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Frequent *PIK3CA* Mutations in Colorectal and Endometrial Cancer with Double Somatic Mismatch Repair Mutations

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Abstract

Background & Aims—Double somatic mutations in mismatch repair (MMR) genes have recently been described in colorectal and endometrial cancers with microsatellite instability (MSI)

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Conflict of Interest: William Grady is a patent holder on an assay for the detection of methylated *MLH1* (US 8,669,060).

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not attributable to *MLH1* hypermethylation or germline mutation. We sought to define the molecular phenotype of this newly recognized tumor subtype.

Methods—From two prospective Lynch syndrome screening studies, we identified patients with colorectal and endometrial tumors harboring 2 somatic MMR mutations, but normal germline MMR testing (“double somatic”). We determined the frequencies of tumor *PIK3CA*, *BRAF*, *KRAS*, *NRAS*, and *PTEN* mutations by targeted next-generation sequencing and used logistic-regression models to compare them to: Lynch syndrome, *MLH1* hypermethylated, and microsatellite stable (MSS) tumors. We validated our findings using independent datasets from The Cancer Genome Atlas (TCGA).

Results—Among colorectal cancer cases, we found that 14/21 (67%) of double somatic cases had *PIK3CA* mutations vs. 4/18 (22%) Lynch syndrome, 2/10 (20%) *MLH1* hypermethylated, and 12/78 (15%) MSS tumors; $p<0.0001$. *PIK3CA* mutations were detected in 100% of 13 double somatic endometrial cancers ($p=0.04$). *BRAF* mutations were absent in double somatic and Lynch syndrome colorectal tumors. We found highly similar results in a validation cohort from TCGA (113 colorectal, 178 endometrial cancer), with 100% of double somatic cases harboring a *PIK3CA* mutation ($p<0.0001$).

Conclusions—*PIK3CA* mutations are present in double somatic mutated colorectal and endometrial cancers at substantially higher frequencies than other MSI subgroups. *PIK3CA* mutation status may better define an emerging molecular entity in colorectal and endometrial cancers, with the potential to inform screening and therapeutic decision making.

Keywords

MSI; MMR; Lynch-like syndrome; PI3K; *PIK3CA*

INTRODUCTION

“Double somatic” colorectal and endometrial cancer is a newly termed entity, describing cases with at least two somatic alterations in the mismatch repair (MMR) genes, which result in a molecular phenotype that mimics Lynch syndrome cancers.¹ Lynch syndrome is due to germline mutation(s) in one of the MMR genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*) and/or *EPCAM*, conferring an increased lifetime risk of colorectal, endometrial, stomach, ovarian, urothelial, and other cancers.^{2, 3} In contrast, double somatic cases have no detectable germline mutation in the MMR genes.

Tumor-based screening for Lynch syndrome has improved sensitivity and specificity compared to previous clinical methods, which focused only on personal and family history features.^{4, 5} The two commonly employed methods are (a) PCR-based microsatellite instability (MSI) testing to identify variation in genomic repeats and (b) immunohistochemistry (IHC) for loss of expression of one or more of the MMR proteins. Either tumor screening test requires confirmation with germline genetic testing of the MMR gene(s) to make the diagnosis of Lynch syndrome.

Most somatic MSI cases are explained by hypermethylation of the *MLH1* promoter and epigenetic silencing of *MLH1*.^{3, 6} *MLH1* hypermethylation is, therefore, generally examined

and excluded prior to recommending germline genetic testing based on abnormal tumor screening. The remainder of cases with abnormal tumor screening that cannot be explained by either germline mutation or *MLH1* hypermethylation have been described as “Lynch-like syndrome.”^{7–11} Some reports suggest that Lynch-like syndrome confers an intermediate risk of cancer between that of Lynch syndrome and people with a personal history of sporadic MSI colon cancer (secondary to *MLH1* hypermethylation).

We, and others, have recently reported that somatic mutations in the MMR genes can also result in the MSI phenotype on tumor screening.^{1, 12–14} These cases have been referred to as double somatic MMR-mutated colorectal/endometrial cancer. Extensive germline and tumor testing is required to more definitively assess if a cancer is due to Lynch syndrome, *MLH1* hypermethylation, or double somatic MMR mutations. The three molecular classes of MMR defects have distinctive practical implications for a patient’s short- and long-term care, future cancer risks, and cancer risk for a patient’s relatives. Somatic MMR gene testing of patients with unexplained abnormal tumor screening has recently been included in the latest NCCN guidelines for high-risk colorectal cancer,¹⁵ supporting the growing recognition of this important entity. Recognition of additional features of double somatic tumors could increase suspicion of this entity and support more comprehensive genetic testing. Molecular characterization may also better differentiate Lynch-like syndrome, a clinically and molecularly defined group that likely includes both Lynch syndrome cases (with a germline mutation not identified by current mutation analysis techniques) and double somatic MMR mutation cases, thus explaining the intermediate cancer risk seen in this group.^{7, 9} Improved understanding of MSI subgroups also has potential therapeutic implications, with MSI serving as a key biomarker in emerging therapies, including programmed-death-receptor-1 (PD-1) targeted immune therapy.¹⁶

In this analysis, we compare the molecular features of double somatic, Lynch syndrome, *MLH1* hypermethylated, and microsatellite stable (MSS) colorectal and endometrial cancers. We focused on mutations in five key genes in the epidermal growth factor receptor (EGFR) and phosphoinositide-3-kinase (PI3K) pathway (*KRAS*, *NRAS*, *BRAF*, *PIK3CA*, and *PTEN*) and compared mutation prevalence among MSI subgroups. We demonstrate that *PIK3CA* mutation prevalence varies significantly by group, with important implications for screening, treatment, and surveillance of double somatic colorectal and endometrial cancers.

MATERIALS AND METHODS

Patient selection

As previously described,¹ the Columbus-area HNPCC study and the Ohio Colorectal Cancer Prevention Initiative are two large population-based studies enrolling unselected consecutive newly diagnosed colorectal and endometrial cancer patients. As part of these studies enrollment, patients underwent Lynch syndrome tumor screening with MSI and MMR IHC, followed by *MLH1* hypermethylation and germline MMR gene testing, as indicated. Both studies identified a group of patients with MMR-deficient tumors without *MLH1* promoter hypermethylation or identified germline MMR gene mutation. These unexplained cases, for which relevant tissue was available, underwent additional tumor genetic testing at the University of Washington (UW).

In addition, a consecutive sample of patients fitting Lynch syndrome, *MLH1* hypermethylation, or MSS tumors (criteria below) were selected for additional tumor genetic testing at UW. Colorectal and endometrial cancer cases were selected from consecutive cases (unselected for age and family history) that were MSS by clinical MSI-PCR testing or had fewer than 20% unstable loci by mSINGS (MSI by next generation sequencing) analysis.¹⁷ Clinical specimens and data were obtained in accordance with the declaration of Helsinki and the ethics guidelines of Human Subjects Division of UW and The Ohio State University (OSU) and with the approval of the local Institutional Review Board (approval numbers: 2012C0123, 1999C0051). Tumor characteristics are summarized in Table 1.

Molecular Testing

UW-OncoPlex was performed on all tumor specimens to identify variations in molecular features.¹⁸ The UW-OncoPlex panel evaluates 194 genes relevant to carcinogenesis in colorectal, endometrial, and other cancers. This analysis focused on mutations in the *KRAS*, *NRAS*, *BRAF*, *PIK3CA* and *PTEN* genes. For *KRAS*, *NRAS*, and *BRAF*, only activating mutations were evaluated.

As previously described for UW-OncoPlex, a shotgun library was generated from 1–3µg of genomic DNA from formalin-fixed paraffin embedded tissue.¹⁹ The library was enriched via hybridization to a custom 950kb Agilent SureSelect XT capture set using the manufacturer's protocol. Following enrichment, the libraries were amplified with barcoded PCR primers, pooled, and sequenced on an Illumina HiSeq with paired 100bp reads. Each library was sequenced to a minimum of 200× average read depth. Sequence data were aligned to the human reference (hg19/GRCh37) with bwa v0.6.1 and samtools v0.1.18. Single nucleotide and small indel variants were called by GATK v2.4 and VarScan2 v2.3.7 and annotated by Annovar. Total mutation burden analysis was performed as previously described.¹

Clinical molecular testing results are summarized in Supplemental Table S1 (online supplement only). For samples without clinical MSI-PCR results, the MSI status of each tumor sample was computationally assessed from the UW-OncoPlex data using the mSINGS algorithm, which detects the fraction of unstable microsatellite loci in next-generation sequence data.¹⁷ Tumors with fewer than 20% unstable loci are designated as MSS; tumors with 20% or greater unstable loci are designated as MSI-H.

Mismatch repair classification

The 174 cases were classified based on their somatic and germline testing. MMR mutations were classified according to American College of Medical Genetics and Genomics (ACMG) standards and guidelines.²⁰ Fifteen cases were excluded due to incomplete data, such as lack of sufficient tumor and/or germline DNA, to be appropriately classified. Additionally, one case was excluded which met criteria for both Lynch syndrome (germline *PMS2* mutation) and double somatic (two somatic *MSH6* mutations).

The remaining 158 cases were categorized into one of four groups. Cases with MSI and/or abnormal MMR IHC were further categorized based on additional testing. (A) Cases with no identified MMR germline mutation, but with two or more somatic pathogenic or likely pathogenic MMR mutations were classified as “double somatic.”¹ (B) Those that had an

identified germline mutation in one of the MMR genes were classified as “Lynch syndrome.” (C) Cases with MSI and/or absence of MLH1 expression based on IHC, but with somatic hypermethylation of the *MLH1* promoter were classified as “*MLH1* hypermethylated.” (D) Cases with normal microsatellite testing and MMR IHC were classified as “MSS.” In total, 34 double somatic, 21 Lynch syndrome, 13 *MLH1* hypermethylated, and 90 MSS cases were included in this analysis.

The Cancer Genome Atlas (TCGA) validation dataset

From the TCGA Colon Adenocarcinoma (COAD) and Uterine Corpus Endometrial Carcinoma (UCEC) projects, we selected consecutive colorectal (n=113) and endometrial (n=178) cancer cases with available microsatellite instability testing data, *MLH1* hypermethylation data, and adequate exome sequencing results on both tumor and germline matched samples. Using the UW-OncoPlex pipeline, we performed variant calling for single nucleotide variants and small indels, then applied a targeted gene-set analysis of *KRAS*, *NRAS*, *BRAF*, *PIK3CA*, *PTEN*, *MLH1*, *MSH2*, *MSH6*, and *PMS2*. According to the criteria described above, we categorized these cases into double somatic (n=8), Lynch syndrome (n=8), *MLH1* hypermethylated (n=73), and MSS (n=202).

Statistical Methods

Colorectal and endometrial cancer cases were categorized in one of four MSI categories: double somatic, Lynch syndrome, *MLH1* hypermethylated, and MSS. The mutation frequencies of each of five molecular features (*PIK3CA*, *KRAS*, *NRAS*, *BRAF*, and *PTEN*) were compared between case groups using the chi-square test (or Fisher’s exact test if the expected number under the null hypothesis of equal frequencies in at least one cell was less than 5). Each of the mutation frequencies were compared among colorectal cancer cases and among endometrial cancer cases. The pattern of molecular features between *PIK3CA*-mutated and *PIK3CA*-wildtype cases was compared descriptively. Similar analysis was performed for the TCGA validation set, using the Fisher’s exact test for comparison of mutation frequencies.

RESULTS

We defined 158 individuals into double somatic, Lynch syndrome, *MLH1* hypermethylated, and MSS colorectal and endometrial cancer. Tumor characteristics of these individuals are summarized in Table 1 and listed individually in Table S1. Double somatic colorectal cancer (19/21 [90%]) and *MLH1* hypermethylated colorectal cancer (9/10 [90%]) were predominantly right-sided, even in comparison to Lynch syndrome cases (11/18 [61%]). All MSI-high colorectal cancers were more right-predominant than MSS cases (24/90 [31%]; $p<0.0001$). The majority of endometrial cancer cases (81%) were endometrioid histology in all subgroups. The family histories for the double somatic tumor cases did not suggest a known cancer predisposition gene to explain these cases (Supplemental Table S2)

We determined the mutation prevalence of *PIK3CA*, *BRAF*, *KRAS*, *NRAS*, and *PTEN* in the four MSI-defined subgroups within each colorectal and endometrial cancer (Figure 1). Recognizing known differences in the molecular profile of colorectal and endometrial

cancer, we analyzed the cases by tumor type (Table 2). All 13 double somatic endometrial cancers harbored a *PIK3CA* mutation, and 67% (14/21) of double somatic colorectal cancers had *PIK3CA* mutations. The frequency of *PIK3CA* mutations was significantly more common in double somatic colorectal cancers compared to the other tumor types, $p<0.0001$. The distribution of *PIK3CA* mutations is shown in Figure 2. The detected *PIK3CA* mutations were largely well-characterized activating somatic hotspot mutations (see Supplemental Tables S3 and S4). We detected *PTEN* mutations in 84% of endometrial cancers but only 12% of colorectal cancers. *PTEN* mutations were least frequent in MSS colorectal cancers ($p=0.01$). The majority of *MLH1* hypermethylated cases (62%) had an activating mutation in *BRAF*, all of which were p.V600E, as has been previously described.²¹ Only one double somatic tumor had a *BRAF* mutation, an endometrial cancer that harbored a *BRAF*p.D594G mutation (Tables 2 and 3). Activating mutations in *KRAS* and *NRAS* did not vary significantly between groups. *PTEN* mutations were detected in 15 (12%) of colorectal cases and 26 (84%) of endometrial cancer cases. The highest frequency was seen in *MLH1* hypermethylated cases (30% colorectal, 100% endometrial), but the difference between case groups was only significant for colorectal cancer ($p=0.01$). *PTEN* and *PIK3CA* mutations overlapped frequently in endometrial cancer cases, but were largely mutually exclusive in colorectal cancers (Figure 1, Supplementary Tables S5 and S6).

An analysis of the publically available TCGA data served to validate the findings made in our cohorts. Among all TCGA cases (Table 3), there was a statistically significant higher proportion of *PIK3CA* mutations among double somatic cases, as compared to Lynch syndrome, *MLH1* hypermethylated, and MSS tumors with a detected *PIK3CA* mutation in 100% of double somatic tumors ($p=0.007$). When evaluating molecular mutation frequency in TCGA within each colorectal and endometrial cancer (Table 3), the highest frequency of *PIK3CA* mutations was observed in double somatic cases ($p=0.09$ colorectal, $p=0.13$ endometrial cancer). There was a statistically significantly higher frequency of activating *BRAF* mutations in *MLH1* hypermethylated colorectal TCGA cases (71%, $p<0.0001$). All double somatic colorectal and endometrial TCGA cases had a *PTEN* mutation, which was significantly different from other MSI subgroups ($p<0.0001$).

DISCUSSION

In this study we provide the first detailed analysis of molecular features of double somatic mismatch-repair-deficient colorectal and endometrial cancers. We found a far higher proportion of *PIK3CA* mutations among double somatic cases compared to Lynch syndrome, *MLH1* hypermethylated, and MSS tumors. This surprising result is unlikely to be attributable to MSI and/or hypermutation alone as the total coding mutation burden was not significantly different from Lynch syndrome or *MLH1* hypermethylated tumors, which are also MSI-high (Supplementary Figure S1). This has important implications for future molecular categorization of this emerging molecular entity. In addition, it could be useful for clinical treatment planning of individuals with double somatic tumors, whose cancer risks are currently being characterized. Future work is needed to characterize the biological mechanism underlying the high prevalence of *PIK3CA* mutations in double somatic cases.

Previous studies have addressed *PIK3CA* mutation prevalence in colorectal and endometrial cancer, including by MSI status, but never before by sub-category of MSI.^{22–25} Previous literature indicates that ~41–71% of endometrial cancers and 14–32% of colorectal cancers have a mutation in any exon of *PIK3CA*.^{26–29} A modestly higher frequency of *PIK3CA* mutations has been reported in MSI colorectal cancers compared to MSS cancers, without differentiation by etiology of MSI.²⁵ We found substantial variability among MSI tumor subtypes: all of the endometrial double somatic cases and about two-thirds of colorectal double somatic cases had a mutation in *PIK3CA*, while Lynch syndrome tumors and those with *MLH1* hypermethylation had much lower *PIK3CA* mutation frequencies. Due to the higher prevalence of *PIK3CA* mutations among all MSI subgroups of endometrial cancer, a mutation in *PIK3CA* appears to be less helpful for classifying deficient MMR cases, such as ruling out Lynch syndrome and/or better understanding whether a detected MMR germline variant of unknown significance might be pathogenic. However, our findings suggest that the very high mutation frequency in the double somatic MSI subgroup may be driving the slightly higher *PIK3CA* mutation frequency previously observed in MSI-high colorectal cancer, which may be relevant to clinical decision-making.

In determining etiology of MSI, screening of the *BRAF* gene is often performed to rule out Lynch syndrome.⁵ In this analysis, *BRAF* mutations were observed only in MSS and *MLH1* hypermethylated colorectal cancers. Thus, the presence of a *BRAF* mutation argues against *MLH1* hypermethylation as the cause of detected MSI, but may suggest either somatic (double somatic) or germline (Lynch syndrome) mutations in the MMR genes. In contrast, in endometrial cancer, *BRAF* mutations are rare (and rarely V600, as in our case), making this an unhelpful marker for differentiating the etiology of MSI for endometrial cancer.³⁰

We sought to validate our finding within independent cases publicly available from the TCGA. This dataset contained a similar number of colorectal cancer cases, but far greater endometrial cancer cases, which explains some of the variability between the overall molecular frequency variability (such as *PTEN* mutations) between the two datasets. Our finding that *PIK3CA* mutations were more prevalent in double somatic cases was confirmed, though this only bordered on statistical significance by individual cancer type (colorectal or endometrial) due to small numbers. However, it is notable that only 2/113 (2%) of colorectal cancer cases and 6/178 (3%) of endometrial cancer cases had double somatic MMR mutations, highlighting the strength of the association, as well as the strength of the numbers in our exploratory dataset.

Although early reports suggested a preponderance of *PIK3CA* mutations in exons 9 and 20, this association was less striking in our cohort.²⁸ We speculate that restricted hotspot analysis may explain the lower overall *PIK3CA* mutation prevalence and stronger predilection for mutation exon 9 and 20 reported in the literature. We cannot rule out that some of the observed mutations are due to mutation burden from a hypermutated phenotype associated with MSI. However, because we are comparing double somatic cases to other MSI-high tumors, the much higher frequency of *PIK3CA* mutations in double somatic cases is unlikely to be explained by hypermutation.³¹

The recognition of *PIK3CA* as a component of an emerging molecular phenotype may also have relevance for cancer treatment. In retrospective studies, patients with colorectal cancers that have *PIK3CA* exon 20 (kinase domain) mutations have worse outcomes with the anti-EGFR therapy cetuximab compared to patients with *PIK3CA*-wildtype colorectal cancers.^{32, 33} PI3K pathway inhibitors have largely failed to show significant clinical benefit in colorectal cancer, even with the relatively high frequency of mutations in the PI3K signaling pathway.^{34, 35} Further study may recommend PI3K inhibitors in combination with other targeted therapies or for specific molecular phenotypes, such as double somatic tumors, though this has not been tested.^{36, 37} Most recently, benefit from programmed-death-receptor-1 (PD-1) targeted immune therapy has been suggested to vary by MSI subgroups, with somatic MSI cases deriving greater benefit than germline MSI (Lynch syndrome), though double somatic cases were not specifically examined.¹⁶ Lastly, the occurrence of *PIK3CA* mutations in double somatic colorectal/endometrial cancers may also highlight relevant secondary prevention options, such as the use of aspirin.^{38, 39} Notably, 17% of participants in the CAPP2 trial, which demonstrated benefit in individuals with Lynch syndrome randomized to daily aspirin or placebo for primary and secondary prevention of colorectal cancer, were clinically diagnosed with Lynch syndrome and may actually be cases of double somatic colorectal cancer.^{40, 41} Thus, while the use of aspirin for secondary prevention of cancer has inherent risks, focusing on double somatic cases in future trials might be considered in future prospective trials to evaluate whether aspirin is an effective prevention tool for these patients. This collectively highlights the need to better recognize key molecular alterations and pathways that are relevant to clinical care, as well as the emerging utility in recognizing tumors with mutations in *PIK3CA*.

A strength of this study is the well-characterized colorectal and endometrial cancer patients with full somatic and germline characterization of MMR genes by NGS. We used a selection of Lynch syndrome, *MLH1* hypermethylated, and MSS cases for comparison with a large number of double somatic MMR cases. Unlike many prior reports,^{22, 24, 42} our analysis was not restricted to certain exons or codons. Even with the relatively small number of cases in this study, we observed multiple significant differences between groups. Some modest associations we observed may be attributable to as yet unknown co-variables. *PIK3CA* mutations, however, seem to be strongly associated with double somatic MMR tumors across colorectal and endometrial cancer, with mutation frequencies substantially higher than literature estimates for both unselected cancer and for MSI-high cancers not analyzed by subgroup.^{26–29} Our findings need to be validated with future, larger studies. In addition, follow-up information from the patients in this study will allow characterization of relevant clinical differences between groups and an assessment of how these molecular features inform clinical outcomes.

In conclusion, we found mutations in *PIK3CA* at significantly higher frequency in double somatic mismatch repair deficient tumors, including 100% of double somatic endometrial cancer cases. In the era of improved definition of cancers by their molecular subtypes, the identification of differences in the frequency of *PIK3CA* mutations among the subtypes has important implications in both tumor biology and the characterization of clinically relevant molecular alterations in these cancers.¹⁵ Our analysis suggests that an individual with an MSI-high or MMR IHC abnormal colorectal cancer, lack of *MLH1* hypermethylation, and

no germline Lynch mutation should be considered for evaluation for double somatic colorectal cancer. In this situation, a mutation in *PIK3CA* has a 67% sensitivity and 83% specificity for identifying the double somatic MMR sub-type of colorectal cancer. The presence of a *BRAF* mutation in a colorectal cancer appears to argue against MSI due to a somatic or germline defect in the MMR genes. In addition, *PIK3CA* may be useful in interpreting the clinical impact of MMR variant of unknown significance since mutations in this gene are less frequent among known Lynch syndrome tumors. Future studies should focus on whether double somatic cases should be treated clinically as sporadic colorectal/endometrial cancer or if more intensive surveillance is required.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Distribution of somatic mutations in colorectal and endometrial cancer cases
 The somatic molecular profile for each tumor is displayed by case sample, with each row representing the profiling of an individual case. Darkly shaded boxes indicate the presence of a mutation in *PIK3CA*, *BRAF*, *KRAS*, *NRAS*, or *PTEN*. Only activating mutations are included for *BRAF*, *KRAS*, and *NRAS*. Colorectal cancers are in blue and endometrial cancers are in yellow.

Figure 2. Mutations in *PIK3CA* classified by MSI status in colorectal cancer and endometrial cancer cases

Mutations were plotted along the *PIK3CA* protein using the cBioPortal visualization engine^{43, 44} and shaded by category.

Table 1

Tumor characteristics

Tumor characteristic, n (%)		All cases (n=158)	Double somatic (n=34)	Lynch syndrome (n=21)	MLH1 methyl (n=13)	Microsatellite stable (n=90)	p-value ^d
Tumor type	Colorectal	127 (80%)	21 (62%)	18 (86%)	10 (77%)	78 (87%)	0.02
	Endometrial	31 (20%)	13 (38%)	3 (14%)	3 (23%)	12 (13%)	
Colorectal site	Left	50 (39%) ^{bc}	2 (10%)	8 (44%) ^b	1 (10%)	39 (50%) ^c	<0.0001
	Right	63 (50%) ^{bc}	19 (90%)	11 (61%) ^b	9 (90%)	24 (31%) ^c	
Endometrial histology	Endometrioid	25 (81%)	8 (62%)	2 (67%)	3 (100%)	12 (100%)	0.06
	Non-endometrioid	6 (19%)	5 (38%)	1 (33%)	0	0	

MLH1 methyl = MLH1 hypermethylation; SD = standard deviation

^a p-value from chi-square test (colorectal site) or Fisher's exact test (tumor type, endometrial histology)

^b One Lynch syndrome case had two synchronous primaries (one on the left and one on the right), which is why the numbers do not add up to 100%.

^c For 15 microsatellite stable cases, the primary site is unknown.

Table 2

Molecular features in MSI-classified cases by cancer type.

	Somatic gene mutation ^a	All cases n=127	Double somatic n=21	Lynch syndrome n=18	<i>MLH1</i> Methyl n=10	Microsatellite stable n=78	p-value ^c
Colorectal cancer (n=127)	<i>PIK3CA</i>	32 (25%)	14 (67%)	4 (22%)	2 (20%)	12 (15%)	<0.0001
	<i>BRAF</i>	13 (10%)	0	0	8 (80%)	5 (6%)	<0.0001
	<i>KRAS</i>	45 (35%)	8 (38%)	7 (39%)	1 (10%)	29 (37%)	0.12
	<i>NRAS</i>	7 (5%)	0	1 (6%)	0	6 (7%)	0.58
	<i>PTEN</i>	15 (12%)	4 (19%)	4 (24%)	3 (30%)	4 (5%)	0.01
Endometrial cancer (n=31)		n=31	n=13	n=3	n=3	n=12	
	<i>PIK3CA</i>	22 (71%)	13 (100%)	2 (67%)	2 (67%)	5 (42%)	0.004
	<i>BRAF</i>	1 (3%) ^b	1 (8%) ^b	0	0	0	1.0
	<i>KRAS</i>	6 (27%)	4 (31%)	0	0	2 (17%)	0.62
	<i>NRAS</i>	1 (3%)	1 (8%)	0	0	0	1.0
	<i>PTEN</i>	26 (84%)	11 (85%)	2 (67%)	3 (100%)	10 (83%)	0.79

MLH1 methyl = *MLH1* hypermethylation^aOnly activating mutations in *BRAF*, *KRAS*, and *NRAS* are included in these analyses.^bThe single double somatic case with a *BRAF* mutation had the mutation p.D594G.^cp-value from chi-square test for *KRAS* in colorectal cancer; all others from Fisher's exact test

Table 3
Molecular features in The Cancer Genome Atlas colorectal and endometrial cancer cases by MSI subgroup.

	Somatic gene mutation ^a	All cases n=291	Double somatic n=8	Lynch syndrome n=8	MLH1 Methyl n=73	Microsatellite stable n=202	p-value ^b
All cases (n=291)	PIK3CA	135 (46%)	8 (100%)	4 (50%)	37 (51%)	86 (43%)	0.007
	BRAF	15 (5%)	0	0	13 (18%)	2 (1%)	<0.0001
	KRAS	79 (27%)	2 (25%)	4 (50%)	24 (33%)	79 (39%)	0.22
	NRAS	10 (3%)	0	0	3 (4%)	7 (3%)	0.85
	PTEN	147 (51%)	8 (100%)	4 (50%)	56 (77%)	79 (39%)	<0.0001
Colorectal cancer (n=113)		n=113	n=2	n=4	n=17	n=90	
	PIK3CA	34 (30%)	2 (100%)	1 (25%)	7 (41%)	24 (27%)	0.09
	BRAF	14 (12%)	0	0	12 (71%)	2 (2%)	<0.0001
	KRAS	43 (38%)	1 (50%)	3 (75%)	3 (18%)	36 (40%)	0.08
	NRAS	6 (5%)	0	0	0	6 (7%)	0.70
Endometrial cancer (n=178)	PTEN	12 (11%)	2 (100%)	1 (25%)	3 (18%)	6 (7%)	0.004
		n=178	n=6	n=4	n=56	n=112	
	PIK3CA	101 (57%)	6 (100%)	3 (75%)	30 (54%)	62 (55%)	0.13
	BRAF	1 (1%)	0	0	1 (2%)	0	0.37
	KRAS	36 (20%)	1 (17%)	1 (25%)	21 (38%)	13 (12%)	0.0009
	NRAS	4 (2%)	0	0	3 (5%)	1 (1%)	0.29
	PTEN	135 (76%)	6 (100%)	3 (75%)	53 (95%)	73 (65%)	<0.0001

MLH1 methyl = MLH1 hypermethylation

^a Only activating mutations in BRAF, KRAS, and NRAS are included in these analyses.

^b p-value from Fisher's exact test