

Clinical Aspects of Microsatellite Instability Testing in Colorectal Cancer

Abstract

Microsatellite instability (MSI) is a molecular hallmark for some colorectal cancers (CRCs) in which short tandem repeats are prone to mutations along with DNA sequences. It is due to DNA-mismatch-repair system deficiency because of a germline/somatic mutation in mismatch-repair (MMR) genes. The germline mutations lead to Lynch syndrome (LS) while epigenetic gene silencing results in sporadic CRC tumors. We discuss in our paper the most important clinical aspects of MSI testing in CRCs. We reviewed the most reliable relevant studies and clinical trials according to their high-quality methods, particularly within two recent decades. MSI testing is used to classify CRC tumors as MSI-high (MSI-H), MSI-low, and microsatellite stable tumors. MSI-H or MMR deficient tumors have shown the best prognosis among all CRCs, so MSI testing is considered as a good prognostic marker. Moreover, it is used to identify LS among familial CRC patients. There is a diagnostic mutation in *BRAF* gene (V600E) by which sporadic CRCs could be distinguished from LS associated CRCs, due to its concordance with sporadic CRCs not LS. Although, some previous studies had demonstrated a predictive role for MSI testing in chemotherapy process, emerging some controversial findings in recent studies has not convinced many authors to recommend it as a routine examination to evaluate therapeutic response. Though emerging new molecular findings have opened novel windows to develop clinical management of CRC, MSI testing has remained as an excellent prognostic and diagnostic tool for CRC tumors.

Keywords: Colorectal cancer, Lynch syndrome, microsatellite instability, microsatellite instability testing, mismatch-repair

What are Molecular Pathways Behind Colorectal Cancer?

Overall, there are at least three main molecular pathways underlying the development of colorectal cancer (CRC): Chromosomal instability (CIN) pathway, microsatellite instability (MSI) pathway, and CpG island methylator phenotype (CIMP) pathway.^[1-3] CIN is the most prevalent molecular cause of genomic instability in CRC, so it is an original genetic basis of about 65%–70% of all sporadic CRC tumors.^[4] CIN is characterized with an imbalance in number of chromosomes (aneuploidy), chromosomal amplification, and a high frequency of loss of heterozygosity resulting in some deleterious mutations in tumor suppressor genes such as *APC* and *TP53*, and oncogenes including *KRAS*.^[5,6]

The second molecular pathway is MSI including about 8%–20% of all CRC tumors which is more common in stage II (20%) than stage III (12%) and stage IV (4%).^[7,8] This genetic change is a

molecular fingerprinting for DNA-mutation in mismatch repair (MMR) system deficiency because of germline mutations or epigenetic changes in MMR genes about which we discuss more in the article.^[2,9]

The last pathway is epigenetic molecular changes leading to alteration in gene expression or gene function without any change in its DNA sequence.^[10] For instance, CIMP within specific sites of promoter could lead to silencing of some vital tumor suppressor genes concluding tumor development which is found in about 35% of CRC tumors.^[11,12]

What is Microsatellite Instability?

MSI is a particular molecular change as a hallmark of averagely 15% of CRCs.^[3,9] At first, these molecular changes were named “dispersed somatic mutations” in simple tandem repeats^[13] or a replication error phenotype (RER).^[14] Due to a defect in DNA-mismatch repair (MMR) system, microsatellites or short tandem repeats, repetitive sequences containing 1–6 nucleotide

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units up to 100 times, are prone to accumulation of mutations. It is mainly attributed to a failure inefficient DNA polymerases attachment to these repetitive sequences during DNA replication.^[14] The most common microsatellite-associated errors are base-base mismatches which escape from internal proofreading function of DNA polymerases resulting in DNA hairpins.^[15] DNA-MMR system is responsible for proofreading of replication errors in microsatellites, including four well-known proteins: MLH1, MSH2, PMS2, and MSH6 interacting with each other as heterodimer complexes.^[13] In MMR-deficient cells, genes including microsatellites in their coding regions, like transforming growth factor (*TGF*)- β R2 gene, are more susceptible to frame shift mutations.^[16] Among MMR genes, two genes including *MSH3* and *MSH6* contain coding microsatellite regions which are prone to mutation in MSI-high (MSI-H) cancers.^[11]

MSI is a molecular change in some different tumors such as colorectal, stomach, endometrium, ovarian, sebaceous carcinoma, glioblastomas, and lymphomas.^[17] Most of MSI CRC tumors are sporadic usually due to epigenetic silencing of *MLH1* promoter because of somatic hypermethylation.^[18] These contain about 12%–15% of all CRCs in which lack of MLH1 function could lead to fast accumulation of mutations in other genes like *TGF*- β and *BAX* resulting in tumor development.^[12] Meanwhile, a somatic heritable hypermethylation of *MSH2* gene promoter has been also recently reported which is rarely occurred by some large deletion mutations in last exon of *EPCAM*, a gene located next to *MSH2*, or *EPCAM-MSH2* locus.^[19] A few of MSI-CRC tumors including about 2%–3% of the all CRC tumors is related to Lynch syndrome (LS), a hereditary predisposing cancer syndrome, which is mainly due to a germline mutation in one of the four DNA-MMR genes: *MLH1*, *MSH2*, *PMS2*, and *MSH6*.^[20,21]

How is Microsatellite Instability Testing Done?

MSI testing is performed by polymerase chain reaction (PCR)-based amplification of microsatellite repeats and comparing their size along with DNA in normal cells versus tumor cells. Currently, it is prepared through fluorescent primers and capillary electrophoresis.^[22] National Cancer Institute has recommended a diagnostic panel for MSI testing in which two mononucleotide markers named BAT-25 and BAT-26, and three dinucleotide markers named D2S123, D5S346, and D17S250 are used. With this panel, MSI-H is defined when at least two markers out of five markers in tumor cells show variability in their size compared to normal cells. Meanwhile, if only one marker present instability in tumor cells, the molecular phenotype is classified as MSI-low (MSI-L). In microsatellite stable (MSS) status, there is no unstable marker in DNA of the tumor cells.^[23]

Afterward, some studies showed an upper specificity and equal or upper sensitivity for mononucleotide markers than dinucleotide markers in MSI testing, a fact according which some commercial kits were developed. These

mononucleotide repeats are quasimonomorphic; hence nearly all people are homozygote for every common allele of a provided marker. Using the monomorphic markers simplifies interpretation of the data.^[24,25] Rather than mononucleotide markers, pentanucleotide markers were also included in a commercial diagnostic MSI kit, Promega MSI analysis system, to identify tissue mix-up.^[24,25] It uses a multiplex fluorescent survey in which PCR of the all five mononucleotide markers and two pentanucleotide markers is done in just a single reaction. The length of the amplified products is easily observable via a capillary electrophoresis method by which the cost of MSI testing has been significantly reduced.^[26,27] Some studies have shown that in MSI-L tumors, instability is usually observed just in dinucleotide markers. Therefore, if MSI analysis is only limited to dinucleotide markers, it might overestimate wrongly MSI-L or MSS tumors as MSI-H tumors.^[28]

Naturally, surgical resected tumors and their adjacent healthy tissues are the best sources to provide samples for MSI testing. Meanwhile, for rectal cancers which are being treated with neoadjuvant therapy to shrink the residual tumor, a presurgical biopsy usually prepares a better sample for MSI testing than surgical tumor [Figure 1].^[11]

What are Clinical Applications of Microsatellite Instability Testing in Colorectal Cancer?

Historically, at least three clinical applications could be considered for MSI testing in CRC: Prognostic, diagnostic, or predictive applications. So, the most important questions according which we designed this article are as following:

1. Can we use MSI testing as a prognostic marker in CRC patients?
2. What is the diagnostic usage of MSI testing in CRC?
3. Can we use MSI testing as a predictive marker to treat CRC patients with different chemotherapy regimens?

Microsatellite instability as a tumor prognostic marker

MSI CRC tumors have presented a better prognosis and a less metastasis compared to MSS-CRC tumors according to different studies.^[13,29,30] The prognostic value of MSI status in stage II CRC patients has been more than in patients with stage III tumors.^[29,31] MSI-CRC tumors contain numerous active, cytotoxic tumor-infiltrating lymphocytes, a reaction independently associated with a better survival.^[32,33] In a meta-analysis included 1277 CRC patients, MSI-CRC tumors had a better survival compared to MSS-CRC tumors.^[7]

Different studies have presented a lower recurrence rate in MSI tumors in comparison to MSS tumors.^[30,34] In a large series on 2141 CRC patients in II or III pathologic stages, lower recurrence rate, delayed time to recurrence, and better survival were reported in MMR deficient patients compared to MMR proficient patients.^[30] Other studies also demonstrated a better prognosis and lower recurrence risk

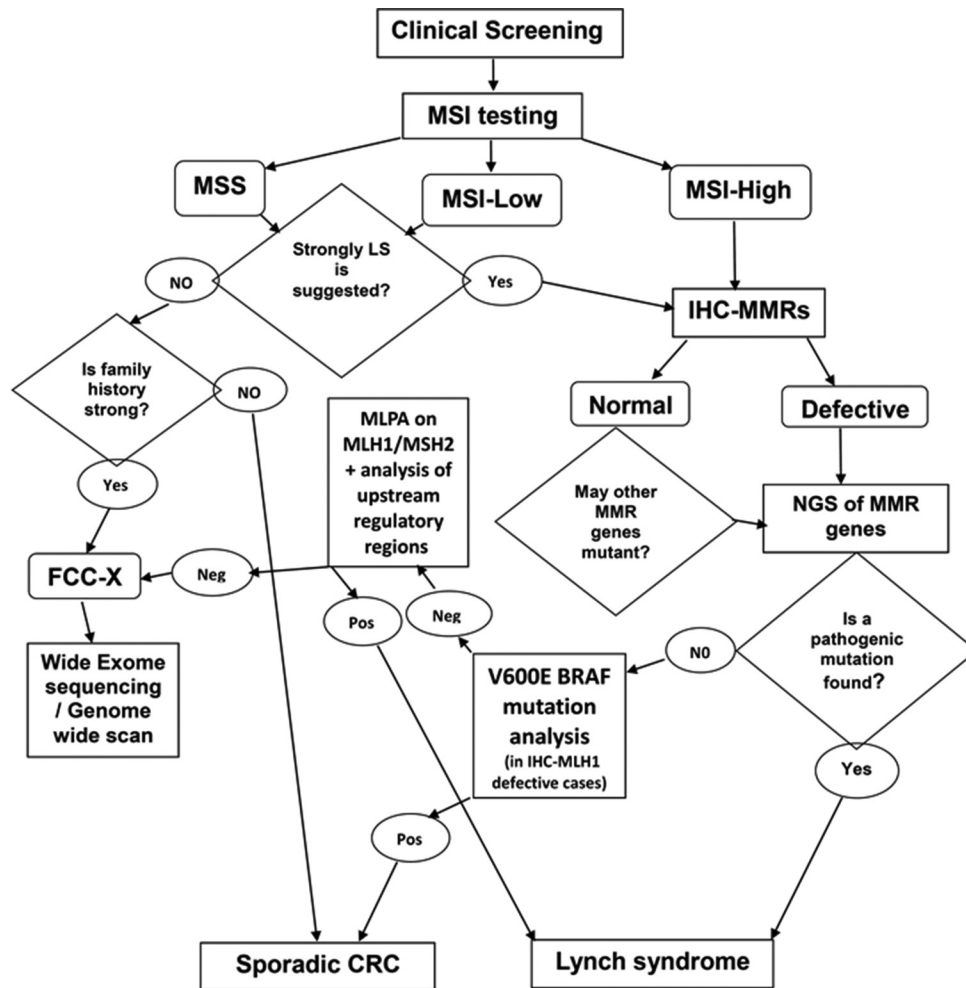


Figure 1: The proposal algorithm for clinical and molecular screening of hereditary non-polyposis colorectal cancer. MSI: Microsatellite instability, MSS: Microsatellite stable, LS: Lynch syndrome, Pos: Positive, Neg: Negative, FCC: Familial colorectal cancer, IHC: Immunohistochemistry

in MSI-H CRC patients with II pathologic stage tumors compared to MSS/MSI-L CRC patients.^[35-37] Moreover, some other studies have reported reduced incidence rate of MSI-H tumors in pathologic stages of III or IV than early stages, indicating a less possibility of metastasis in these cases.^[38,39] Meanwhile in a recent study, it was, interestingly, determined that MSI-H CRCs in stage II had a poorer prognosis and 3-year survival in comparison to MSS and MSI-L CRCs. Multivariate analysis of the results confirmed MSI-H phenotype as a poor independent prognostic marker [Table 1].^[40]

Genetic diagnosis of Lynch syndrome

DNA-MSI is a molecular demonstration of MMR deficient tumors through which human MMR genes and their important role in pathogenesis of LS was explored.^[9,31,41] Since this achievement in 1993, MSI testing has remained as a main method in research and clinical interventions associated to hereditary nonpolyposis CRC (HNPCC).^[42]

There are some clinical criteria for primary screening of patients at risk for HNPCC. Amsterdam I and II criteria,

and revised Bethesda guidelines, historically, have been used to clinical selection of these patients. Sensitivity of these triple criteria increases, respectively, so the revised Bethesda guidelines has the most sensitivity among them.^[43-45] After clinical screening of at risk patients, some molecular approaches are considered to evaluate what proportion of them are affected to LS.

Although both techniques including MSI testing and immunohistochemistry (IHC) have high efficacy in screening of at risk patients for genetic testing of germline mutations in MMR genes, given the some properties of MSI testing which in IHC are not seen, MSI testing is considered as an excellent and accessible method to identify LS patients.^[26,41,46] There are usually some deleterious mutations in at least half of the microsatellites or more in MMR deficient tumor cells, leading to instability of their sequences. Therefore, MSI status provides an outstanding and accessible marker to evaluate MMR deficiency. Since that both MSI status and LS are made by MMR deficiencies, MSI testing can be used to identify LS as a surrogate marker for MMR deficiency.^[26]

Table 1: Some important published studies within 1993-2014 according which MMR deficiency has been proposed as a positive prognostic marker in colorectal cancer patients

Publication year	Author/s	Trial groups		The group with increased survival	P
		Group 1	Group 2		
2002	Liang JT, et al.	MSI-H(+) HDFL(+), n=35; MSI-H(-) HDFL(+), n=134	MSI-H(+) HDFL(-), n=17; MSI-H(-) HDFL(-), n=58	MSI-H(+) HDFL(+)	0.0001
2003	Brueckl WM, et al.	MSI-H(+) HDFU(+), n=7	MSI-H(-) HDFU(+), n=36	MSI-H(+) HDFU(+)	0.021
2003	Ribic CM, et al.	MSI-H(+) HDFU(-), MSI-H(-) HDFU(-), n=287	MSI-H(+) HDFU(+), MSI-H(-) HDFU(+), n=283	MSI-H(+) HDFU(-)	0.004
2004	Carethers JM, et al.	MSI-H(+) HDFU(-), MSI-H(-) HDFU(-), n=138	MSI-H(+) HDFU(+), MSI-H(-) HDFU(+), n=66	MSI-H(-) HDFU(+)	<0.05
2008	Muller CI, et al.	MSI-H(+) FUFOX(+), MSI-H(-) FUFOX(+), n=474	MSI-H(+) CAPOX(+), MSI-H(-) CAPOX(+), n=474	MSI-H(-) FUFOX(+)	0.02
2009	Bertagnolli MM, et al.	MSI-H(+) IFL(+), MSI-H(-) IFL(-), n=629	MSI-H(+) FU/LV(+), MSI-H(-) FU/LV(-), n=635	MSI-H(+) IFL(+)	0.03
2010	Sargent DJ, et al.	MSI-H(+) HDFU(-), MSI-H(-) HDFU(-), n=228	MSI-H(+) HDFU(+), MSI-H(-) HDFU(+), n=229	MSI-H(-) HDFU(+)	0.02
2010	Kim ST, et al.	MSI-H(+) FUFOX(+), MSI-H(-) FUFOX(+), n=75	MSI-H(+) CAPOX(+), MSI-H(-) CAPOX(+), n=96	none	0.95
2016	Tougeron D, et al.	MSI-H(+) surgery alone, n=263	MSI-H(+) surgery/FUFOX, n=119; MSI-H(+) surgery/FU, n=51	MSI-H(+) surgery/ FUFOX	<0.001

MMR: Mismatch-repair

MSI testing has some advantages compared to IHC in LS diagnosis, including convenience of doing and interpreting the results, high rate of reproducibility, identification of nontruncating missense mutations in normal IHC, and also sufficiency of just one tumor section for MSI testing instead four sections in IHC. Meanwhile, MSI testing has also some limitations in LS diagnosis. MSI is not LS-specific and it is demonstrated in about 10%–15% of the sporadic CRCs too. In these cases, MMR deficiency is almost due to CpG island hypermethylation of the *MLH1* promoter and associated gene silencing.^[18,47] Although *MLH1* hypermethylation could be rarely observed with germline MMR genes mutations, it can also be considered as a second hit to inactivate of the *MLH1* wild allele in LS tumors.^[48,49]

Given the concordance of V600E mutation of *BRAF* with sporadic MSI-CRC tumors which is associated with *MLH1* hypermethylation and lack of this mutation in LS tumors, according to many studies,^[18,50-52] *BRAF* V600E mutation could be used as a surrogate marker to distinguish of sporadic MSI-CRC tumors from LS tumors [Figure 1].^[48,53]

Some different studies have presented that CRC tumors due to a germline mutation of *MSH6* could demonstrate MSI-L instead MSI-H phenotype. It means that some *MSH6* mutant CRC tumors did not show MSI-H status.^[54,55] According to these studies, *MSH2/MSH3* protein dimer remains active in *MSH6* mutant cells and MSI may be limited only to mononucleotide repeats.^[55] It seems more *MSH6* mutant tumors would be detectable with MSI-H phenotype instead MSS or MSI-L if enough mononucleotide markers are used for MSI testing.^[26]

Predicting response to chemotherapy

Although some previous studies had indicated improved response of MSI tumors to chemotherapy with 5-fluorouracil (5-FU),^[56-58] later studies showed a weak response of locally advanced MSI-H CRC tumors to 5-FU-based regimens in adjuvant therapy,^[59,60] indicating no benefit from single-agent fluoropyrimidine therapy in MMR-deficient CRC tumors.^[36,61-63] Further, some studies indicate not only resistance of MSI-H CRC patients to treatment with 5-FU, but also lower survival of them after receiving 5-FU in comparison with the patients who did not receive 5-FU.^[59,63] Ignoring lack of enough samples and some methodological problems as significant limitations to interpret the results, next studies demonstrated improved response of MSI-CRC tumors to combination chemotherapy with oxaliplatin and irinotecan in comparison to 5-FU based agents.^[37,64-66] Also in a recent retrospective multicenter study on 433 MMR deficient CRC patients, more disease-free survival was observed using adjuvant oxaliplatin-based chemotherapy in comparison to adjuvant fluoropyrimidine alone.^[67] Apparently, MSI-H CRC cells have been more sensitive to irinotecan, a topoisomerase inhibitor, compared to MSS CRC cells. So, complete response rate to neoadjuvant therapy with irinotecan was about 60% in MSI-H CRC patients versus 20% in MSS CRC patients.^[68] Simultaneously, in a meta-analysis study including 964 metastatic CRC patients, 91 patients with MSI-H tumors presented no distinct benefit from chemotherapy.^[69] Some clinical trials such as the Quick and Simple and Reliable suggest that MSI status cannot predict who may benefit from chemotherapy [Table 2].^[70]

Table 2: Some important clinical trials within 2002–2016 evaluating predictive value of microsatellite instability state in different chemotherapy regimens for colorectal cancer patients

Publication year	Author/s	Type of study	Number of cases		The group with increased survival	P
			MMR deficient	MMR proficient		
1993	Thibodeau B, <i>et al.</i>	Case series	25	65	MMR deficient	0.02
2005	Popat S, <i>et al.</i>	Meta-analysis	1277	6365	MMR deficient	0.16
2005	Benatti P, <i>et al.</i>	Case series	256	1007	MMR deficient	<0.01
2009	Ligtenberg K, <i>et al.</i>	Case series	344	1797	MMR deficient	0.035
2009	Koopman M, <i>et al.</i>	Case series	18	532	MMR deficient	<0.001
2010	Guastadisegni C, <i>et al.</i>	Meta-analysis	1278	7965	MMR deficient	<0.0001
2011	Sinicrope FA, <i>et al.</i>	Case series	344	1797	MMR deficient	<0.001
2013	Phipps AI, <i>et al.</i>	Cohort	460	2774	MMR deficient	<0.001
2014	Inamura K, <i>et al.</i>	Case series	190	1021	MMR deficient	<0.001

MSI-H: Microsatellite instability-high, HDFL: High-dose 5-fluorouracil plus leucovorin chemotherapy, HDFU: High-dose fluorouracil-based chemotherapy, FUFOX: Fluorouracil plus oxaliplatin chemotherapy, CAPOX: Capecitabine plus oxaliplatin chemotherapy, IFL: Irinotecan/fluorouracil/levoleucovorin, FU/LV: 5-fluorouracil/leucovorin

Due to some controversial findings in the studies related to predicting role of MSI status in chemotherapy response, recently European Society for Medical Oncology has not considered MSI as a predictive marker for chemotherapy.^[71] Anyway, the association between MSI status and response to chemotherapy has been still remained as an active area in clinical and molecular cancer research.

Summary

Despite of so much molecular findings about CRC tumorigenesis, MSI testing is being still used as an excellent accessible prognostic and diagnostic marker in CRC patients. According to MSI analysis, CRC tumors are classified to MSI-H, MSI-L, and MSS tumors. MSI-H CRC tumors have shown the best prognosis and a better survival in comparison with the two others. MSI testing has been also used to identify MMR deficiency in CRC tumors due to a germline mutation in MMR genes, leading to LS, or epigenetic gene silencing leading to sporadic CRC tumors. Since *BRAF* mutation is observed with CIMP in CRC tumors without existing in LS CRC tumors, it can be used to distinguish sporadic CRC tumors from hereditary ones. Although there are some evidences for poor response of MMR deficient CRC tumors to chemotherapy with 5-FU based regimens, recent studies have explored some different features. Therefore, application of MSI testing for predicting response to chemotherapy has remained ambiguous as an active field for more investigation.

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Conflicts of interest

There are no conflicts of interest.

References

- Dawson H, Lugli A. Molecular and pathogenetic aspects of tumor budding in colorectal cancer. *Front Med (Lausanne)* 2015;2:11.
- Neumann JH, Jung A, Kirchner T. Molecular pathology of colorectal cancer. *Pathologie* 2015;36:137-44.
- Zeinalian M, Emami MH, Salehi R, Naimi A, Kazemi M, Hashemzadeh-Chaleshtori M. Molecular analysis of Iranian colorectal cancer patients at risk for Lynch syndrome: A new molecular, clinicopathological feature. *J Gastrointest Cancer* 2015;46:118-25.
- Al-Sohaily S, Biankin A, Leong R, Kohonen-Corish M, Warusavitarne J. Molecular pathways in colorectal cancer. *J Gastroenterol Hepatol* 2012;27:1423-31.
- Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, *et al.* Genetic alterations during colorectal-tumor development. *N Engl J Med* 1988;319:525-32.
- Grady WM, Markowitz SD. The molecular pathogenesis of colorectal cancer and its potential application to colorectal cancer screening. *Dig Dis Sci* 2015;60:762-72.
- Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol* 2005;23:609-18.
- Vilar E, Gruber SB. Microsatellite instability in colorectal cancer-the stable evidence. *Nat Rev Clin Oncol* 2010;7:153-62.
- Lynch HT, Lynch PM, Lanspa SJ, Snyder CL, Lynch JF, Boland CR. Review of the Lynch syndrome: History, molecular genetics, screening, differential diagnosis, and medicolegal ramifications. *Clin Genet* 2009;76:1-18.
- van Engeland M, Derks S, Smits KM, Meijer GA, Herman JG. Colorectal cancer epigenetics: Complex simplicity. *J Clin Oncol* 2011;29:1382-91.
- Shi C, Washington K. Molecular testing in colorectal cancer: Diagnosis of Lynch syndrome and personalized cancer medicine. *Am J Clin Pathol* 2012;137:847-59.
- Ahnen DJ. The American College of gastroenterology emily couric lecture – The adenoma-carcinoma sequence revisited: Has the era of genetic tailoring finally arrived? *Am J Gastroenterol* 2011;106:190-8.
- Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. *Science* 1993;260:816-9.
- Aaltonen LA, Peltomäki P, Leach FS, Sistonen P, Pylkkänen L,

- Mecklin JP, *et al.* Clues to the pathogenesis of familial colorectal cancer. *Science* 1993;260:812-6.
15. Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature* 1993;363:558-61.
 16. Guanti G, Resta N, Simone C, Cariola F, Demma I, Fiorente P, *et al.* Involvement of PTEN mutations in the genetic pathways of colorectal cancerogenesis. *Hum Mol Genet* 2000;9:283-7.
 17. Aaltonen LA, Salovaara R, Kristo P, Canzian F, Hemminki A, Peltomäki P, *et al.* Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. *N Engl J Med* 1998;338:1481-7.
 18. Kim SJ, Kim HR, Kim SH, Han JH, Cho YB, Yun SH, *et al.* hMLH1 promoter methylation and BRAF mutations in high-frequency microsatellite instability colorectal cancers not fulfilling the revised Bethesda guidelines. *Ann Surg Treat Res* 2014;87:123-30.
 19. Kempers MJ, Kuiper RP, Ockeloen CW, Chappuis PO, Hutter P, Rahner N, *et al.* Risk of colorectal and endometrial cancers in EPCAM deletion-positive Lynch syndrome: A cohort study. *Lancet Oncol* 2011;12:49-55.
 20. Hendriks YM, de Jong AE, Morreau H, Tops CM, Vasen HF, Wijnen JT, *et al.* Diagnostic approach and management of Lynch syndrome (hereditary nonpolyposis colorectal carcinoma): A guide for clinicians. *CA Cancer J Clin* 2006;56:213-25.
 21. Lynch HT, Lynch JF, Attard TA. Diagnosis and management of hereditary colorectal cancer syndromes: Lynch syndrome as a model. *CMAJ* 2009;181:273-80.
 22. Kloor M, Staffa L, Ahadova A, von Knebel Doeberitz M. Clinical significance of microsatellite instability in colorectal cancer. *Langenbecks Arch Surg* 2014;399:23-31.
 23. CAP Technology Assessment Committee: Northfield, IL: College of American Pathologists. POET Report: Prognostic Uses of MSI Testing, VI, Perspectives on Emerging Technology. 2011, available from: http://www.cap.org/apps/docs/committees/technology/microsatellite_testing.pdf.
 24. Suraweera N, Duval A, Reperant M, Vaury C, Furlan D, Leroy K, *et al.* Evaluation of tumor microsatellite instability using five quasimonomorphic mononucleotide repeats and pentaplex PCR. *Gastroenterology* 2002;123:1804-11.
 25. Bacher JW, Flanagan LA, Smalley RL, Nassif NA, Burgart LJ, Halberg RB, *et al.* Development of a fluorescent multiplex assay for detection of MSI-High tumors. *Dis Markers* 2004;20:237-50.
 26. Zhang L. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part II. The utility of microsatellite instability testing. *J Mol Diagn* 2008;10:301-7.
 27. Murphy KM, Zhang S, Geiger T, Hafez MJ, Bacher J, Berg KD, *et al.* Comparison of the microsatellite instability analysis system and the Bethesda panel for the determination of microsatellite instability in colorectal cancers. *J Mol Diagn* 2006;8:305-11.
 28. Yuan L, Chi Y, Chen W, Chen X, Wei P, Sheng W, *et al.* Immunohistochemistry and microsatellite instability analysis in molecular subtyping of colorectal carcinoma based on mismatch repair competency. *Int J Clin Exp Med*. 2015;8(11):20988-1000.
 29. Ligtenberg MJ, Kuiper RP, Chan TL, Goossens M, Hebeda KM, Voorendt M, *et al.* Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3' exons of TACSTD1. *Nat Genet* 2009;41:112-7.
 30. Sinicrope FA, Foster NR, Thibodeau SN, Marsoni S, Monges G, Labianca R, *et al.* DNA mismatch repair status and colon cancer recurrence and survival in clinical trials of 5-fluorouracil-based adjuvant therapy. *J Natl Cancer Inst* 2011;103:863-75.
 31. Zeinalian M, Hashemzadeh-Chaleshtori M, Salehi R, Kazemi M, Emami MH. Tumor microsatellite instability and clinicopathologic features in Iranian colorectal cancer patients at risk for Lynch syndrome. *J Res Med Sci* 2015;20:154-60.
 32. Phillips SM, Banerjea A, Feakins R, Li SR, Bustin SA, Dorudi S. Tumour-infiltrating lymphocytes in colorectal cancer with microsatellite instability are activated and cytotoxic. *Br J Surg* 2004;91:469-75.
 33. Phipps AI, Lindor NM, Jenkins MA, Baron JA, Win AK, Gallinger S, *et al.* Colon and rectal cancer survival by tumor location and microsatellite instability: The colon cancer family registry. *Dis Colon Rectum* 2013;56:937-44.
 34. Tejpar S, Saridaki Z, Delorenzi M, Bosman F, Roth AD. Microsatellite instability, prognosis and drug sensitivity of stage II and III colorectal cancer: More complexity to the puzzle. *J Natl Cancer Inst* 2011;103:841-4.
 35. Benatti P, Gafà R, Barana D, Marino M, Scarselli A, Pedroni M, *et al.* Microsatellite instability and colorectal cancer prognosis. *Clin Cancer Res* 2005;11:8332-40.
 36. Guastadisegni C, Colafranceschi M, Ottini L, Dogliotti E. Microsatellite instability as a marker of prognosis and response to therapy: A meta-analysis of colorectal cancer survival data. *Eur J Cancer* 2010;46:2788-98.
 37. Müller CI, Schulmann K, Reinacher-Schick A, Andre N, Arnold D, Tannapfel A, *et al.* Predictive and prognostic value of microsatellite instability in patients with advanced colorectal cancer treated with a fluoropyrimidine and oxaliplatin containing first-line chemotherapy. A report of the AIO Colorectal Study Group. *Int J Colorectal Dis* 2008;23:1033-9.
 38. Roth AD, Tejpar S, Delorenzi M, Yan P, Fiocca R, Klingbiel D, *et al.* Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: Results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. *J Clin Oncol* 2010;28:466-74.
 39. Koopman M, Kortman GA, Mekenkamp L, Ligtenberg MJ, Hoogerbrugge N, Antonini NF, *et al.* Deficient mismatch repair system in patients with sporadic advanced colorectal cancer. *Br J Cancer* 2009;100:266-73.
 40. Kim NK. Is a microsatellite instability still useful for tailored treatment in stage II and III colon cancer? *Ann Coloproctol* 2014;30:5-6.
 41. Shia J. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome: Part I. The utility of immunohistochemistry. *J Mol Diagn* 2008;10:293-300.
 42. Leach FS, Nicolaides NC, Papadopoulos N, Liu B, Jen J, Parsons R, *et al.* Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. *Cell* 1993;75:1215-25.
 43. Laghi L, Bianchi P, Roncalli M, Malesci A. Re: Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst* 2004;96:1402-3.
 44. Serrano M, Lage P, Belga S, Filipe B, Francisco I, Rodrigues P, *et al.* Bethesda criteria for microsatellite instability testing: Impact on the detection of new cases of Lynch syndrome. *Fam Cancer* 2012;11:571-8.
 45. Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Rüschoff J, *et al.* Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst* 2004;96:261-8.

46. Lindor NM, Burgart LJ, Leontovich O, Goldberg RM, Cunningham JM, Sargent DJ, *et al.* Immunohistochemistry versus microsatellite instability testing in phenotyping colorectal tumors. *J Clin Oncol* 2002;20:1043-8.
47. Joo YE. Prognostic significance of CpG island methylator phenotype in colorectal cancer. *Gut Liver* 2015;9:139-40.
48. Deng G, Bell I, Crawley S, Gum J, Terdiman JP, Allen BA, *et al.* BRAF mutation is frequently present in sporadic colorectal cancer with methylated hMLH1, but not in hereditary nonpolyposis colorectal cancer. *Clin Cancer Res* 2004;10(1 Pt 1):191-5.
49. Young J, Simms LA, Biden KG, Wynter C, Whitehall V, Karamatic R, *et al.* Features of colorectal cancers with high-level microsatellite instability occurring in familial and sporadic settings: Parallel pathways of tumorigenesis. *Am J Pathol* 2001;159:2107-16.
50. Kambara T, Simms LA, Whitehall VL, Spring KJ, Wynter CV, Walsh MD, *et al.* BRAF mutation is associated with DNA methylation in serrated polyps and cancers of the colorectum. *Gut* 2004;53:1137-44.
51. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, *et al.* Mutations of the BRAF gene in human cancer. *Nature* 2002;417:949-54.
52. Asl JM, Almasi S, Tabatabaiefar MA. High frequency of BRAF proto-oncogene hot spot mutation V600E in cohort of colorectal cancer patients from Ahvaz City, Southwest Iran. *Pak J Biol Sci* 2014;17:565-9.
53. Thiel A, Heinonen M, Kantonen J, Gylling A, Lahtinen L, Korhonen M, *et al.* BRAF mutation in sporadic colorectal cancer and Lynch syndrome. *Virchows Arch* 2013;463:613-21.
54. Berends MJ, Wu Y, Sijmons RH, Mensink RG, van der Sluis T, Hordijk-Hos JM, *et al.* Molecular and clinical characteristics of MSH6 variants: An analysis of 25 index carriers of a germline variant. *Am J Hum Genet* 2002;70:26-37.
55. Hendriks YM, Wagner A, Morreau H, Menko F, Stormorken A, Quehenberger F, *et al.* Cancer risk in hereditary nonpolyposis colorectal cancer due to MSH6 mutations: Impact on counseling and surveillance. *Gastroenterology* 2004;127:17-25.
56. Liang JT, Huang KC, Lai HS, Lee PH, Cheng YM, Hsu HC, *et al.* High-frequency microsatellite instability predicts better chemosensitivity to high-dose 5-fluorouracil plus leucovorin chemotherapy for stage IV sporadic colorectal cancer after palliative bowel resection. *Int J Cancer* 2002;101:519-25.
57. Brueckl WM, Moesch C, Brabletz T, Koebnick C, Riedel C, Jung A, *et al.* Relationship between microsatellite instability, response and survival in palliative patients with colorectal cancer undergoing first-line chemotherapy. *Anticancer Res* 2003;23:1773-7.
58. Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, *et al.* Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med* 2003;349:247-57.
59. Sargent DJ, Marsoni S, Monges G, Thibodeau SN, Lbianca R, Hamilton SR, *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;28:3219-26.
60. Bertagnolli MM, Niedzwiecki D, Compton CC, Hahn HP, Hall M, Damas B, *et al.* Microsatellite instability predicts improved response to adjuvant therapy with irinotecan, fluorouracil, and leucovorin in stage III colon cancer: Cancer and Leukemia Group B Protocol 89803. *J Clin Oncol* 2009;27:1814-21.
61. Des Guetz G, Schischmanoff O, Nicolas P, Perret GY, Morere JF, Uzzan B. Does microsatellite instability predict the efficacy of adjuvant chemotherapy in colorectal cancer? A systematic review with meta-analysis. *Eur J Cancer* 2009;45:1890-6.
62. Watanabe T, Wu TT, Catalano PJ, Ueki T, Satriano R, Haller DG, *et al.* Molecular predictors of survival after adjuvant chemotherapy for colon cancer. *N Engl J Med* 2001;344:1196-206.
63. Carethers JM, Smith EJ, Behling CA, Nguyen L, Tajima A, Doctolero RT, *et al.* Use of 5-fluorouracil and survival in patients with microsatellite-unstable colorectal cancer. *Gastroenterology* 2004;126:394-401.
64. des Guetz G, Mariani P, Cucherousset J, Benamoun M, Lagorce C, Sastre X, *et al.* Microsatellite instability and sensitivity to FOLFOX treatment in metastatic colorectal cancer. *Anticancer Res* 2007;27:2715-9.
65. Kim ST, Lee J, Park SH, Park JO, Lim HY, Kang WK, *et al.* The effect of DNA mismatch repair (MMR) status on oxaliplatin-based first-line chemotherapy as in recurrent or metastatic colon cancer. *Med Oncol* 2010;27:1277-85.
66. Kim JE, Hong YS, Ryu MH, Lee JL, Chang HM, Lim SB, *et al.* Association between deficient mismatch repair system and efficacy to irinotecan-containing chemotherapy in metastatic colon cancer. *Cancer Sci* 2011;102:1706-11.
67. Tougeron D, Mouillet G, Trouilloud I, Lecomte T, Coriat R, Aparicio T, *et al.* Efficacy of adjuvant chemotherapy in colon cancer with microsatellite instability: A large multicenter AGEO study. *J Natl Cancer Inst* 2016;108. pii: Djv438.
68. Fallik D, Borrini F, Boige V, Viguier J, Jacob S, Miquel C, *et al.* Microsatellite instability is a predictive factor of the tumor response to irinotecan in patients with advanced colorectal cancer. *Cancer Res* 2003;63:5738-44.
69. Des Guetz G, Uzzan B, Nicolas P, Schischmanoff O, Perret GY, Morere JF. Microsatellite instability does not predict the efficacy of chemotherapy in metastatic colorectal cancer. A systematic review and meta-analysis. *Anticancer Res* 2009;29:1615-20.
70. Hutchins G, Southward K, Handley K, Magill L, Beaumont C, Stahlschmidt J, *et al.* Value of mismatch repair, KRAS, and BRAF mutations in predicting recurrence and benefits from chemotherapy in colorectal cancer. *J Clin Oncol* 2011;29:1261-70.
71. Schmoll HJ, Van Cutsem E, Stein A, Valentini V, Glimelius B, Haustermans K, *et al.* ESMO consensus guidelines for management of patients with colon and rectal cancer: a personalized approach to clinical decision making. *Ann Oncol* 2012;23:2479-516.