming increasingly adopted for optimal care and outcome for colon cancer patients. Gastroenterologists will need to be increasingly aware.

Keywords

Genetics; Heredity; Colon Cancer; Fecal DNA Test; Polyposis

The use of molecular screening, that is, the assessment of DNA (for direct mutation or epigenetic alterations without a change in sequence), RNA, and/or protein from normal tissue (blood or buccal swab), feces, or tumor, is becoming more commonplace to help screen, diagnose, prognosticate, and perform personalized and precise care and treatment plans for patients at risk or with colorectal cancer (CRC). Knowledge of these tests by the gastroenterologist will be necessary. Molecular screening, in particular DNA testing, can

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Reprint requests: Address requests for reprints to: John M. Carethers, MD, Department of Internal Medicine, University of Michigan, 3101 Taubman Center, 1500 East Medical Center Drive, Ann Arbor, Michigan 48109. jcarethe@umich.edu; fax: (734) 232-3838.

Conflicts of interest

The author discloses no conflicts.

assess the genetic background of a patient (passed through the germline from either parent) or characterize the genetic makeup of a tumor and, on the basis of knowledge gained from published human tumor literature for specific DNA changes, inform the physician regarding patient outcome and recurrence, as well as inform an optimal approach to surveillance and/or treatment that will afford the patient the best outcome.

Germline DNA Testing for Hereditary Colon Cancer

Consideration for germline genetic analysis starts with the clinician being observant for predictors of high risk. Family or personal history of cancer, younger age for cancer, and possessing clinical features of a syndrome are major clues.^{1,2} Genetic testing for germline mutations is a team approach and is performed in at-risk individuals. Patient health records are obtained to verify cancers with suspicion of a syndrome, and only after genetic counseling does the patient submit to genetic testing. Genetic testing must be properly interpreted, and the patient must be advised on appropriate therapy and surveillance. Once a germline mutation is found, other family members can elect to undergo genetic counseling and testing through a targeted approach because the mutation in the family is now known.¹ However, suspicion of a syndrome, even when genetic testing is not conclusive, should drive therapy and surveillance for these patients.

Technological Primer

Molecular screening involving DNA can range from examining a single suspected gene to fully examining the entire genome. At present for germline testing of mutations, genomic DNA is sent for individual gene evaluation.^{1,2} For instance, high-risk family members or patients with young-onset CRC may be tested for individual genes that are known to presumably fit the phenotype presented, such as familial adenomatous polyposis, *MYH*-associated polyposis, Lynch syndrome, or *PTEN* hamartoma syndrome (including Cowden disease) as examples. After genetic counseling, the patient's or family member's genomic DNA (via blood or buccal swab) is amplified in the test laboratory to assess the sequence of the *APC*, *MYH*, DNA mismatch repair (MMR), or *PTEN* genes, respectively. However, whole exome sequencing, in which the entire human coding DNA is selected, or whole genome sequencing, in which the entire genome (coding and non-coding regions) is sequenced via DNA chip arrays, is progressively being used and will eventually become the norm.³ This is due to falling costs for these technologies, the ease of automation for the process, and the ability for broad coverage of the entire genome. Whole genome sequencing can also provide information on chromosome or gene copy number (amplifications or deletions of genomic DNA) and translocations (where portions of DNA have moved from their normal chromosomal location to another chromosomal location).^{3,4} Because the entire genome or exome is sequenced, there is the potential for incidental findings (eg, mutation in another gene that was not intentionally tested for), and at present, it is controversial and is an open discussion on how ordering physicians and genetic counselors might deal with such incidental findings.^{5,6}

Findings, Importance, and Translation

Our knowledge of the genetics of adenomatous and hamartomatous polyposis syndromes, which have a high risk for colon and other cancers, has grown during the past 2 decades, linking genes and epigenetic changes in the germline to channeling care to modify the natural history of these syndromes in patients for cancer onset. The adenomatous polyposis syndromes include familial adenomatous polyposis (*APC* mutation), the autosomal recessive *MYH*-associated polyposis (*MYH* mutations), Lynch syndrome (DNA MMR gene mutation), syndrome X (unknown mutation), and the recently described polymerase proofreading-associated polyposis (*POLE* or *POLD1* mutation) that is relatively rare.³ Similarly with the hamartomatous polyposis syndromes, we now understand the genetics for Cowden syndrome, Bannayan–Riley–Ruvulcaba syndrome and Lhermitte–Duclos syndrome, collectively known as the PTEN hamartoma syndrome (*PTEN* mutation), Peutz–Jeghers (*STK11* mutation), juvenile polyposis (*SMAD4*, *BMPRIA*, or *ENG* mutation), hyperplastic/serrated polyposis (unknown mutation), and a recently described novel genetic mechanism for hereditary mixed polyposis syndrome (overexpression of *GREM1*)^{7,8} (Table 1). Knowing that a patient carries a germline mutation alters the physician's and patient's care approach from screening to constant proactive surveillance and can accurately identify other carriers of the mutation in the family for targeted clinical surveillance. This approach minimizes the individual's chances for subsequent cancer development and optimizes the patient's longevity and quality of life. This approach also focuses surveillance resources on those who carry germline mutations, while alleviating family members from unnecessary surveillance tests if they do not carry the germline mutation.

Roadblocks and/or Limitations

Limitations of germline testing include finding variants of uncertain significance, which are not predicted or defined as pathogenic mutations within the gene tested, and the absence of any mutation found in any of the genes tested for a patient or family with a phenotype. The physician's continued clinical suspicion, along with careful proactive surveillance of patients and family members, is likely the more prudent approach as new genes are identified or novel types of mutations are discovered as causal in the colon cancer syndromes. Patients and families can be tested later as new information is published and new germline testing becomes available.

Fecal DNA Testing

Fecal DNA examination is one suggested test by the U.S. Multi-Society Task Force for CRC screening in asymptomatic individuals age 50 years or older (others include flexible sigmoidoscopy, colonoscopy, fecal immunochemical testing [FIT]/fecal occult blood testing [FOBT], computed tomographic colonography, and barium enema).⁹

Technological Primer

Human tumor DNA, which needs to be identified separately from the more abundant microbial DNA in feces, is assessed for mutation of specific genes that are involved in the pathogenesis or are markers of CRC. The fecal DNA can be assessed for integrity (meaning the DNA from the tumor is intact and not disintegrated) and assessed for epigenetic changes

such as methylation of specific human genes. The idea is that tumor cells containing altered DNA are shed into the feces where it can be detected and, if present, subsequently trigger the utilization of colonoscopy to search and identify the source of the altered DNA.¹⁰

Findings, Importance, and Translation

Only 2 of 6 developed fecal DNA tests, one based on multiple genetic markers and one based on the methylation of vimentin, have reached the status of a Clinical Laboratories Improvement Act (CLIA) (regulated by the Centers for Medicare and Medicaid Services) laboratory test, with only one in current commercial use.¹¹ These tests hold promise, but the diagnostic accuracy for screening is not known for any of the fecal DNA tests. Only 2 studies have had performance in CRC screening settings, each with different fecal DNA mutation panels assessed, with sensitivities for cancer detection from 25%–52%, but with improvement on the sensitivity of FOBT.¹² The U.S. Multi-Society Task Force on CRC listed fecal DNA testing among tests that primarily detect cancer (as opposed to detecting adenomas and cancer), but interval use was uncertain.⁹ At present, fecal DNA tests are not widely used, and its intended use would optimally be for individuals who are not eligible, unwilling, or unable to be screened by one of the more invasive screening tests.

Roadblocks and/or Limitations

Major barriers for adoptive general use of fecal DNA tests include (1) unknown diagnostic accuracy, (2) current lack of standardization or optimization of fecal DNA panels for high sensitivity and specificity, (3) unclear ease of use of test, (4) unclear acceptability of fecal DNA testing, (5) current lack of standardization for stool collection for fecal DNA testing, (6) no defined optimal interval for screening individuals, and (7) higher cost of fecal DNA tests relative to FIT and FOBT.^{11,13} Overall, fecal DNA tests are cost-effective when compared with no screening, but at present when compared with other colorectal screening modalities, it is not cost-effective largely because of test performance.¹⁴ New fecal DNA panels, including the use of proprietary quantitative allele-specific real-time target and signal amplification technology, are being tested to improve sensitivity of fecal DNA tests.^{11,12} A large prospective trial in average-risk individuals for CRC screening is ongoing, which uses the only commercial CLIA-approved fecal DNA test,¹¹ and should provide information on accuracy of this test. In a similar way that FIT has improved sensitivity and specificity for CRC detection over FOBT and has largely replaced FOBT in the clinical setting for screening, fecal DNA testing will likely replace FIT as its sensitivity and accuracy elevate and its cost comes down.

DNA Testing and Molecular Screening Directly From the Tumor

Technological Primer

Knowledge of molecular changes from within the tumor has grown tremendously. DNA changes from within the tumor are typically somatic, meaning they are not carried through the germline, and occur spontaneously and intrinsically as part of the natural genetic pathogenesis of the colon tumor.¹⁵ These DNA changes can act as tumor biomarkers that allow the physician to diagnose, predict tumor behavior, predict recurrence or metastasis, assess response to chemotherapy, and precisely understand the biology as to best approach

care for the patient.¹⁶ Some of the genetic changes as biomarkers have as good or better predictability as stage.

Findings, Importance, and Translation

The Cancer Genome Atlas Network published comprehensive mutation data from 224 sporadic CRCs.¹⁷ This work confirmed many previous mutational findings within colon cancers during the past quarter century of research, plus it identified new findings such as sporadic polymerase epsilon (*POLE*) mutations. Two groups of tumors emerged from their analysis, hypermutated colon cancers, consisting of DNA MMR inactivation or *POLE* mutation, and non-hypermutated colon cancers. The mutation profiles of these 2 groups are different, with hypermutated tumors targeting mutations in *ACVR2*, *TGFBR2*, *BRAF*, and DNA MMR genes, whereas non-hypermutated tumors follow the well-described Fearon and Vogelstein model with *APC*, *P53*, *KRAS*, and *PIK3CA* mutations.^{2,15,17} These 2 characterized pathways are the major genetic signatures found in colon cancers, but other signatures are likely present.¹⁸ These distinct groups of colon cancer show different biological behavior in patients. For instance, hypermutable tumors (as a consequence of loss of DNA MMR function) have a predilection for the proximal colon, confer better patient survival when matched for stage against patients with non-hypermutable tumors, and are more resistant to 5-fluorouracil-based chemotherapy (Table 2).^{15,19} Functional DNA MMR can recognize certain chemotherapeutic agents that intercalate into DNA, such as 5-fluorouracil, in addition to its normal function of recognizing and directing repair of polymerase mistakes after DNA is replicated.^{20–22} Recognition of 5-fluorouracil by DNA MMR then can trigger cell death.²³ When DNA MMR is not functional, cell death does not occur in the colon cancer cells, the overall tumor does not respond, and thus 5-fluorouracil is predicted ineffective for the patient with an advanced hypermutable tumor, and this has been confirmed in retrospective trials.^{24,25} The DNA MMR status can be assessed in many pathology laboratories via immunohistochemistry, because the presence of all of the DNA MMR proteins is a surrogate that generally signifies MMR functionality.^{15,25} Absence of any DNA MMR protein could represent a sporadic (somatic) or germline cause, but either would represent an MMR-deficient tumor that would not have any patient survival benefit to 5-fluorouracil-based chemotherapy. Thus, biological information (eg, DNA MMR status), in addition to stage, is considered in the approach to treatment of patients with advanced colon cancer.

The use of colon tumor DNA for patient care is in practice today and will become much more frequent in the future. For instance, drugs such as cetuximab and panitumumab, monoclonal antibodies that block epidermal growth factor receptor (EGFR) and are used to treat patients with stage IV colon cancer, block downstream signaling through the *KRAS*/*BRAF*/MAPK pathway as well through PI3 kinase, slowing cell proliferation and enhancing cell death.^{2,26} Colon tumors may overexpress EGFR, and in addition, tumors may exhibit mutational activation of *KRAS*, *BRAF*, and *PIK3CA*. This mutational activation, now independent from the effects of EGFR, makes cetuximab and panitumumab ineffective because of incessant downstream signaling below EGFR even when EGFR is inhibited.²⁶ Genotyping *KRAS*/*BRAF* before using these drugs is now required standard clinical

practice, and these drugs are approved for those who are wild-type (non-mutated) for KRAS/BRAF.

Tumor DNA can be predictive for outcome with aspirin use in patients with colon cancer. Aspirin is a cyclooxygenase-2 inhibitor that can down-regulate the expression of PI3 kinase (the protein made by *PIK3CA*). Among patients whose tumors exhibit mutation of *PIK3CA*, regular aspirin use reduced their probability of death compared with non-aspirin users. This effect was not seen in patients whose tumors had wild-type *PIK3CA*.²⁷ Thus, knowing whether a colon cancer patient has mutant *PIK3CA* predicts their response to aspirin to improve chances of long-term survival. This molecular pathologic epidemiology study will need to be confirmed in a large prospective study that verifies aspirin usage as an adjuvant targeted therapy for patients with mutant *PIK3CA* cancer.

Roadblocks and/or Limitations

Limitations of tumor DNA information for clinical decision-making include (1) tumor material availability is required for genetic analysis, (2) acquired additional genetic mechanisms with or without treatment may bypass or trump the predicted effects of a mutation, (3) information on genetic information for validity of a purported genetic biomarker may not have been tested in clinical trials, and (4) genetic biomarker information in a patient might not fully predict what occurs in another patient because of non-tumor mechanisms such as drug metabolism differences, microbiome makeup, or other factors. The development of blood-based tests that can detect what is reflected from within a tumor when present in a patient might mitigate some of these limitations.

Conclusions

The increased knowledge of the genetics and epigenetics of sporadic colon cancer combined with advances in molecular technology will direct diagnostic, preventive, prognosis, and therapeutic approaches for colon cancer for the foreseeable future. These include whole genome or exome sequencing of at-risk or colon cancer patient DNA to identify germline mutations, future fecal DNA tests that accurately increase the early diagnosis of colon tumors, the prognostic use of tumor molecular and genetic information that is predictive of outcome or chemotherapy response, such as the DNA MMR protein status,²⁴ and prevention of recurrent cancer with aspirin when knowing the *PIK3CA* mutational status of the patient's tumor.²⁷ The gastroenterologist will need to become more familiar with DNA tests because the rapid increase in knowledge of germline and somatic molecular changes in colon cancer has therapeutic implications, and this information will be routinely used for patient testing and precision medicine, directly affecting patient outcome.

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Abbreviations used in this paper

CLIA	Clinical Laboratories Improvement Act
CRC	colorectal cancer
EGFR	epidermal growth factor receptor
FIT	fecal immunochemical test
FOBT	fecal occult blood test
MMR	mismatch repair

References

1. Stoffel EM, Kastrinos F. Familial CRC: beyond the Lynch syndrome. *Clin Gastroenterol Hepatol*. 2013 Aug 17. Epub ahead of print.
2. Carethers JM. Proteomics, genomics and molecular biology in the personalized treatment of colorectal cancer. *J Gastrointest Surg*. 2012; 16:1648–1650. [PubMed: 22760966]
3. Palles C, Cazier JB, Howarth KM, et al. Germline mutations affecting the proofreading domains of POLE and POLD1 predispose to colorectal adenomas and carcinomas. *Nat Genet*. 2013; 45:136–144. [PubMed: 23263490]
4. Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. *Nature*. 2012; 487:330–337. [PubMed: 22810696]
5. Green RC, Berg JS, Grody WW, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet Med*. 2013; 15:565–574. [PubMed: 23788249]
6. Burke W, Matheny Antommara AH, Bennett R, et al. Recommendations for returning genomic incidental findings? We need to talk! *Genet Med*. 2013; 15:854–859. [PubMed: 23907645]
7. Jaeger E, Leedham S, Lewis A, et al. Hereditary mixed polyposis syndrome is caused by a 40-kb upstream duplication that leads to increased and ectopic expression of the BMP antagonist GREM1. *Nat Genet*. 2012; 44:699–703. [PubMed: 22561515]
8. Carethers JM. Unwinding the heterogeneous nature of hamartomatous polyposis syndromes. *JAMA*. 2005; 294:2498–2500. [PubMed: 16287964]
9. Levin B, Lieberman DA, McFarland B, et al. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. *Gastroenterology*. 2008; 134:1570–1595. [PubMed: 18384785]
10. Lidgard GP, Domanico MJ, et al. Clinical performance of an automated stool DNA assay for detection of colorectal neoplasia. *Clin Gastroenterol Hepatol*. 2013; 11:1313–1318. [PubMed: 23639600]
11. Lin, JS.; Webber, EM.; Beil, TL., et al. Fecal DNA testing in screening for colorectal cancer in average-risk adults. Rockville, MD: Agency for Healthcare Research and Quality (US); 2012.
12. Imperiale TF. Noninvasive screening tests for colorectal cancer. *Dig Dis*. 2012; 30(Suppl 2):16–26. [PubMed: 23207928]
13. Bosch LJ, Carvalho B, Fijneman RJ, et al. Molecular tests for colorectal cancer screening. *Clin Colorectal Cancer*. 2011; 10:8–23. [PubMed: 21609931]
14. Skally M, Hanly P, Sharp L. Cost effectiveness of fecal DNA screening for colorectal cancer: a systematic review and quality appraisal of the literature. *Appl Health Econ Health Policy*. 2013; 11:181–192. [PubMed: 23549792]
15. Grady WM, Carethers JM. Genomic and epigenetic instability in colorectal cancer pathogenesis. *Gastroenterology*. 2008; 135:1079–1099. [PubMed: 18773902]
16. Venook AP, Niedzwiecki D, Lopatin M, et al. Biologic determinants of tumor recurrence in stage II colon cancer: validation study of the 12-gene recurrence score in cancer and leukemia group B (CALGB) 9581. *J Clin Oncol*. 2013; 31:1775–1781. [PubMed: 23530100]

17. Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. *Nature*. 2012; 487:330–337. [PubMed: 22810696]
18. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. *Nature*. 2013; 500:415–421. [PubMed: 23945592]
19. Jo W-S, Carethers JM. Chemotherapeutic implications in microsatellite unstable colorectal cancer. *Cancer Biomarkers*. 2006; 2:51–60. [PubMed: 17192059]
20. Carethers JM, Chauhan DP, Fink D, et al. Mismatch repair proficiency and in vitro response to 5-fluorouracil. *Gastroenterology*. 1999; 117:123–131. [PubMed: 10381918]
21. Tajima A, Hess MT, Cabrera BL, et al. The mismatch repair complex hMutS α recognizes 5-fluorouracil-modified DNA: implications for chemosensitivity and resistance. *Gastroenterology*. 2004; 127:1678–1684. [PubMed: 15578504]
22. Tajima A, Iwaizumi M, Tseng-Rogenski S, et al. Both hMutS α and hMutS β complexes participate in 5-fluorouracil cytotoxicity. *PLoS One*. 2011; 6:e28117. [PubMed: 22164234]
23. Iwaizumi M, Tseng-Rogenski S, Carethers JM. DNA mismatch repair proficiency executing 5-fluorouracil cytotoxicity in colorectal cancer cells. *Cancer Biol Ther*. 2011; 12:756–764. [PubMed: 21814034]
24. Carethers JM, Smith EJ, Behling CA, et al. Use of 5-fluorouracil and survival in patients with microsatellite unstable colorectal cancer. *Gastroenterology*. 2004; 126:394–401. [PubMed: 14762775]
25. Boland CR, Goel A. Microsatellite instability in colorectal cancer. *Gastroenterology*. 2010; 138:2073–2087. [PubMed: 20420947]
26. Van Cutsem E, Köhne CH, Hitre E, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med*. 2009; 360:1408–1417. [PubMed: 19339720]
27. Liao X, Lochhead P, Nishihara R, et al. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. *N Engl J Med*. 2012; 367:1596–1606. [PubMed: 23094721]

Table 1**Genetics of Adenomatous and Hamartomatous Polyposis Syndromes**

Adenomatous syndrome	Chromosomal location(s)	Mutated gene(s)	Inheritance pattern	Year gene(s) or syndrome discovered
Familial adenomatous polyposis, Gardner's variant, Turcot's variant	5q21	<i>APC</i>	Autosomal dominant	1991
<i>MYH</i> -associated polyposis	1p32–34	<i>MYH</i>	Autosomal recessive	2003
Polymerase proofreading-associated polyposis	12q24.3 19q13.3	<i>POLE (L424V)</i> <i>POLD1 (S478N)</i>	Autosomal dominant	2013
Lynch syndrome, Muir-Torre variant, Turcot's variant	2p16, 3p21, 2q32, 7p22, 2p16, 3p22	<i>hMSH2</i> , <i>hMLH1</i> , <i>hPMS2</i> , <i>hMSH6</i> , <i>EPCAM</i> (<i>TACSTD1</i>)	Autosomal dominant	1993–1997, 2009
Syndrome X	Unknown	Unknown	Possibly autosomal dominant	2005

Hamartomatous syndrome	Chromosomal location(s)	Mutated gene(s)	Frequency in germline	Year gene(s) or syndrome discovered
<i>PTEN</i> hamartoma syndrome	10q22–23	<i>PTEN/MMAC1/TEP1</i>	>80%	1997
Cowden disease, Lhermitte–Duclos variant, Bannayan–Riley–Ruvalcaba syndrome	10q22–23	<i>PTEN/MMAC1/TEP1</i>	~60%	
Juvenile polyposis syndrome (with hereditary hemorrhagic telangiectasia overlap)	18q21.1 10q22–23 9q34	<i>SMAD4</i> <i>BMPRIA/ALK3</i> <i>ENG</i>	~20% ~25% Unknown	1998, 2001, 2005
Peutz–Jeghers syndrome	19p13.3	<i>STK11/LKB1</i>	70%–90%	1997
Hereditary mixed polyposis syndrome	15q13–q14 duplication	<i>GREM1</i> overexpression	Unknown	2003/2012
Hyperplastic (serrated) polyposis syndrome	1p, unknown	Unknown	Unknown	1996

Table 2

Comparison of Clinicopathologic Characteristics Between DNA MMR-deficient and DNA MMR-proficient Colon Tumors

DNA MMR-deficient tumors (MSI-H)	DNA MMR-proficient tumors (MSS)
Microsatellite instability	Loss of heterozygosity (LOH)
Diploid	Aneuploid
Frequently mucinous	Few mucinous tumors
Poor differentiation	Well differentiated
Proximal colon	Fewer proximal tumors
Young (germline)/old (hypermethylated <i>hMLH1</i>) patients	Few young patients
Few p53 mutation/LOH	p53 mutation/LOH
Lymphoid Crohn's-like reaction	No lymphoid Crohn's-like reaction
Better survival matched for stage	Poorer survival matched for stage
No response to 5-fluorouracil chemotherapy	Many respond to 5-fluorouracil chemotherapy

MSI-H, microsatellite unstable-high; MSS, microsatellite stable.