A Case of Microsatellite Instability–High Colon Cancer in a Young Woman With Familial Adenomatous Polyposis

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ABSTRACT

Two major molecular pathways of colorectal carcinogenesis, chromosomal instability (CIN) and microsatellite instability (MSI), are considered to be mutually exclusive. Distinguishing CIN from MSI-high tumors has considerable therapeutic implications, because patients with MSI-high tumors can derive considerable benefit from immune checkpoint inhibitors, and tumors that evolved through the CIN pathway do not respond to these agents. Familial adenomatous polyposis (FAP) is a genetic syndrome that is defined by a mutation in the *APC* gene and is thought to lead to carcinogenesis through the CIN pathway. Here, we report a case of a young woman with FAP who was treated for medulloblastoma as a child and developed advanced MSI-high colon cancer as a young adult. Her response to second-line immunotherapy enabled resection of her colon cancer, and she is free of disease >10 months after surgery. This case highlights the potential for overlap between the CIN and MSI carcinogenic pathways and associated therapeutic implications.

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Familial adenomatous polyposis (FAP) and Lynch syndrome embody the 2 major molecular pathways of colorectal carcinogenesis: chromosomal instability (CIN) and microsatellite instability (MSI), respectively.^{1,2} CIN and MSI are typically considered to be mutually exclusive biologic mechanisms. The identification of tumors driven by the MSI pathway has significant therapeutic implications, because colorectal cancers (CRCs) driven by this mechanism respond to immune checkpoint inhibitors (ICIs), whereas tumors driven by the CIN pathway do not.³ This report presents a case involving a young woman with FAP who was treated for medulloblastoma as a child and developed MSI-high colon cancer as a young adult. Her case highlights the possibility for overlap between the CIN and MSI carcinogenic pathways and its potential clinical ramifications.

Case Report

In December 2018, a 24-year-old woman presented to our institution with severe iron deficiency anemia and rectal bleeding. Her medical history was notable for medulloblastoma treated at the age of 6 years with craniospinal irradiation at a total dose of 2,340 cGy and chemotherapy with lomustine, vincristine, and cisplatin. These treatments left her with growth hormone deficiency, hypothyroidism, ataxia, and mild neurocognitive impairment. She also has a history of a benign chest wall desmoid tumor.

As part of her workup for rectal bleeding, esophago-gastroduodenoscopy was performed, which revealed numerous small fundic gland polyps. Colonoscopy identified a large rectal tubulovillous adenoma, numerous small and large adenomas in the sigmoid colon, and an obstructing mass at 38 cm from the anal verge, biopsy of which was nondiagnostic. Imaging (Figure 1A) revealed a lobulated, mucin-containing mass arising from the sigmoid colon with invasion of the bladder and cecum.

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Figure 1. Radiographic and pathologic correlates from a case of microsatellite instability-high colon cancer in a woman with familial adenomatous polyposis. **(A)** MRI of the pelvis at the time of diagnosis showing a mass arising from the sigmoid colon and invading the bladder. **(B)** MRI of the pelvis after combined therapy with ipilimumab and nivolumab showing evidence of a response. **(C)** Sigmoid colon resection specimen with pT4b adenocarcinoma (bracket) and numerous polyps (arrows).

(continued)

However, she did not have evidence of distant metastases. Germline genetic testing performed on peripheral blood using a commercial 47-gene panel (Invitae Corp) revealed a pathogenic *APC* c.3180_3184delAAAC (p.Q1062*) variant (supplemental eTable 1, available with this article at JNCCN.org). No germline variants were identified in the other 46 genes examined, including the mismatch repair genes, and germline testing of her parents and siblings has not yet been performed. The patient has no known family history of malignancy or polyposis in her parents, siblings, or other more distant relatives. Surgical exploration in February 2019 revealed a perforated sigmoid colon mass with an abscess cavity involving the bladder, cecum, and terminal ileum. A total colectomy with end ileostomy and Hartmann's pouch were performed with incomplete resection of gross tumor at the bladder dome.

Pathologic examination revealed an 8.8-cm, perforated, moderately differentiated mucinous adenocarcinoma arising from the sigmoid colon with direct extension into the cecum, small bowel, and associated abscess cavity (pT4b) (Figure 1C, D). Nineteen tubular adenomas were identified in the colectomy specimen, and a second focus of pT1 mucinous adenocarcinoma was identified in association with a sigmoid colon adenoma (Figure 1E, F). At gross examination, the polyp-containing carcinoma was grossly distinct from the invasive tumor, with intervening normal mucosa and no underlying connection between the 2

lesions. None of 62 lymph nodes were involved with carcinoma (pN0). Immunohistochemistry revealed absent expression of MSH2 and MSH6 (with intact expression of MLH1 and PMS2) in both the pT4b (Figure 1G) and pT1 adenocarcinomas (Figure 1H), whereas expression of all proteins was intact in nonneoplastic cells.

Somatic genetic analyses of both the pT4b and pT1 tumors was performed using exonic sequencing of 447 cancer genes and rearrangement detection from 191 regions across 60 genes, as previously described (Table 1, supplemental eTable 1).^{4,5} Somatic mutations are determined by a bioinformatics pipeline intended to remove likely germline variants present in local patient populations and public databases.^{6,7} This analysis identified the known germline *APC* variant; another pathogenic *APC* variant, c.4660_4661insA (p.T1556N*3); a pathogenic *MSH2* variant, c.560delT (p.E188Rfs*26); and other somatic variants shared between the 2 tumors (supplemental eTable 2, supplemental eFigure 1). The pT1 cancer harbored a third pathogenic somatic *APC* variant (c.4461delT [p.L1488Yfs*19]) not seen in the pT4b tumor, whereas the pT4b cancer harbored a somatic *APC* variant of uncertain significance (c.775C>T [p.R259W]) not seen in the pT1 tumor. In both tumors, the only significant copy number alteration identified was a deletion of 2p16.3-p22.1, inclusive of the entire *EPCAM*, *MSH2*, and *MSH6* genes (Figure 1I, J). Both tumors were MSI-high with markedly high tumor mutational burdens.

Figure 1 (cont.). Radiographic and pathologic correlates from a case of microsatellite instability-high colon cancer in a woman with familial adenomatous polyposis. **(D)** Histologic evaluation of the pT4b adenocarcinoma shows a moderately differentiated adenocarcinoma. **(E)** Low-magnification histologic view of the polyp, which was grossly distinct from the invasive tumor. Inset square indicates region shown in F. **(F)** High-magnification histologic view of the polyp shows a tubular adenoma (right-sided glandular elements) with underlying moderately differentiated pT1 adenocarcinoma, which was thought to possibly represent a metastasis from the pT4b adenocarcinoma (left-sided glandular elements). **(G, H)** Immunohistochemical evaluation of mismatch repair protein expression shows loss of MSH2 and MSH6 expression within the pT4b adenocarcinoma **(G)** and within the polyp-associated pT1 adenocarcinoma **(H)**. Expression of MLH1 and PMS2 was intact in both specimens (not shown). **(I)** Genome-wide copy number profiles of the pT4b adenocarcinoma and the polyp-associated pT1 adenocarcinoma tumor show matched, segmental single-copy deletions of chromosome 2p and an otherwise flat copy number landscape. **(J)** Copy number profile of chromosome 2p in the metastatic tumor shows a segmental deletion encompassing *EPCAM*, *MSH2*, and *MSH6*. Scale bars in D, F, G, and H indicate 100 μ m. Scale bar in E indicates 5 mm. Original magnification for all, $\times 20$.

Because the patient had gross residual disease, the decision was made to treat her with FOLFOX (5-FU/leucovorin/oxaliplatin) chemotherapy to potentially reduce her tumor burden and enable completion of resection of her remaining cancer. She received eight 14-day cycles of FOLFOX, with mild progression of the residual pelvic mass seen on imaging. Because of the MSI-high biology, treatment was changed to ipilimumab, 1 mg/kg and nivolumab, 3 mg/kg for four 21-day cycles, followed by 2 doses of nivolumab, 240 mg monotherapy administered 14 days apart.⁸ Repeat imaging showed an excellent response to therapy but a large residual mass (Figure 1B), so the

patient underwent en bloc resection with Hartmann's pouch resection, partial cystectomy, partial hysterectomy, and unilateral salpingo-oophorectomy in November 2019. Pathologic evaluation identified a 6.2-cm tumor bed consisting predominantly of mucin with only minute microscopic foci of residual adenocarcinoma and negative margins. CT imaging performed 4 and 10 months later showed no evidence of recurrent/metastatic disease.

Discussion

Hereditary cancer syndromes can offer critical insights into the pathobiology of human oncogenesis.⁹ In this

Table 1. Summary of Somatic Sequencing Results

pT4b Sigmoid Colon Adenocarcinoma		pT1 Sigmoid Colon Adenocarcinoma	
Tumor mutational burden: 74.52 per megabase Private somatic variants (n=80)		Tumor mutational burden: 66.156 per megabase Private somatic variants (n=76)	
Notable Mutations Among the 43 Shared Variants			
Gene	Variant		
APC (germline)	c.3180_3184delAAAAC (p.Q1062*), exon 16		
APC	c.4660_4661insA (p.T1556Nfs*3), exon 16		
MSH2	c.560delT (p.E188Rfs*26), exon 3		
2p16.3-p22.1	Single copy deletion		
KRAS	c.38G>A (p.G13D), exon 2		
PIK3CA	c.3140A>G (p.H1047R), exon 21		

Both the pT4b and pT1 sigmoid colon adenocarcinomas underwent somatic sequencing for 447 cancer genes and rearrangement detection from 191 regions across 60 genes.^{4,5} The tumors both had high tumor mutational burdens and shared 47 somatic variants. These variants included a known germline mutation in APC as well as a second mutation in APC; a deletion of 2p16.3-p22.1; and acquired mutations in MSH2, KRAS, and PIK3CA. Each tumor also had private, presumed to be passenger, somatic variants. A comprehensive summary of the mutations is available in supplemental eTable 2.

case, the unique finding of MSI-high in FAP-associated colon cancer shows the potential for overlap between the CIN and MSI carcinogenic pathways, which are typically considered to be mutually exclusive.^{2,10,11} The overlapping CIN and MSI biologies had clinical relevance for this individual, as evidenced by the profound response to ICI therapy leading to an eventual R0 resection. Data have consistently shown ICIs to be highly effective treatments for MSI-high CRCs,^{3,8,12} whereas those arising through the CIN pathway do not benefit from immune checkpoint blockade.³

Intriguingly, this individual's pT1 adenocarcinoma was also MSI-high with identical oncogenic mutations in MSH2, KRAS, and PIK3CA and numerous shared variants presumed to be "passenger" mutations from the underlying MSI. One notable exception, however, is that this pT1 adenocarcinoma had a third inactivating c.4461delT APC variant that was not seen in the pT4b adenocarcinoma, suggesting that this pT1 cancer may have actually represented a metastasis from the dominant pT4b cancer to a nearby adenoma.

Based on the available germline and somatic data, we hypothesize that this patient's dominant pT4b adenocarcinoma arose from a traditional FAP adenoma with biallelic APC inactivation and somatic mutational activation of KRAS. Although the patient's family has not yet completed germline sequencing, the lack of a family history of colon cancer or polyps leads us to believe that she has a de novo germline mutation in APC, which occurs in approximately one-third of FAP cases.¹ Given the paucity of other chromosomal copy number alterations, however, we hypothesize that the large chromosome 2p16.3-p22.1 deletion (inclusive of EPCAM, MSH2, and MSH6) was an early molecular event in this cancer that led to expansion of an MSI-high clone, rather than a CIN cancer, and subsequent

hypermethylation after acquisition of a somatic c.560delT "second hit" in MSH2. Interestingly, recent case series of Hodgkin lymphoma survivors have suggested that treatment-induced CRCs are disproportionately MSI-high with somatic biallelic mismatch repair gene inactivation.^{13,14}

Conclusions

Although we are only able to speculate regarding the process through which this individual with FAP developed MSI-high cancer, this case provides novel insight about the potential for intersection between CIN and MSI colorectal carcinogenesis. Such biology is of critical therapeutic relevance because of the efficacy of immune checkpoint blockade for MSI-driven cancers, and this case further supports the importance of testing for MSI status in all cases of CRC, as suggested by the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines).¹⁵ Interestingly, this patient's experience suggests that MSI-high biology and vulnerability to checkpoint blockade can emerge even in the setting of conditions that can predispose patients to other, theoretically distinct pathways of carcinogenesis. Finally, the study highlights the critical importance of screening survivors of childhood malignancies for secondary cancers and the opportunities to learn about how therapeutic interventions may impact survivorship.

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eFigure 1: Dendrogram of Mutations Observed in the T4b and T1 Adenocarcinoma

eTable 1: Genes Assessed on Germline and Somatic Testing for a Young Woman With Colon Cancer

eTable 2: Somatic Sequencing Results From 2 Sigmoid Colon Adenocarcinomas

eFigure 1. Dendrogram of mutations observed in the T4b adenocarcinoma and the T1 adenocarcinoma observed in a polyp. The T1 lesion is proposed to be a metastasis of the T4b lesion due to the number of shared somatic mutations.

eTable 1. Genes Assessed on Germline and Somatic Testing for a Young Woman With Colon Cancer

Germline genes included in the Invitae Common Hereditary Cancers Panel (n=47)	Somatic genes included in the DFCI Somatic Sequencing Panel (n=447)
<i>APC</i>	<i>ABCB11</i>
<i>ATM</i>	<i>ABL1</i>
<i>AXIN2</i>	<i>ACVR1</i>
<i>BARD1</i>	<i>AKT1</i>
<i>BMPR1A</i>	<i>AKT2</i>
<i>BRCA1</i>	<i>AKT3</i>
<i>BRCA2</i>	<i>ALK</i>
<i>BRIP1</i>	<i>APC</i>
<i>CDH1</i>	<i>AR</i>
<i>CDK4</i>	<i>ARAF</i>
<i>CDKN2A</i>	<i>ARHGAP35</i>
<i>CHEK2</i>	<i>ARHGEF12</i>
<i>CTNNA1</i>	<i>ARID1A</i>
<i>DICER1</i>	<i>ARID1B</i>
<i>EPCAM</i>	<i>ARID2</i>
<i>GREM1</i>	<i>ASXL1</i>
<i>HOXB13</i>	<i>ATM</i>
<i>KIT</i>	<i>ATR</i>
<i>MEN1</i>	<i>ATRX</i>
<i>MLH1</i>	<i>AURKA</i>
<i>MSH2</i>	<i>AURKB</i>
<i>MSH3</i>	<i>AXIN2</i>
<i>MSH6</i>	<i>AXL</i>
<i>MUTYH</i>	<i>B2M</i>
<i>NBN</i>	<i>BABAM1</i>
<i>NF1</i>	<i>BAP1</i>
<i>NTHL1</i>	<i>BARD1</i>
<i>PALB2</i>	<i>BCL11B</i>
<i>PDGFRA</i>	<i>BCL2</i>
<i>PMS2</i>	<i>BCL2L1</i>
<i>POLD1</i>	<i>BCL2L12</i>
<i>POLE</i>	<i>BCL6</i>
<i>PTEN</i>	<i>BCOR</i>
<i>RAD50</i>	<i>BCORL1</i>
<i>RAD51C</i>	<i>BLM</i>
<i>RAD51D</i>	<i>BMPR1A</i>
<i>SDHA</i>	<i>BRAF</i>
<i>SDHB</i>	<i>BRCA1</i>
<i>SDHC</i>	<i>BRCA2</i>
<i>SDHD</i>	<i>BRCC3</i>

(continued)

eTable 1. Genes Assessed on Germline and Somatic Testing for a Young Woman With Colon Cancer (cont.)

Germline genes included in the Invitae Common Hereditary Cancers Panel (n=47)	Somatic genes included in the DFCI Somatic Sequencing Panel (n=447)
<i>SMAD4</i>	<i>BRD3</i>
<i>SMARCA4</i>	<i>BRD4</i>
<i>STK11</i>	<i>BRE</i>
<i>TP53</i>	<i>BRIP1</i>
<i>TSC1</i>	<i>BUB1B</i>
<i>TSC2</i>	<i>C17ORF70</i>
<i>VHL</i>	<i>C19ORF40</i>
	<i>C10RF86</i>
	<i>CALR</i>
	<i>CARD11</i>
	<i>CASP8</i>
	<i>CBFA2T3</i>
	<i>CBFB</i>
	<i>CBL</i>
	<i>CBLB</i>
	<i>CCND1</i>
	<i>CCND2</i>
	<i>CCND3</i>
	<i>CCNE1</i>
	<i>CD274</i>
	<i>CD79B</i>
	<i>CDC73</i>
	<i>CDH1</i>
	<i>CDH4</i>
	<i>CDK12</i>
	<i>CDK4</i>
	<i>CDK6</i>
	<i>CDK8</i>
	<i>CDKN1A</i>
	<i>CDKN1B</i>
	<i>CDKN1C</i>
	<i>CDKN2A</i>
	<i>CDKN2B</i>
	<i>CDKN2C</i>
	<i>CEBPA</i>
	<i>CHEK1</i>
	<i>CHEK2</i>
	<i>CIC</i>
	<i>CIITA</i>
	<i>COL7A1</i>

(continued)

eTable 1. Genes Assessed on Germline and Somatic Testing for a Young Woman With Colon Cancer (cont.)

Germline genes included in the Invitae Common Hereditary Cancers Panel (n=47)	Somatic genes included in the DFCI Somatic Sequencing Panel (n=447)
	<i>CREBBP</i>
	<i>CRKL</i>
	<i>CRLF2</i>
	<i>CRTC1</i>
	<i>CSF3R</i>
	<i>CTCF</i>
	<i>CTLA4</i>
	<i>CTNNA1</i>
	<i>CTNB1</i>
	<i>CUX1</i>
	<i>CXCR4</i>
	<i>CYLD</i>
	<i>DAXX</i>
	<i>DCLRE1C</i>
	<i>DDB1</i>
	<i>DDB2</i>
	<i>DDR2</i>
	<i>DICER1</i>
	<i>DIS3</i>
	<i>DIS3L2</i>
	<i>DKC1</i>
	<i>DMC1</i>
	<i>DNMT3A</i>
	<i>DOCK8</i>
	<i>EGFR</i>
	<i>EGLN1</i>
	<i>ELANE</i>
	<i>EME1</i>
	<i>ENG</i>
	<i>EP300</i>
	<i>EPCAM</i>
	<i>ERBB2</i>
	<i>ERBB3</i>
	<i>ERBB4</i>
	<i>ERCC1</i>
	<i>ERCC2</i>
	<i>ERCC3</i>
	<i>ERCC4</i>
	<i>ERCC5</i>
	<i>ERCC6</i>

(continued)

eTable 1. Genes Assessed on Germline and Somatic Testing for a Young Woman With Colon Cancer (cont.)

Germline genes included in the Invitae Common Hereditary Cancers Panel (n=47)	Somatic genes included in the DFCI Somatic Sequencing Panel (n=447)
	<i>ERG</i>
	<i>ESR1</i>
	<i>ETV1</i>
	<i>ETV4</i>
	<i>ETV5</i>
	<i>ETV6</i>
	<i>EWSR1</i>
	<i>EXO1</i>
	<i>EXT1</i>
	<i>EXT2</i>
	<i>EZH2</i>
	<i>FAH</i>
	<i>FAM175A</i>
	<i>FAM46C</i>
	<i>FAN1</i>
	<i>FANCA</i>
	<i>FANCB</i>
	<i>FANCC</i>
	<i>FANCD2</i>
	<i>FANCE</i>
	<i>FANCF</i>
	<i>FANCG</i>
	<i>FANCI</i>
	<i>FANCL</i>
	<i>FANCM</i>
	<i>FAS</i>
	<i>FAT1</i>
	<i>FBXW7</i>
	<i>FGFR1</i>
	<i>FGFR2</i>
	<i>FGFR3</i>
	<i>FGFR4</i>
	<i>FH</i>
	<i>FLCN</i>
	<i>FLT1</i>
	<i>FLT3</i>
	<i>FLT4</i>
	<i>FOXA1</i>
	<i>FOXL2</i>
	<i>FUS</i>

(continued)

eTable 1. Genes Assessed on Germline and Somatic Testing for a Young Woman With Colon Cancer (cont.)

Germline genes included in the Invitae Common Hereditary Cancers Panel (n=47)	Somatic genes included in the DFCI Somatic Sequencing Panel (n=447)
	<i>GALNT12</i>
	<i>GATA2</i>
	<i>GATA3</i>
	<i>GATA4</i>
	<i>GATA6</i>
	<i>GBA</i>
	<i>GEN1</i>
	<i>GLI1</i>
	<i>GLI2</i>
	<i>GNA11</i>
	<i>GNAQ</i>
	<i>GNAS</i>
	<i>GPC3</i>
	<i>GREM1</i>
	<i>H19</i>
	<i>H3F3A</i>
	<i>H3F3B</i>
	<i>HABP2</i>
	<i>HELQ</i>
	<i>HFE</i>
	<i>HIST1H3B</i>
	<i>HIST1H3C</i>
	<i>HMBS</i>
	<i>HNF1A</i>
	<i>HOXB13</i>
	<i>HRAS</i>
	<i>ID3</i>
	<i>ID4</i>
	<i>IDH1</i>
	<i>IDH2</i>
	<i>IGF1R</i>
	<i>IGF2</i>
	<i>IKZF1</i>
	<i>IL7R</i>
	<i>ITK</i>
	<i>JAK1</i>
	<i>JAK2</i>
	<i>JAK3</i>
	<i>JAZF1</i>
	<i>KAT6A</i>

(continued)

eTable 1. Genes Assessed on Germline and Somatic Testing for a Young Woman With Colon Cancer (cont.)

Germline genes included in the Invitae Common Hereditary Cancers Panel (n=47)	Somatic genes included in the DFCI Somatic Sequencing Panel (n=447)
	<i>KAT6B</i>
	<i>KCNQ1</i>
	<i>KDM5A</i>
	<i>KDM5C</i>
	<i>KDM6A</i>
	<i>KDR</i>
	<i>KEAP1</i>
	<i>KIF1B</i>
	<i>KIT</i>
	<i>KLF2</i>
	<i>KLF4</i>
	<i>KLLN</i>
	<i>KMT2A</i>
	<i>KMT2D</i>
	<i>KRAS</i>
	<i>LIG4</i>
	<i>LMO1</i>
	<i>LMO2</i>
	<i>MAF</i>
	<i>MAFB</i>
	<i>MAP2K1</i>
	<i>MAP2K2</i>
	<i>MAP2K4</i>
	<i>MAP3K1</i>
	<i>MAPK1</i>
	<i>MAX</i>
	<i>MBD4</i>
	<i>MCL1</i>
	<i>MCM8</i>
	<i>MDM2</i>
	<i>MDM4</i>
	<i>MECOM</i>
	<i>MED12</i>
	<i>MEF2B</i>
	<i>MEN1</i>
	<i>MET</i>
	<i>MGA</i>
	<i>MITF</i>
	<i>MLH1</i>
	<i>MLH3</i>

(continued)

eTable 1. Genes Assessed on Germline and Somatic Testing for a Young Woman With Colon Cancer (cont.)

Germline genes included in the Invitae Common Hereditary Cancers Panel (n=47)	Somatic genes included in the DFCI Somatic Sequencing Panel (n=447)
	<i>MPL</i>
	<i>MRE11A</i>
	<i>MSH2</i>
	<i>MSH6</i>
	<i>MTA1</i>
	<i>MTAP</i>
	<i>MTOR</i>
	<i>MUS81</i>
	<i>MUTYH</i>
	<i>MYB</i>
	<i>MYBL1</i>
	<i>MYC</i>
	<i>MYCL1</i>
	<i>MYCN</i>
	<i>MYD88</i>
	<i>NBN</i>
	<i>NEIL1</i>
	<i>NEIL2</i>
	<i>NEIL3</i>
	<i>NF1</i>
	<i>NF2</i>
	<i>NFE2L2</i>
	<i>NFKBIA</i>
	<i>NFKBIE</i>
	<i>NFKBIZ</i>
	<i>NKX2-1</i>
	<i>NKX3-1</i>
	<i>NOTCH1</i>
	<i>NOTCH2</i>
	<i>NOTCH3</i>
	<i>NPM1</i>
	<i>NR0B1</i>
	<i>NRAS</i>
	<i>NRG1</i>
	<i>NSD1</i>
	<i>NT5C2</i>
	<i>NTHL1</i>
	<i>NTRK1</i>
	<i>NTRK2</i>
	<i>NTRK3</i>

(continued)

eTable 1. Genes Assessed on Germline and Somatic Testing for a Young Woman With Colon Cancer (cont.)

Germline genes included in the Invitae Common Hereditary Cancers Panel (n=47)	Somatic genes included in the DFCI Somatic Sequencing Panel (n=447)
	<i>OGG1</i>
	<i>PALB2</i>
	<i>PARK2</i>
	<i>PAX5</i>
	<i>PAXIP1</i>
	<i>PBRM1</i>
	<i>PDCD1LG2</i>
	<i>PDGFRA</i>
	<i>PDGFRB</i>
	<i>PHF6</i>
	<i>PHOX2B</i>
	<i>PIK3C2B</i>
	<i>PIK3CA</i>
	<i>PIK3R1</i>
	<i>PIM1</i>
	<i>PML</i>
	<i>PMS1</i>
	<i>PMS2</i>
	<i>PNKP</i>
	<i>POLB</i>
	<i>POLD1</i>
	<i>POLE</i>
	<i>POLH</i>
	<i>POLQ</i>
	<i>POT1</i>
	<i>PPARG</i>
	<i>PPM1D</i>
	<i>PPP2R1A</i>
	<i>PRDM1</i>
	<i>PRF1</i>
	<i>PRKAR1A</i>
	<i>PRKCI</i>
	<i>PRKDC</i>
	<i>PRSS1</i>
	<i>PTCH1</i>
	<i>PTEN</i>
	<i>PTK2B</i>
	<i>PTPN11</i>
	<i>PTPN14</i>
	<i>PVRL4</i>

(continued)

eTable 1. Genes Assessed on Germline and Somatic Testing for a Young Woman With Colon Cancer (cont.)

Germline genes included in the Invitae Common Hereditary Cancers Panel (n=47)	Somatic genes included in the DFCI Somatic Sequencing Panel (n=447)
	<i>QKI</i>
	<i>RAC1</i>
	<i>RAD21</i>
	<i>RAD50</i>
	<i>RAD51</i>
	<i>RAD51C</i>
	<i>RAD51D</i>
	<i>RAD52</i>
	<i>RAD54B</i>
	<i>RAF1</i>
	<i>RARA</i>
	<i>RASA1</i>
	<i>RB1</i>
	<i>RBBP8</i>
	<i>RBM10</i>
	<i>RECQL4</i>
	<i>REL</i>
	<i>RELA</i>
	<i>RET</i>
	<i>RHBDF2</i>
	<i>RHEB</i>
	<i>RHOA</i>
	<i>RHOH</i>
	<i>RHOT1</i>
	<i>RICTOR</i>
	<i>RIF1</i>
	<i>RINT1</i>
	<i>RIT1</i>
	<i>RMRP</i>
	<i>RNF43</i>
	<i>RNF8</i>
	<i>ROS1</i>
	<i>RPA1</i>
	<i>RPTOR</i>
	<i>RSP02</i>
	<i>RSP03</i>
	<i>RUNX1</i>
	<i>RUNX1T1</i>
	<i>SBDS</i>
	<i>SDHA</i>

(continued)

eTable 1. Genes Assessed on Germline and Somatic Testing for a Young Woman With Colon Cancer (cont.)

Germline genes included in the Invitae Common Hereditary Cancers Panel (n=47)	Somatic genes included in the DFCI Somatic Sequencing Panel (n=447)
	<i>SDHAF2</i>
	<i>SDHB</i>
	<i>SDHC</i>
	<i>SDHD</i>
	<i>SERPINA1</i>
	<i>SETBP1</i>
	<i>SETD2</i>
	<i>SF3B1</i>
	<i>SH2B3</i>
	<i>SH2D1A</i>
	<i>SLC25A13</i>
	<i>SLC34A2</i>
	<i>SLX1A</i>
	<i>SLX1B</i>
	<i>SLX4</i>
	<i>SMAD2</i>
	<i>SMAD4</i>
	<i>SMARCA4</i>
	<i>SMARCB1</i>
	<i>SMARCE1</i>
	<i>SMC3</i>
	<i>SMO</i>
	<i>SOCS1</i>
	<i>SOS1</i>
	<i>SOX2</i>
	<i>SOX9</i>
	<i>SPOP</i>
	<i>SRSF2</i>
	<i>SRY</i>
	<i>SS18</i>
	<i>STAG2</i>
	<i>STAT3</i>
	<i>STAT6</i>
	<i>STK11</i>
	<i>SUFU</i>
	<i>SUZ12</i>
	<i>TAL1</i>
	<i>TAL2</i>
	<i>TAZ</i>
	<i>TCEB1</i>

(continued)

eTable 1. Genes Assessed on Germline and Somatic Testing for a Young Woman With Colon Cancer (cont.)

Germline genes included in the Invitae Common Hereditary Cancers Panel (n=47)	Somatic genes included in the DFCI Somatic Sequencing Panel (n=447)
	<i>TCF3</i>
	<i>TCF7L2</i>
	<i>TDG</i>
	<i>TERC</i>
	<i>TERT</i>
	<i>TET1</i>
	<i>TET2</i>
	<i>TFE3</i>
	<i>TLX3</i>
	<i>TMEM127</i> <i>TMPRSS2</i>
	<i>TNFAIP3</i>
	<i>TOPBP1</i>
	<i>TP53</i>
	<i>TP53BP1</i>
	<i>TRAF3</i>
	<i>TRAF7</i>
	<i>TRIM37</i>
	<i>TSC1</i>
	<i>TSC2</i>
	<i>TSHR</i>
	<i>U2AF1</i>
	<i>UBE2T</i>
	<i>UIMC1</i>
	<i>UROD</i>
	<i>USP28</i>
	<i>USP8</i>
	<i>VEGFA</i>
	<i>VHL</i>
	<i>WAS</i>
	<i>WHSC1</i>
	<i>WHSC1L1</i>
	<i>WRN</i>
	<i>WT1</i>
	<i>XPA</i>
	<i>XPC</i>
	<i>XPO1</i>
	<i>XRCC1</i>
	<i>XRCC2</i>
	<i>XRCC3</i>

(continued)

eTable 1. Genes Assessed on Germline and Somatic Testing for a Young Woman With Colon Cancer (cont.)

Germline genes included in the Invitae Common Hereditary Cancers Panel (n=47)	Somatic genes included in the DFCI Somatic Sequencing Panel (n=447)
	<i>XRCC4</i>
	<i>XRCC5</i>
	<i>XRCC6</i>
	<i>YAP1</i>
	<i>ZNF217</i>
	<i>ZNRF3</i>
	<i>ZRSR2</i>

cTable 2: Somatic sequencing results from two sigmoid colon adenocarcinomas in a 24-year-old female with FAP

pT4b sigmoid colon adenocarcinoma				pT1 sigmoid colon adenocarcinoma			
Tumor Mutational Burden: 74.52 per Megabase		Tumor Mutational Burden: 66.156 per Megabase					
Gene	Variant	Coverage	Notable oncogenic variants	Gene	Variant	Coverage	Notable oncogenic variants
APC (germline)	c.3180_3184delAAAAA (p.Q1062*), exon 16	39% of 231 reads		APC (germline)	c.3180_3184delAAAAA (p.Q1062*), exon 16	50% of 146 reads	
APC	c.4660_4661insA (p.T1556Nfs*3), exon 16	29% of 329 reads		APC	c.4660_4661insA (p.T1556Nfs*3), exon 16	15% of 136 reads	
MSH2	c.560delTT (p.E188Rfs*26), exon 3	58% of 229 reads		MSH2	c.560delTT (p.E188Rfs*26), exon 3	23% of 160 reads	
2p16.3-p22.1	Single copy deletion			2p16.3-p22.1	Single copy deletion		
KRAS	c.38G>A (p.G13D), exon 2	31% of 309 reads		KRAS	c.38G>A (p.G13D), exon 2	15% of 198 reads	
PIK3CA	c.3140A>G (p.H1047R), exon 21	37% of 256 reads		PIK3CA	c.3140A>G (p.H1047R), exon 21	12% of 166 reads	
Other shared somatic variants (presumed passenger variants)				Other shared somatic variants (presumed passenger variants)			
ALK	c.2548G>A (p.G850R), exon 15	37% of 291 reads		ALK	c.2548G>A (p.G850R), exon 15	18% of 143 reads	
ARID1A	c.6415delC (p.F2141Sfs*59), exon 20	35% of 493 reads		ARID1A	c.6415delC (p.F2141Sfs*59), exon 20	18% of 259 reads	
ARID1B	c.643G>A (p.A215T), exon 1	34% of 35 reads		ARID1B	c.643G>A (p.A215T), exon 1	22% of 22 reads	
ARHGGEF12	c.3341C>T (p.A1114V), exon 34	33% of 331 reads		ARHGGEF12	c.3341C>T (p.A1114V), exon 34	14% of 151 reads	
ATRX	c.6111-3delT ()	35% of 167 reads		ATRX	c.6111-3delT ()	26% of 68 reads	
AXL	c.2399G>A (p.R800Q), exon 20	34% of 250 reads		AXL	c.2399G>A (p.R800Q), exon 20	17% of 151 reads	
BMPRI1A	c.1419_1420delTTG (p.V476Gfs*9), exon 12	31% of 493 reads		BMPRI1A	c.1419_1420delTTG (p.V476Gfs*9), exon 12	13% of 340 reads	
BRCA2	c.2441C>T (p.P814L), exon 11	35% of 257 reads		BRCA2	c.2441C>T (p.P814L), exon 11	21% of 130 reads	
EP300	c.6673C>T (p.Q2225*), exon 31	35% of 391 reads		EP300	c.6673C>T (p.Q2225*), exon 31	15% of 153 reads	
ERBB3	c.850G>A (p.G284R), exon 7	40% of 320 reads		ERBB3	c.850G>A (p.G284R), exon 7	12% of 232 reads	
CITTA	c.13G>A (p.A5T), exon 1	49% of 189 reads		CITTA	c.13G>A (p.A5T), exon 1	7% of 296 reads	
DAXX	c.1318G>A (p.D440N), exon 5	37% of 161 reads		DAXX	c.1318G>A (p.D440N), exon 5	42% of 100 reads	
DICER1	c.904-5delT ()	16% of 309 reads		DICER1	c.904-5delT ()	21% of 76 reads	
EGLN1	c.935G>A (p.R312H), exon 2	35% of 280 reads		EGLN1	c.935G>A (p.R312H), exon 2	6% of 109 reads	
ERBB2	c.3208G>A (p.A1070T), exon 26	38% of 120 reads		ERBB2	c.3208G>A (p.A1070T), exon 26	6% of 109 reads	
FANCA	c.2447C>T (p.A816V), exon 26	42% of 277 reads		FANCA	c.2447C>T (p.A816V), exon 26	14% of 63 reads	
FANCM	c.4515+6_4515+7insT ()	33% of 148 reads		FANCM	c.4515+6_4515+7insT ()	14% of 290 reads	
FAT1	c.5885G>C (p.R1953T), exon 10	48% of 532 reads		FAT1	c.5885G>C (p.R1953T), exon 10	21% of 133 reads	
MAP3K1	c.3748G>T (p.G1250C), exon 15	38% of 302 reads		MAP3K1	c.3748G>T (p.G1250C), exon 15	20% of 108 reads	
MET	c.3028-55delAA ()	36% of 353 reads		MET	c.3028-55delAA ()	8% of 160 reads	
MTA1	c.1344-55delG ()	29% of 277 reads		MTA1	c.1344-55delG ()	7% of 122 reads	
MYCN	c.802_813delGATGAAGAGGAA (p.D268_E271del), exon 3	27% of 363 reads		MYCN	c.802_813delGATGAAGAGGAA (p.D268_E271del), exon 3	13% of 168 reads	
NOTCH1	c.2644G>A (p.A882T), exon 17	37% of 77 reads		NOTCH1	c.2644G>A (p.A882T), exon 17	14% of 198 reads	
NOTCH3	c.5913-92dupA ()	27% of 204 reads		NOTCH3	c.5913-92dupA ()	8% of 122 reads	
PAX5	c.81dupT (p.V28Cfs*47), exon 2	34% of 391 reads		PAX5	c.81dupT (p.V28Cfs*47), exon 2	11% of 67 reads	
PDGFRA	c.629-4A>G ()	49% of 230 reads		PDGFRA	c.629-4A>G ()	8% of 68 reads	
PHF6	c.585+7delA ()	34% of 172 reads		PHF6	c.585+7delA ()	8% of 162 reads	
POLD1	c.2953C>T (p.R985W), exon 23	33% of 99 reads		POLD1	c.2953C>T (p.R985W), exon 23	53% of 105 reads	
POLO	c.7680-7delT ()	31% of 240 reads		POLO	c.7680-7delT ()	14% of 132 reads	
PRKDC	c.1109A>G (p.N399S), exon 85	51% of 405 reads		PRKDC	c.1109A>G (p.N399S), exon 85	13% of 190 reads	
RAF1	c.952C>T (p.R318W), exon 9	35% of 424 reads		RAF1	c.952C>T (p.R318W), exon 9	4% of 176 reads	
REL	c.1262G>A (p.R421H), exon 11	32% of 461 reads		REL	c.1262G>A (p.R421H), exon 11	7% of 51 reads	
SETD2	c.7162delA (p.T238Pfs*23), exon 17	35% of 403 reads		SETD2	c.7162delA (p.T238Pfs*23), exon 17	15% of 144 reads	
TSC2	c.1654G>A (p.A552T), exon 16	40% of 195 reads		TSC2	c.1654G>A (p.A552T), exon 16	7% of 254 reads	
USP28	c.1283-6T>A ()	47% of 121 reads		USP28	c.1283-6T>A ()	5% of 116 reads	
VEGFA	c.658+7delC ()	33% of 186 reads		VEGFA	c.658+7delC ()	13% of 193 reads	
WHSC1	c.3647delA (p.K1217Rfs*112), exon 21	30% of 152 reads		WHSC1	c.3647delA (p.K1217Rfs*112), exon 21	22% of 97 reads	
Private Somatic Variants (Presumed Passenger Variants)				Private Somatic Variants (Presumed Passenger Variants)			
ABL1	c.1069delA (p.N358Tfs*14), exon 6	9% of 357 reads		ABL1	c.3109G>A (p.E1037K), exon 11	16% of 178 reads	
AKT2	c.1228C>A (p.L410I), exon 12	13% of 241 reads		AKT2	c.2291G>T (p.R764L), exon 11	6% of 162 reads	
APC	c.775C>T (p.R269W), exon 8	22% of 259 reads		APC	c.4461delT (p.L1489Yfs*19), exon 16	9% of 253 reads	
ARAF	c.758delC (p.R255Gfs*37), exon 9	21% of 343 reads		ARID1A	c.1373G>A (p.G458D), exon 3	17% of 133 reads	
ARID1B	c.4226A>G (p.Y1409C), exon 18	2% of 383 reads		ARID1B	c.1437C>C (p.L48P), exon 1	6% of 89 reads	
ATR	c.7895T>C (p.L2632S), exon 47	36% of 355 reads		ATM	c.7927-6delT ()	5% of 127 reads	
BCL11B	c.2618T>C (p.M873T), exon 4	20% of 348 reads		BCL11B	c.1427C>T (p.T476M), exon 4	9% of 66 reads	
BCOR	c.235C>T (p.R79W), exon 4	3% of 285 reads		BCOR	c.1837delA (p.T613Qfs*39), exon 11	7% of 177 reads	
C17orf70	c.574delC (p.H192Tfs*62), exon 3	13% of 235 reads		BLM	c.2261_2262insA (p.D757Rfs*18), exon 10	8% of 81 reads	
CBFA2T3	c.1541G>A (p.R514H), exon 11	23% of 254 reads		BLM	c.2261_2262insA (p.D757Rfs*18), exon 10	8% of 81 reads	
CBL	c.747-2T>C ()	16% of 255 reads		BLM	c.2556-3T>A ()	7% of 91 reads	
COL1A1	c.5261delA (p.P1754Qfs*87), exon 59	15% of 213 reads		BRCC3	c.147C>C (p.V5A), exon 1	4% of 125 reads	
CSF3R	c.728delC (p.P243Lfs*20), exon 7	9% of 142 reads		BRCC3	c.191delA (p.K65Rfs*26), exon 3	21% of 130 reads	
CUX1	c.530-4A>G ()	3% of 166 reads		BRCC3	c.147C>C (p.V5A), exon 1	4% of 125 reads	
CYLD	c.1438G>A (p.E480K), exon 8	15% of 393 reads		BRCC3	c.191delA (p.K65Rfs*26), exon 3	16% of 174 reads	
CYLD	c.1684-2T>C ()	39% of 305 reads		CASP8	c.9711T>C (p.M324T), exon 9	4% of 125 reads	
DICER1	c.4692A>G (p.D1561G), exon 24	8% of 491 reads		CASP8	c.9711T>C (p.M324T), exon 9	16% of 174 reads	
EP300	c.4229G>T (p.R1410M), exon 26	5% of 201 reads		CBLL	c.5727C>T (p.H191T), exon 5	10% of 190 reads	
ERCC4	c.2321C>T (p.A774V), exon 11	4% of 489 reads		CBLL	c.5727C>T (p.H191T), exon 5	10% of 190 reads	
EXO1	c.2386delA (p.N797Tfs*27), exon 15	18% of 166 reads		CDK4	c.753delC (p.R252Efs*25), exon 7	10% of 168 reads	
EZH2	c.455delA (p.N152Hfs*15), exon 5	23% of 256 reads		CDK4	c.753delC (p.R252Efs*25), exon 7	10% of 168 reads	
FAT1	c.13138-2531delT ()	20% of 316 reads		FAT1	c.3028-55delAA ()	7% of 296 reads	
FBXW7	c.2001delG (p.S66Vfs*39), exon 12	17% of 607 reads		CRTC1	c.1706delC (p.S572Afs*6), exon 14	5% of 73 reads	
FLCN	c.1285dupC (p.H429Pfs*27), exon 11	12% of 179 reads		DIS3	c.1504-4delT ()	10% of 130 reads	
FUS	c.175G>A (p.G59S), exon 3	20% of 394 reads		DIS3	c.1504-4delT ()	10% of 130 reads	
GNA11	c.194G>A ()	24% of 294 reads		DIS3L2	c.1459A>G (p.I486V), exon 13	9% of 144 reads	
IGF2	c.140C>T (p.A1121V), exon 4	18% of 228 reads		DIS3L2	c.1459A>G (p.I486V), exon 13	10% of 144 reads	
IGF2	c.140C>T (p.T471), exon 2	21% of 236 reads		EP300	c.6673C>T (p.Q2225*), exon 31	15% of 153 reads	
ILTR	c.793delA (p.R267Gfs*28), exon 6	17% of 424 reads		ERG	c.1359delT (p.F453Lfs*59), exon 12	13% of 180 reads	
JAK1	c.2580delA (p.K860Nfs*16), exon 19	21% of 408 reads		EWSR1	c.226-398C>T ()	14% of 290 reads	
KAT16A	c.2221A>G (p.S741G), exon 13	18% of 266 reads		FANCA	c.426+2T>C ()	5% of 164 reads	
KEAP1	c.778C>T (p.R260*), exon 3	14% of 312 reads		FANCA	c.922_923insC (p.V311Sfs*2), exon 4	11% of 200 reads	
KIF1B	c.1977-695delA ()	4% of 326 reads		FGR2	c.781G>A (p.G261R), exon 7	5% of 164 reads	
KMT2A	c.7031A>T (p.N2344), exon 27	3% of 387 reads		FLT1	c.970A>G (p.T324A), exon 7	9% of 181 reads	
KMT2D	c.2657delC (p.P886Lfs*44), exon 10	38% of 242 reads		GNAS	c.342_343insC (p.V117Rfs*23), exon 5	11% of 62 reads	
KMT2D	c.5317C>T (p.Q1773*), exon 22	5% of 375 reads		KDM5A	c.4522C>T (p.R1508W), exon 27	56% of 289 reads	
KMT2D	c.3890G>A (p.R1259H), exon 11	42% of 204 reads		KEAP1	c.1112G>C (p.G371D), exon 3	9% of 150 reads	
MED12	c.807G>T (p.E269D), exon 6	3% of 334 reads		KMT2A	c.5947G>T (p.G1983C), exon 22	9% of 194 reads	
MTA1	c.724G>A (p.A242T), exon 9	12% of 120 reads		KMT2D	c.3730delG (p.V1244Sfs*86), exon 11	8% of 280 reads	
MTOR	c.4918C>T (p.R1640W), exon 35	17% of 330 reads		KMT2D	c.10694A>G (p.K3565R), exon 38	17% of 164 reads	
MYBL1	c.494C>T (p.A165V), exon 5	15% of 246 reads		MED4	c.1058delT (p.L353*), exon 3	8% of 156 reads	
NBN	c.1301C>T (p.F341), exon 10	3% of 349 reads		MCL1	c.480C>A (p.P164T), exon 1	8% of 260 reads	
NFI	c.8190C>T (p.F271L), exon 56	4% of 195 reads		MCL1	c.950A>T (p.E317V), exon 3	9% of 229 reads	
NFKBIE	c.750A>T (p.E250D), exon 1	14% of 67 reads		MDM2	c.840-7delT ()	17% of 195 reads	
NOTCH1	c.247G>A (p.A83T), exon 3	4% of 124 reads		MED12	c.1731G>T (p.Q577H), exon 12	9% of 277 reads	
NR0B1	c.197G>A (p.C66Y), exon 1	20% of 424 reads		MED12	c.2873G>T (p.G958V), exon 21	4% of 263 reads	
NRG1	c.266delA (p.K915Sfs*24), exon 2	20% of 190 reads		MLH3	c.1755delA (p.E568Nfs*24), exon 2	8% of 325 reads	
NSD1	c.1217delA (p.G408Rfs*11), exon 4	18% of 197 reads		MTAP	c.737A>G (p.T253A), exon 7	8% of 179 reads	
NTRK2	c.1745G>A (p.R582H), exon 17	3% of 451 reads		NOTCH2	c.7219C>T (p.H2407Y), exon 34	19% of 151 reads	
PBRM1	c.996-5_996	34% of 246 reads		NRG1	c.1933G>A (p.V645I), exon 13	23% of 149 reads	
PDGFRB	c.821C>T (p.S274F), exon 6	3% of 333 reads		NSD1	c.5934A>T (p.E1978D), exon 19	19% of 36 reads	
PIK3CB	c.859_859dupCC (p.R287Pfs*93), exon 3	14% of 202 reads		NTRK3	c.2494C>G (p.P832S), exon 19	8% of 225 reads	
PMS1	c.2635-1G>T ()	16% of 232 reads		PIK3CB2B	c.859dupCC (p.R287Pfs*38), exon 3	15% of 138 reads	
POLE	c.6053G>T (p.S2018), exon 44	22% of 316 reads		POLB	c.261+6A>T ()	12% of 157 reads	
POLD1	c.2717-20G>A ()	6% of 136 reads		POLD1	c.2620A>G (p.N874D), exon 21	16% of 105 reads	
POLH	c.1148G>A (p.R383H), exon 10	19% of 366 reads		PRDM1	c.2056C>T (p.P868S), exon 7	14% of 35 reads	
PTCH1	c.3605delC (p.S1203Afs*52), exon 22	33% of 213 reads		PRKCI	c.819delA (p.T726Qfs*7), exon 9	12% of 65 reads	
PTPN11	c.1174G>A (p.A392T), exon 10	23% of 239 reads		PRKDC	c.11533delA (p.N384Afs*12), exon 82	10% of 140 reads	
RAD21	c.1286A>G (p.D429G), exon 10	16% of 379 reads		PRKDC	c.8860_8861delAG (p.S2954Cfs*9), exon 65	6% of 261 reads	
RAD50	c.2157delA (p.K722Rfs*14), exon 13	17% of 240 reads		PTK2B	c.1816A>T (p.S606C), exon 25	9% of 184 reads	
RBBP8	c.1745C>T (p.A552T),						