DOI:10.1093/jnci/djt173
Advance Access publication July 22, 2013

BRIEF COMMUNICATION

Microsatellite Instability and *BRAF* Mutation Testing in Colorectal Cancer Prognostication

Paul Lochhead, Aya Kuchiba, Yu Imamura, Xiaoyun Liao, Mai Yamauchi, Reiko Nishihara, Zhi Rong Qian, Teppei Morikawa, Jeanne Shen, Jeffrey A. Meyerhardt, Charles S. Fuchs, Shuji Ogino

Manuscript received November 14, 2012; revised March 9, 2013; accepted May 30, 2013.

Correspondence to: Shuji Ogino, MD, PhD, Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, 450 Brookline Ave, Rm M422, Boston, MA 02215 (e-mail: shuji_ogino@dfci.harvard.edu).

***BRAF* mutation in colorectal cancer is associated with microsatellite instability (MSI) through its relationship with high-level CpG island methylator phenotype (CIMP) and *MLH1* promoter methylation. MSI and *BRAF* mutation analyses are routinely used for familial cancer risk assessment. To clarify clinical outcome associations of combined MSI/*BRAF* subgroups, we investigated survival in 1253 rectal and colon cancer patients within the Nurses' Health Study and Health Professionals Follow-up Study with available data on clinical and other molecular features, including CIMP, LINE-1 hypomethylation, and *KRAS* and *PIK3CA* mutations. Compared with the majority subtype of microsatellite stable (MSS)/*BRAF*–wild-type, MSS/*BRAF*-mutant, MSI-high/*BRAF*-mutant, and MSI-high/*BRAF*–wild-type subtypes showed multivariable colorectal cancer-specific mortality hazard ratios of 1.60 (95% confidence interval [CI] =1.12 to 2.28; *P* = .009), 0.48 (95% CI = 0.27 to 0.87; *P* = .02), and 0.25 (95% CI = 0.12 to 0.52; *P* < .001), respectively. No evidence existed for a differential prognostic role of *BRAF* mutation by MSI status ($P_{interaction}$ > .50). Combined *BRAF*/MSI status in colorectal cancer is a tumor molecular biomarker for prognosic risk stratification.**

J Natl Cancer Inst;2013;105:1151–1156

High-level microsatellite instability (MSI-high) is present in approximately 15% of colorectal cancers and is associated with superior survival (1–9). *BRAF* mutation, present in 10% to 20% of colorectal cancers, is associated with MSI-high through its relationship to high-level CpG island methylator phenotype (CIMP) (10–14) and is generally associated with inferior prognosis (15–28). Because the presence of *BRAF* mutation in MSI-high colorectal cancer decreases the likelihood of Lynch syndrome, MSI and *BRAF* analyses have an established clinical utility (29–31). Clinicians are therefore increasingly availed of MSI/*BRAF* status in colorectal cancer (29–31); however, outcomes for combined MSI/*BRAF* subgroups have not been clearly defined. It remains uncertain whether the prognostic role of *BRAF* mutation depends on MSI status (15–18).

Using the database of two US nationwide prospective cohort studies, the Nurses' Health Study and the Health Professionals Follow-up Study (32–34), we tested the hypothesis that combined MSI/*BRAF* status could serve as a prognostic molecular biomarker.

Rectal and colon cancer cases were identified through reporting by participants or next-of-kin and by searching the National Death Index for unreported lethal cases. The National Death Index was used to ascertain deaths (32–34). Cause of death was determined by study physicians. Informed consent was obtained from all study subjects. This study was approved by the Human Subjects Committees of Harvard School of Public Health and Brigham and Women's Hospital.

DNA was extracted from formalin-fixed paraffin-embedded specimens, collected from hospitals across the United States where participants had undergone tumor resection or diagnostic biopsy (33). No statistically significant demographic differences existed between case subjects with and without available tissue (33). Tumor molecular biomarkers (including MSI, CIMP, LINE-1 hypomethylation, and *KRAS*, *BRAF*, and *PIK3CA* mutations) were analyzed as previously described (35–41) (details provided in Supplementary Methods, available online).

All statistical analyses were performed using SAS (version 9.2; SAS Institute, Cary, NC). All statistical tests were two-sided. Survival time was assessed using the Kaplan–Meier and log-rank methods. Cox proportional hazards models were used to estimate mortality hazard ratios (HRs), adjusting for potential confounders (details provided in Supplementary Methods).

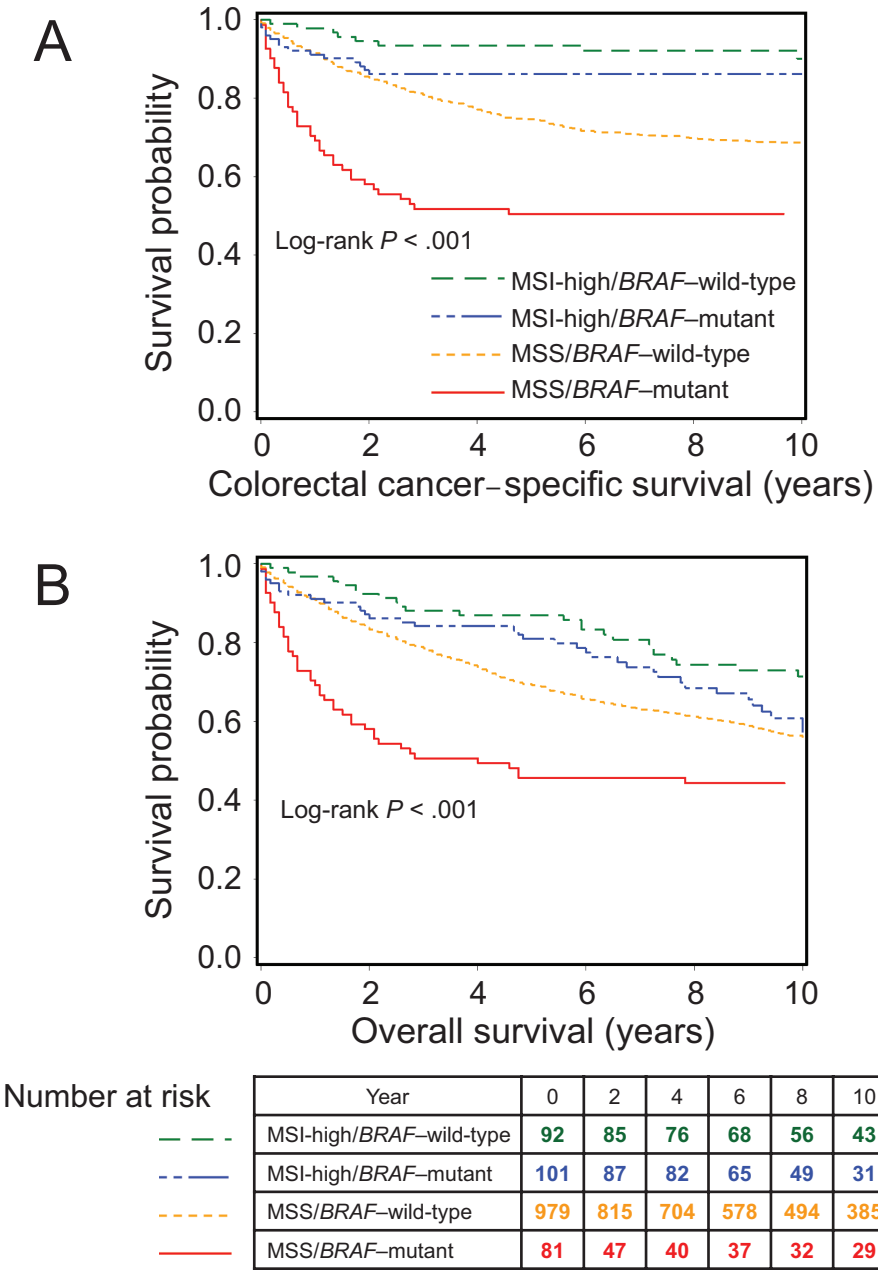
Characteristics of 1253 colorectal cancer case subjects are summarized in Supplementary Table 1 (available online). During follow-up (median = 8.2 years; interquartile range = 3.5–13.1 years), there were 608 deaths, including 361 colorectal cancer–specific deaths. We first analyzed *BRAF* mutation and MSI status as independent variables in survival analyses (Supplementary Figures 1 and 2, Supplementary Table 2, available online). In multivariable analyses, *BRAF* mutation was associated with statistically significantly higher colorectal cancer–specific mortality (multivariable HR = 1.64, 95% confidence interval [CI] = 1.18 to 2.27; *P* = .003). MSI-high was associated with statistically significantly lower colorectal cancer–specific mortality (multivariable HR = 0.28, 95% CI = 0.17 to 0.46; *P* < .001). MSI status was a confounder for *BRAF* mutation; when we simply adjusted for MSI status, the colorectal cancer–specific hazard ratio for *BRAF* mutation was 2.05 (compared with univariate HR estimate of 1.14).

Increased colorectal cancer–specific mortality appeared to be associated with *BRAF* mutation in both MSS (multivariable HR = 1.60, 95% CI = 1.12 to 2.28; *P* = .009) and MSI-high tumor strata (multivariable HR = 1.90, 95% CI = 0.79 to 4.57; *P* = .15) (Supplementary Table 3, available online). Lower colorectal cancer–specific mortality

was associated with MSI-high in both *BRAF*–wild-type (multivariable HR = 0.25, 95% CI = 0.12 to 0.52; $P < .001$) and *BRAF*-mutant strata (multivariable HR = 0.30, 95% CI = 0.16 to 0.58; $P < .001$).

For combined MSI/*BRAF* subgroups, 5-year colorectal cancer–specific survival was 46% for MSS/*BRAF*-mutant, 65% for MSS/*BRAF*–wild-type, 73% for MSI-high/*BRAF*-mutant, and 79% for MSI-high/*BRAF*–wild-type (log-rank $P < .001$) (Figure 1). In multivariable analyses (Table 1), compared with the majority subtype of MSS/*BRAF*–wild-type, MSS/*BRAF*-mutant, MSI-high/*BRAF*–mutant and MSI-high/*BRAF*–wild-type subtypes showed colorectal cancer-specific mortality hazard ratios of 1.60 (95% CI = 1.12 to 2.28; $P = .009$), 0.48 (95% CI = 0.27 to 0.87; $P = .02$), and 0.25 (95% CI = 0.12 to 0.52; $P < .001$), respectively. We found no evidence of interaction between MSI and *BRAF* status in survival models (all $P_{interaction} > .50$).

Tumor molecular classification has become crucial for clinical, translational, and epidemiologic research (42–49) because of uniqueness of each tumor and the continuum of colorectal biogeography influencing tumor characteristics (50–52). Despite their frequent coexistence as a result of their associations with high-level CIMP (CIMP-high) (53–58), we found MSI-high and *BRAF* mutation in colorectal cancer to have divergent associations with patient survival. Our findings are compatible with previous



Year	0	2	4	6	8	10
MSI-high/*BRAF*–wild-type	92	85	76	68	56	43
MSI-high/*BRAF*–mutant	101	87	82	65	49	31
MSS/*BRAF*–wild-type	979	815	704	578	494	385
MSS/*BRAF*–mutant	81	47	40	37	32	29

Figure 1. Kaplan–Meier survival plots for colorectal cancer according to combined MSI/*BRAF* subgroup. **A**) Colorectal cancer–specific survival. **B**) Overall survival. Multi-group log-rank *P* values demonstrate statistically significant deviation of any one of the survival curves from the null hypothesis. MSI = microsatellite instability; MSS = microsatellite stable.

Table 1. Colorectal cancer–specific and overall mortality according to combined microsatellite instability (MSI)/*BRAF* subgroup*

		Colorectal cancer–specific mortality					Overall mortality				
Subgroup	**No. of cases (%)**	**No. of events**	**Univariate HR (95% CI)**	***P***	**Multivariable HR (95% CI)**	***P***	**No. of events**	**Univariate HR (95% CI)**	***P***	**Multivariable HR (95% CI)**	***P***
MSS/*BRAF*–wild-type	979 (78)	299	1 (referent)		1 (referent)		485	1 (referent)		1 (referent)	
MSS/*BRAF*–mutant	81 (6.5)	40	2.10 (1.51 to 2.93)	<.001	1.60 (1.12 to 2.28)	.009	49	1.53 (1.14 to 2.06)	.005	1.36 (1.00 to 1.84)	.052
MSI-high/*BRAF*–mutant	101 (8.1)	14	0.44 (0.26 to 0.75)	.003	0.48 (0.27 to 0.87)	.02	42	0.86 (0.63 to 1.18)	.36	0.84 (0.59 to 1.19)	.32
MSI-high *BRAF*–wild-type	92 (7.3)	8	0.26 (0.13 to 0.52)	<.001	0.25 (0.12 to 0.52)	<.001	32	0.63 (0.44 to 0.90)	.01	0.58 (0.40 to 0.85)	.005
$P_{interaction}$ between MSI and *BRAF*			.67		.72			.70		.83	

* The multivariable Cox regression models were stage-stratified. In addition to MSI/*BRAF* subgroup, covariables initially included: age at diagnosis (continuous), sex, year of diagnosis (continuous), body mass index (≥30 vs <30 kg/m^2), tumor location (proximal vs distal colorectum), tumor differentiation (poor vs well-moderately differentiated), family history of colorectal cancer in any first degree relative (present vs absent), CIMP status (CIMP-high vs CIMP-low/0), LINE-1 methylation (continuous), and *KRAS* and *PIK3CA* mutations (present vs absent). A backward elimination with threshold of *P* equal to .10 was used to select covariables. Age, year of diagnosis, body mass index, tumor differentiation, and LINE-1 methylation remained in the colorectal cancer–specific survival model. The same covariables, with the exception of LINE-1 methylation, remained in the overall survival model. CI = confidence interval; HR = hazard ratio; MSS = microsatellite stable.

studies that have found MSI-high to be associated with favorable outcome (2–8,15,17) and *BRAF* mutation to be associated with poor survival (16–28) [except for (59)]. MSI status is an established prognostic biomarker and is associated with host–tumor immune response (60–65).

Concordant with several other studies (16–20,66,67) [except for (15)], MSS/*BRAF*-mutant tumors were associated with the highest mortality. Patients with MSI-high/*BRAF*–wild-type tumors experienced the lowest mortality, consistent with a number of previous reports (15–20,67). Although we found MSI-high/*BRAF*-mutant tumors to be associated with favorable prognosis (vs MSS/*BRAF*–wild-type), confirmation in other populations is required.

Although some studies (17–19,68) suggest that the adverse prognostic association of *BRAF* mutation is limited to MSS tumors, other studies (15,16) and our analysis suggest that *BRAF* mutation remains prognostic among MSI-high cancers. We found no evidence for a differential prognostic role of *BRAF* mutation according to MSI status, consistent with a large population-based study (18). Taking into account existing literature, our data justify stratifying patients into poor (MSS/*BRAF*-mutant), intermediate (MSS/*BRAF*–wild-type), and favorable (MSI-high/*BRAF*–wild-type) prognostic groups (Supplementary Figure 4, available online).

Limitations of our study include its observational nature and lack of treatment data, and thus unknown bias, including differential treatment assignment, might confound results. Nevertheless, our regression analyses were adjusted for disease stage, on which treatment decisions are largely based, and our findings are consistent with data from independent clinical trials of colon cancer patients (15,16).

Strengths of our study include use of a molecular pathological epidemiology (69–79) database containing more than 1200 colorectal cancer cases characterized for key tumor molecular features. MSI-high and *BRAF*-mutant tumors represent a minority of colorectal cancers. The size and comprehensiveness of this population-based, molecular pathological epidemiology database enabled us to estimate an effect size for each tumor subtype while controlling for multiple potential confounders, including disease stage, age at diagnosis, body mass index, tumor differentiation, and tumor LINE-1 methylation level.

In conclusion, our data support a prognostic role for combined MSI/*BRAF* testing in colorectal cancer. Future studies should examine the predictive role of MSI/*BRAF* classification for response to therapeutic and lifestyle interventions.

References

1. Vilar E, Tabernero J. Molecular dissection of microsatellite instable colorectal cancer. *Cancer Discov*. 2013;3(5):502–511.
2. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol*. 2005;23(3):609–618.
3. Roth AD, Delorenzi M, Tejpar S, et al. Integrated analysis of molecular and clinical prognostic factors in stage II/III colon cancer. *J Natl Cancer Inst*. 2012;104(21):1635–1646.
4. Sinicrope FA, Foster NR, Yoon HH, et al. Association of obesity with DNA mismatch repair status and clinical outcome in patients with stage II or III colon carcinoma participating in NCCTG and NSABP adjuvant chemotherapy trials. *J Clin Oncol*. 2012;30(4):406–412.
5. Bertagnolli MM, Redston M, Compton CC, et al. Microsatellite instability and loss of heterozygosity at chromosomal location 18q: prospective evaluation of biomarkers for stages II and III colon cancer—a study of CALGB 9581 and 89803. *J Clin Oncol*. 2011;29(23):3153–3162.
6. Kim GP, Colangelo LH, Wieand HS, et al. Prognostic and predictive roles of high-degree microsatellite instability in colon cancer: a National Cancer Institute-National Surgical Adjuvant Breast and Bowel Project Collaborative Study. *J Clin Oncol*. 2007;25(7):767–772.
7. 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.
8. Sinicrope FA, Foster NR, Thibodeau SN, 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(11):863–875.
9. 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(15):2788–2798.
10. Lao VV, Grady WM. Epigenetics and colorectal cancer. *Nat Rev Gastroenterol Hepatol*. 2011;8(12):686–700.
11. Goel A, Boland CR. Epigenetics of colorectal cancer. *Gastroenterology*. 2012;143(6):1442–1460 e1441.
12. Wong JJ, Hawkins NJ, Ward RL, Hitchins MP. Methylation of the 3p22 region encompassing MLH1 is representative of the CpG island methylator phenotype in colorectal cancer. *Mod Pathol*. 2011;24(3):396–411.
13. Ogino S, Goel A. Molecular classification and correlates in colorectal cancer. *J Mol Diagn*. 2008;10(1):13–27.
14. Weisenberger DJ, Siegmund KD, Campan M, et al. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with *BRAF* mutation in colorectal cancer. *Nat Genet*. 2006;38(7):787–793.
15. French AJ, Sargent DJ, Burgart LJ, et al. Prognostic significance of defective mismatch repair and *BRAF* V600E in patients with colon cancer. *Clin Cancer Res*. 2008;14(11):3408–3415.
16. Ogino S, Shima K, Meyerhardt JA, et al. Predictive and prognostic roles of *BRAF* mutation in stage III colon cancer: results from intergroup trial CALGB 89803. *Clin Cancer Res*. 2012;18(3):890–900.
17. Samowitz WS, Sweeney C, Herrick J, et al. Poor survival associated with the *BRAF* V600E mutation in microsatellite-stable colon cancers. *Cancer Res*. 2005;65(14):6063–6069.
18. Phipps AI, Buchanan DD, Makar KW, et al. *BRAF* mutation status and survival after colorectal cancer diagnosis according to patient and tumor characteristics. *Cancer Epidemiol Biomarkers Prev*. 2012;21(10):1792–1798.
19. Roth AD, Tejpar S, Delorenzi M, 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(3):466–474.
20. Zlobec I, Kovac M, Erzberger P, et al. Combined analysis of specific *KRAS* mutation, *BRAF* and microsatellite instability identifies prognostic subgroups of sporadic and hereditary colorectal cancer. *Int J Cancer*. 2010;127(11):2569–2575.
21. Hutchins G, Southward K, Handley K, 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(10):1261–1270.
22. Ogino S, Nosho K, Kirkner GJ, et al. CpG island methylator phenotype, microsatellite instability, *BRAF* mutation and clinical outcome in colon cancer. *Gut*. 2009;58(1):90–96.
23. Popovici V, Budinska E, Tejpar S, et al. Identification of a poor-prognosis *BRAF*-mutant-like population of patients with colon cancer. *J Clin Oncol*. 2012;30(12):1288–1295.
24. Richman SD, Seymour MT, Chambers P, et al. *KRAS* and *BRAF* mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: results from the MRC FOCUS trial. *J Clin Oncol*. 2009;27(35):5931–5937.
25. Farina-Sarasqueta A, van Lijnschoten G, Moerland E, et al. The *BRAF* V600E mutation is an independent prognostic factor for survival in stage II and stage III colon cancer patients. *Ann Oncol*. 2010;21(12):2396–2402.

26. Yokota T, Ura T, Shibata N, et al. *BRAF* mutation is a powerful prognostic factor in advanced and recurrent colorectal cancer. *Br J Cancer*. 2011;104(5):856–862.
27. Price TJ, Hardingham JE, Lee CK, et al. Impact of *KRAS* and *BRAF* gene mutation status on outcomes from the phase III AGITG MAX trial of capecitabine alone or in combination with bevacizumab and mitomycin in advanced colorectal cancer. *J Clin Oncol*. 2011;29(19):2675–2682.
28. Souglakos J, Philips J, Wang R, et al. Prognostic and predictive value of common mutations for treatment response and survival in patients with metastatic colorectal cancer. *Br J Cancer*. 2009;101(3):465–472.
29. Funkhouser WK, Lubin IM, Monzon FA, et al. Relevance, pathogenesis, and testing algorithm for mismatch repair-defective colorectal carcinomas: a report of the Association for Molecular Pathology. *J Mol Diagn*. 2012;14(2):91–103.
30. Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group. Recommendations from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives. *Genet Med*. 2009;11(1):35–41.
31. Febbo PG, Ladanyi M, Aldape KD, et al. NCCN Task Force report: evaluating the clinical utility of tumor markers in oncology. *J Natl Compr Canc Netw*. 2011;9(Suppl 5):S1–S32; quiz S33.
32. Chan AT, Ogino S, Fuchs CS. Aspirin and the risk of colorectal cancer in relation to the expression of COX-2. *N Engl J Med*. 2007;356(21):2131–2142.
33. Morikawa T, Kuchiba A, Yamauchi M, et al. Association of CTNNB1 (beta-catenin) alterations, body mass index, and physical activity with survival in patients with colorectal cancer. *JAMA*. 2011;305(16):1685–1694.
34. Liao X, Lochhead P, Nishihara R, et al. Aspirin use, tumor *PIK3CA* mutation status, and colorectal cancer survival. *N Engl J Med*. 2012;367(17):1596–1606.
35. Ogino S, Kawasaki T, Brahmandam M, et al. Sensitive sequencing method for *KRAS* mutation detection by pyrosequencing. *J Mol Diagn*. 2005;7(3):413–421.
36. Ogino S, Kawasaki T, Kirkner GJ, Loda M, Fuchs CS. CpG island methylator phenotype-low (CIMP-low) in colorectal cancer: possible associations with male sex and *KRAS* mutations. *J Mol Diagn*. 2006;8(5):582–588.
37. Liao X, Morikawa T, Lochhead P, et al. Prognostic role of *PIK3CA* mutation in colorectal cancer: cohort study and literature review. *Clin Cancer Res*. 2012;18(8):2257–2268.
38. Ogino S, Kawasaki T, Brahmandam M, et al. Precision and performance characteristics of bisulfite conversion and real-time PCR (MethyLight) for quantitative DNA methylation analysis. *J Mol Diagn*. 2006;8(2):209–217.
39. Nosho K, Irahara N, Shima K, et al. Comprehensive biostatistical analysis of CpG island methylator phenotype in colorectal cancer using a large population-based sample. *PloS One*. 2008;3(11):e3698.
40. Ogino S, Nosho K, Kirkner GJ, et al. A cohort study of tumoral LINE-1 hypomethylation and prognosis in colon cancer. *J Natl Cancer Inst*. 2008;100(23):1734–1738.
41. Irahara N, Nosho K, Baba Y, et al. Precision of pyrosequencing assay to measure LINE-1 methylation in colon cancer, normal colonic mucosa and peripheral blood cells. *J Mol Diagn*. 2010;12(2):177–183.
42. Wang D, Dubois RN. Associations between obesity and cancer: the role of fatty acid synthase. *J Natl Cancer Inst*. 2012;104(5):343–345.
43. Kuchiba A, Morikawa T, Yamauchi M, et al. Body mass index and risk of colorectal cancer according to fatty acid synthase expression in the nurses' health study. *J Natl Cancer Inst*. 2012;104(5):415–420.
44. Ballard-Barbash R, Friedenreich CM, Courneya KS, Siddiqi SM, McTiernan A, Alfano CM. Physical activity, biomarkers, and disease outcomes in cancer survivors: a systematic review. *J Natl Cancer Inst*. 2012;104(11):815–840.
45. Ogino S, Nishihara R, Lochhead P, et al. Prospective study of family history and colorectal cancer risk by tumor LINE-1 methylation level. *J Natl Cancer Inst*. 2013;105(2):130–140.
46. Buchanan DD, Win AK, Walsh MD, et al. Family history of colorectal cancer in *BRAF* p.V600E mutated colorectal cancer cases. *Cancer Epidemiol Biomarkers Prev*. 2013;22(5):917–926.
47. Rex DK, Ahnen DJ, Baron JA, et al. Serrated lesions of the colorectum: review and recommendations from an expert panel. *Am J Gastroenterol*. 2012;107(9):1315–1329.
48. Kelley RK, Wang G, Venook AP. Biomarker use in colorectal cancer therapy. *J Natl Compr Canc Netw*. 2011;9(11):1293–1302.
49. Razzak AA, Oxentenko AS, Vierkant RA, et al. Associations between intake of folate and related micronutrients with molecularly defined colorectal cancer risks in the Iowa Women's Health Study. *Nutr Cancer*. 2012;64(7):899–910.
50. Ogino S, Fuchs CS, Giovannucci E. How many molecular subtypes? Implications of the unique tumor principle in personalized medicine. *Expert Rev Mol Diagn*. 2012;12(6):621–628.
51. Yamauchi M, Lochhead P, Morikawa T, et al. Colorectal cancer: a tale of two sides or a continuum? *Gut*. 2012;61(6):794–797.
52. Yamauchi M, Morikawa T, Kuchiba A, et al. Assessment of colorectal cancer molecular features along bowel subsites challenges the conception of distinct dichotomy of proximal versus distal colorectum. *Gut*. 2012;61(6):847–854.
53. Hinoue T, Weisenberger DJ, Lange CP, et al. Genome-scale analysis of aberrant DNA methylation in colorectal cancer. *Genome Res*. 2012;22(2):271–282.
54. Zlobec I, Bihl M, Foerster A, Rufle A, Lugli A. Comprehensive analysis of CpG island methylator phenotype (CIMP)-high, -low, and -negative colorectal cancers based on protein marker expression and molecular features. *J Pathol*. 2011;225(3):336–343.
55. Beggs AD, Jones A, El-Bahwary M, Abulafi M, Hodgson SV, Tomlinson IP. Whole-genome methylation analysis of benign and malignant colorectal tumours. *J Pathol*. 2013;229(5):697–704.
56. Dahlin AM, Palmqvist R, Henriksson ML, et al. The role of the CpG island methylator phenotype in colorectal cancer prognosis depends on microsatellite instability screening status. *Clin Cancer Res*. 2010;16(6):1845–1855.
57. Bae JM, Kim JH, Kang GH. Epigenetic alterations in colorectal cancer: the CpG island methylator phenotype. *Histol Histopathol*. 2013;28(5):585–595.
58. Yang Q, Dong Y, Wu W, et al. Detection and differential diagnosis of colon cancer by a cumulative analysis of promoter methylation. *Nature Commun*. 2012;3:1206.
59. Barault L, Charon-Barra C, Jooste V, et al. Hypermethylator phenotype in sporadic colon cancer: study on a population-based series of 582 cases. *Cancer Res*. 2008;68(20):8541–8546.
60. Ogino S, Galon J, Fuchs CS, Dranoff G. Cancer immunology-analysis of host and tumor factors for personalized medicine. *Nature Rev Clin Oncol*. 2011;8(12):711–719.
61. Dahlin AM, Henriksson ML, Van Guelpen B, et al. Colorectal cancer prognosis depends on T-cell infiltration and molecular characteristics of the tumor. *Mod Pathol*. 2011;24(5):671–682.
62. Galon J, Franck P, Marincola FM, et al. Cancer classification using the immunoscore: a worldwide task force. *J Transl Med*. 2012;10(1):205.
63. Nosho K, Baba Y, Tanaka N, et al. Tumour-infiltrating T-cell subsets, molecular changes in colorectal cancer, and prognosis: cohort study and literature review. *J Pathol*. 2010;222(4):350–366.
64. Edin S, Wikberg ML, Dahlin AM, et al. The distribution of macrophages with a m1 or m2 phenotype in relation to prognosis and the molecular characteristics of colorectal cancer. *PloS One*. 2012;7(10):e47045.
65. Ogino S, Nosho K, Irahara N, et al. Lymphocytic reaction to colorectal cancer is associated with longer survival, independent of lymph node count, microsatellite instability, and CpG island methylator phenotype. *Clin Cancer Res*. 2009;15(20):6412–6420.
66. Gavin PG, Colangelo LH, Fumagalli D, et al. Mutation profiling and microsatellite instability in stage II and III colon cancer: an assessment of their prognostic and oxaliplatin predictive value. *Clin Cancer Res*. 2012;18(23):6531–6541.
67. Pai RK, Jayachandran P, Koong AC, et al. *BRAF*-mutated, microsatellite-stable adenocarcinoma of the proximal colon: an aggressive adenocarcinoma with poor survival, mucinous differentiation, and adverse morphologic features. *Am J Surg Pathol*. 2012;36(5):744–752.

68. Bond CE, Umapathy A, Buttenshaw RL, Wockner L, Leggett BA, Whitehall VL. Chromosomal instability in *BRAF* mutant, microsatellite stable colorectal cancers. *PloS One*. 2012;7(10):e47483.
69. Ogino S, Stampfer M. Lifestyle factors and microsatellite instability in colorectal cancer: the evolving field of molecular pathological epidemiology. *J Natl Cancer Inst*. 2010;102(6):365–367.
70. Ogino S, Chan AT, Fuchs CS, Giovannucci E. Molecular pathological epidemiology of colorectal neoplasia: an emerging transdisciplinary and interdisciplinary field. *Gut*. 2011;60(3):397–411.
71. Ogino S, Lochhead P, Chan AT, et al. Molecular pathological epidemiology of epigenetics: emerging integrative science to analyze environment, host, and disease. *Mod Pathol*. 2013;26(4):465–484.
72. Hughes LA, Khalid-de Bakker CA, Smits KM, et al. The CpG island methylator phenotype in colorectal cancer: progress and problems. *Biochim Biophys Acta*. 2012;1825(1):77–85.
73. Hughes LA, Williamson EJ, van Engeland M, et al. Body size and risk for colorectal cancers showing *BRAF* mutation or microsatellite instability: a pooled analysis. *Int J Epidemiol*. 2012;41(4):1060–1072.
74. Curtin K, Slattery ML, Samowitz WS. CpG island methylation in colorectal cancer: past, present and future. *Patholog Res Int*. 2011;2011:902674.
75. Gay LJ, Mitrou PN, Keen J, et al. Dietary, lifestyle and clinico-pathological factors associated with *APC* mutations and promoter methylation in colorectal cancers from the EPIC-Norfolk Study. *J Pathol*. 2012;228(3):405–415.
76. Rosty C, Young JP, Walsh MD, et al. Colorectal carcinomas with KRAS mutation are associated with distinctive morphological and molecular features. *Mod Pathol*. 2013;26(6):825–834.
77. Ku CS, Cooper DN, Wu M, et al. Gene discovery in familial cancer syndromes by exome sequencing: prospects for the elucidation of familial colorectal cancer type X. *Mod Pathol*. 2012;25(8):1055–1068.
78. Chia WK, Ali R, Toh HC. Aspirin as adjuvant therapy for colorectal cancer-reinterpreting paradigms. *Nat Rev Clin Oncol*. 2012;9(10):561–570.
79. Spitz MR, Caporaso NE, Sellers TA. Integrative cancer epidemiology—the next generation. *Cancer Discov*. 2012;2(12):1087–1090.

Funding

This work was supported by the US National Institutes of Health (P01 CA87969 to S.E. Hankinson; P01 CA55075 and UM1 CA167552 to W.C. Willett; P50 CA127003 to CSF; and R01 CA151993 to SO); by the Bennett Family Fund and the Entertainment Industry Foundation through the National Colorectal Cancer Research Alliance; by the Frank Knox Memorial Fellowship at Harvard University (to PL); by a fellowship from the Chief Scientist Office of the Scottish Government (to PL); and by a fellowship from the Japan Society for Promotion of Science (to TM).

Notes

P. Lochhead, A. Kuchiba, Y. Imamura, and X. Liao contributed equally. C.S. Fuchs and S. Ogino contributed equally.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

We would like to thank the participants and staff of the Nurses' Health Study and the Health Professionals Follow-up Study for their valuable contributions as well as the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY.

Affiliations of authors: Department of Medical Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA (PL, AK, YI, XL, MY, RN, ZRQ, TM, JAM, CSF, SO); Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK (PL); Department of Pathology (JS, SO) and Channing Division of Network Medicine, Department of Medicine (CSF), Brigham and Women's Hospital and Harvard Medical School, Boston, MA; Department of Epidemiology, Harvard School of Public Health, Boston, MA (SO).