PATIENT

DISEASE Colon adenocarcinoma (CRC)
NAME
DATE OF BIRTH
SEX
MEDICAL RECORD #

PHYSICIAN

ORDERING PHYSICIAN
MEDICAL FACILITY
ADDITIONAL RECIPIENT
MEDICAL FACILITY ID
PATHOLOGIST

SPECIMEN

SPECIMEN SITE
SPECIMEN ID
SPECIMEN TYPE
DATE OF COLLECTION
SPECIMEN RECEIVED

Companion Diagnostic (CDx) Associated Findings

GENOMIC FINDINGS DETECTED	FDA-APPROVED THERAPEUTIC OPTIONS
KRAS wildtype (codons 12 & 13)	Erbitux® (Cetuximab)
KRAS/NRAS wildtype (codons 12, 13, 59, 61, 117, & 146 in exons 2, 3, & 4)	Vectibix® (Panitumumab)

For Microsatellite Instability (MSI) results, confirmatory testing using a validated orthogonal method should be performed.

OTHER ALTERATIONS & BIOMARKERS IDENTIFIED

Results reported in this section are not prescriptive or conclusive for labeled use of any specific therapeutic product. See *professional services* section for additional information.

Microsatellite status MSI-High §
Tumor Mutational Burden 35 Muts/Mb §
ASXL1 G645fs*58
ASXL1 S1335fs*115
ATM R3047*
BAP1 I191fs*2
CDH1 P127fs*41
CDH1 S70fs*13
CIC P1597fs*23
CTNNB1 W383R
FAM123B E370fs*8
MLL2 P2354fs*30
NTRK1 TPM3(NM_152263)-NTRK1(NM_002529) fusion (T10*; N9) §
PALB2 M296fs*1
RNF43 G659fs*41
SUFU A25fs*23
TP53 R273C

§ Refer to appendix for limitation statements related to detection of any copy number alterations, gene rearrangements, BRCA1/2 alterations, LOH, MSI, or TMB results in this section.

Please refer to appendix for Explanation of Clinical Significance Classification and for variants of unknown significance (VUS).

Note: The intended use (IU) statement and claims made on this sample report may not be up to date. For the latest version of the FoundationOne CDx claims and IU, please see the current label: www.foundationmedicine.com/f1cdx

ABOUT THE TEST FoundationOne®CDx is the first FDA-approved broad companion diagnostic for solid tumors.

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FoundationOne®CDx (F1CDx) is a next generation sequencing based in vitro diagnostic device for detection of substitutions, insertion and deletion alterations (indels), and copy number alterations (CNAs) in 324 genes and select gene rearrangements, as well as genomic signatures including microsatellite instability (MSI) and tumor mutational burden (TMB) using DNA isolated from formalin-fixed paraffin embedded (FFPE) tumor tissue specimens. The test is intended as a companion diagnostic to identify patients who may benefit from treatment with the targeted therapies listed in Table 1 in accordance with the approved therapeutic product labeling. Additionally, F1CDx is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with solid malignant neoplasms. Genomic findings other than those listed in Table 1 are not prescriptive or conclusive for labeled use of any specific therapeutic product.

The test is also used for detection of genomic loss of heterozygosity (LOH) from FFPE ovarian tumor tissue. Positive homologous recombination deficiency (HRD) status (F1CDx HRD defined as tBRCA-positive and/or LOH high) in ovarian cancer patients is associated with improved progression-free survival (PFS) from Rubraca (rucaparib) maintenance therapy in accordance with the RUBRACA product label.

The F1CDx assay will be performed at Foundation Medicine, Inc. sites located in Cambridge, MA and Morrisville, NC.

TABLE 1: COMPANION DIAGNOSTIC INDICATIONS

INDICATION	BIOMARKER	THERAPY
Non-small cell lung cancer (NSCLC)	*EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations	Gilotrif® (Afatinib), Iressa® (Gefitinib), Tagrisso® (Osimertinib), or Tarceva® (Erlotinib)
	EGFR exon 20 T790M alterations	Tagrisso® (Osimertinib)
	ALK rearrangements	Alecensa® (Alectinib), Xalkori® (Crizotinib), or Zykadia® (Ceritinib)
	BRAF V600E	Tafinlar® (Dabrafenib) in combination with Mekinist® (Trametinib)
Melanoma	*BRAF* V600E	Tafinlar® (Dabrafenib) or Zelboraf® (Vemurafenib)
	BRAF V600E and V600K	Mekinist® (Trametinib) or Cotellic® (Cobimetinib) in combination with Zelboraf® (Vemurafenib)
Breast cancer	*ERBB2* (HER2) amplification	Herceptin® (Trastuzumab), Kadcyla® (Ado-trastuzumab emtansine), or Perjeta® (Pertuzumab)
	PIK3CA C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R, H1047L, H1047R, and H1047Y alterations	Piqray® (Alpelisib)
Colorectal cancer	*KRAS* wild-type (absence of mutations in codons 12 and 13)	Erbitux® (Cetuximab)
	KRAS wild-type (absence of mutations in exons 2, 3, and 4) and *NRAS* wild type (absence of mutations in exons 2, 3, and 4)	Vectibix® (Panitumumab)
Ovarian cancer	*BRCA1/2* alterations	Lynparza® (Olaparib) or Rubraca® (Rucaparib)

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Interpretive content on this page and subsequent pages is provided as a professional service, and is not reviewed or approved by the FDA.

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Biomarker Findings

Microsatellite status - MSI-High
Tumor Mutational Burden - 35 Muts/Mb

Genomic Findings

For a complete list of the genes assayed, please refer to the Appendix.

KRAS wildtype
NRAS wildtype
NTRK1 TPM3-NTRK1 fusion
ATM R3047*
PALB2 M296fs*1
CTNNB1 W383R
RNF43 G659fs*41
SUFU A25fs*23
ASXL1 G645fs*58, S1335fs*115
BAP1 I191fs*2
CDH1 S70fs*13, P127fs*41
CIC P1597fs*23
FAM123B E370fs*8
MLL2 P2354fs*30
TP53 R273C

3 Disease relevant genes with no reportable alterations: ***BRAF, KRAS, NRAS***

15 Therapies with Clinical Benefit
0 Therapies with Lack of Response
47 Clinical Trials

BIOMARKER FINDINGS	THERAPIES WITH CLINICAL BENEFIT (IN PATIENT'S TUMOR TYPE)	THERAPIES WITH CLINICAL BENEFIT (IN OTHER TUMOR TYPE)
Microsatellite status - MSI-High	Nivolumab 2A	Atezolizumab
	Pembrolizumab 2A	Avelumab
		Cemiplimab
10 Trials *see p. 19*		Durvalumab
Tumor Mutational Burden - 35 Muts/Mb	Nivolumab	Atezolizumab
	Pembrolizumab	Avelumab
		Cemiplimab
10 Trials *see p. 21*		Durvalumab

GENOMIC FINDINGS	THERAPIES WITH CLINICAL BENEFIT (IN PATIENT'S TUMOR TYPE)	THERAPIES WITH CLINICAL BENEFIT (IN OTHER TUMOR TYPE)
KRAS - wildtype	Cetuximab 2A	none
0 Trials	Panitumumab 2A	
NRAS - wildtype	Cetuximab 2A	none
0 Trials	Panitumumab 2A	
NTRK1 - TPM3-NTRK1 fusion	Entrectinib 2A	Crizotinib
6 Trials *see p. 27*	Larotrectinib 2A	
ATM - R3047*	none	Niraparib
		Olaparib
		Rucaparib
10 Trials *see p. 23*		Talazoparib
PALB2 - M296fs*1	none	Niraparib
		Olaparib
		Rucaparib
10 Trials *see p. 28*		Talazoparib
CTNNB1 - W383R	none	none
10 Trials *see p. 25*		
RNF43 - G659fs*41	none	none
2 Trials *see p. 30*		
SUFU - A25fs*23	none	none
5 Trials *see p. 31*		

☐ *NCCN category*

GENOMIC FINDINGS WITH NO REPORTABLE THERAPEUTIC OR CLINICAL TRIAL OPTIONS

For more information regarding biological and clinical significance, including prognostic, diagnostic, germline, and potential chemosensitivity implications, see the Genomic Findings section.

NOTE Genomic alterations detected may be associated with activity of certain FDA-approved drugs; however, the agents listed in this report may have varied clinical evidence in the patient's tumor type.

Neither the therapeutic agents nor the trials identified are ranked in order of potential or predicted efficacy for this patient, nor are they ranked in order of level of evidence for this patient's tumor type.

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BIOMARKER

Microsatellite status

RESULT
MSI-High

POTENTIAL TREATMENT STRATEGIES

On the basis of prospective clinical evidence in multiple solid tumor types, MSI and associated increased mutational burden[1-2] may predict sensitivity to anti-PD-1 and anti-PD-L1 immune checkpoint inhibitors[2-6], including the approved therapies nivolumab[7-8], pembrolizumab[9-10], atezolizumab, avelumab, and durvalumab[3-5] . Pembrolizumab therapy resulted in a significantly higher objective response rate in MSI-H CRC compared with MSS CRC (40% vs. 0%)[9]. Similarly, a clinical study of nivolumab, alone or in combination with ipilimumab, in patients with CRC reported a significantly higher response rate in patients with tumors with high MSI than those without[7]. An earlier case study reported that nivolumab therapy resulted in a complete response in a patient with MSI-H CRC[8]. A Phase 1b trial of atezolizumab combined with bevacizumab reported PRs for 40% (4/10) of patients with MSI-H CRC[3]. MSI has not been found to be a predictive biomarker for combination chemotherapy regimens, including FOLFOX[11-12] and FOLFIRI[13-14]. MSI and deficient MMR are associated with lack of benefit of postsurgical fluorouracil (FU)-based adjuvant therapy[15-16] but may predict benefit from irinotecan chemotherapy[17].

FREQUENCY & PROGNOSIS

MSI-H colorectal cancers (CRCs) make up 10-15% of CRC cases[2,18-21]. Multiple studies have shown that MSI-H CRCs have a better prognosis than MSI-low (MSI-L) or microsatellite stable (MSS) tumors[18,22-28]. MSI-H CRCs are associated with certain pathologic and molecular features, including poor differentiation, right-sided and mucinous tumors, increased numbers of tumor infiltrating lymphocytes, diploidy, and a relatively high frequency of BRAF mutations[19-20,29].

FINDING SUMMARY

Microsatellite instability (MSI) is a condition of genetic hypermutability that generates excessive amounts of short insertion/deletion mutations in the genome; it generally occurs at microsatellite DNA sequences and is caused by a deficiency in DNA mismatch repair (MMR) in the tumor[20]. Defective MMR and consequent MSI occur as a result of genetic or epigenetic inactivation of one of the MMR pathway proteins, primarily MLH1, MSH2, MSH6, or PMS2[20,30-31]. This sample has a high level of MSI, equivalent to the clinical definition of an MSI-high (MSI-H) tumor: one with mutations in >30% of microsatellite markers[19,29,32]. MSI-H status indicates high-level deficiency in MMR and typically correlates with loss of expression of at least one, and often two, MMR family proteins[19-20,29,31]. While approximately 80% of MSI-H tumors arise due to somatic inactivation of an MMR pathway protein, about 20% arise due to germline mutations in one of the MMR genes[20], which are associated with a condition known as Lynch syndrome (also known as hereditary nonpolyposis colorectal cancer or HNPCC)[33]. Lynch syndrome leads to an increased risk of colorectal, endometrial, gastric, and other cancers[33-35] and has an estimated prevalence in the general population ranging from 1:600 to 1:2000[36-38]. Therefore, in the appropriate clinical context, germline testing of MLH1, MSH2, MSH6, and PMS2 is recommended.

BIOMARKER

Tumor Mutational Burden

RESULT
35 Muts/Mb

POTENTIAL TREATMENT STRATEGIES

On the basis of clinical evidence in solid tumors, increased TMB may be associated with greater sensitivity to immunotherapeutic agents, including anti-PD-L1[39-41] and anti-PD-1 therapies[39-42]. A large-scale retrospective analysis of immune checkpoint inhibitor efficacy in CRC reported significantly improved OS for patients with tumors harboring TMB ≥ 12 Muts/Mb compared to those with tumors with TMB < 12 Muts/Mb[39]. Another study reported that a TMB ≥ 12 Muts/Mb cutoff identifies >99% of MSI-High CRC cases but only 3% of MSS cases, indicating the utility of this cutoff for identification of patients with CRC likely to benefit from treatment with immune checkpoint inhibitors[43].

FREQUENCY & PROGNOSIS

Elevated TMB has been reported in 8-25% of colorectal cancer (CRC) samples[21,44-46]. Multiple studies have reported that the majority (up to 90%) of hypermutant CRC cases exhibit high levels of microsatellite instability (MSI-H) and mismatch repair deficiency (MMR-D)[21,46]. Increased TMB is significantly associated with MSI-H and MMR-D, with studies reporting that 100% of MSI-H CRCs harbor elevated TMB and, conversely, that 100% of tumors with low TMB harbor intact MMR[44-46]. A subset of CRCs that harbor increased TMB but not MSI-H are driven by mutations in POLE, which lead to an "ultramutated" phenotype with especially high TMB[21,46]. Tumors with increased TMB harbor BRAF V600E mutations more frequently than those with low TMB[21,46], whereas TMB-low tumors more frequently harbor mutations in TP53 and APC[21]. Although direct associations between blood or tissue TMB and prognosis of patients with CRC have not been reported, multiple studies have shown that MSI-H CRCs have a better prognosis than MSI-low (MSI-L) or microsatellite stable (MSS) tumors[18,22-28].

FINDING SUMMARY

Tumor mutational burden (TMB, also known as mutation load) is a measure of the number of somatic protein-coding base substitution and insertion/deletion mutations occurring in a tumor specimen. TMB is affected by a variety of causes, including exposure to mutagens such as ultraviolet light in melanoma[47-48] and cigarette smoke in lung cancer[10,49], mutations in the proofreading domains of DNA polymerases encoded by the POLE and POLD1 genes[21,50-53], and microsatellite instability (MSI)[21,50,53]. This sample harbors a TMB level that may be associated with sensitivity to PD-1- or PD-L1-targeting immune checkpoint inhibitors, alone or in combination with other agents[39,43].

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GENE
KRAS

ALTERATION
wildtype

POTENTIAL TREATMENT STRATEGIES
Lack of mutations in KRAS or NRAS is associated with clinical benefit of treatment with EGFR-targeting antibodies cetuximab[54-57] or panitumumab[58-60] in patients with CRC. Therefore, these agents are indicated for treatment of patients with CRC lacking such mutations (NCCN Guidelines v2.2019).

FREQUENCY & PROGNOSIS
Approximately 50-65% of colorectal cancers (CRCs) have been reported to lack KRAS mutations[61-69]. Numerous studies have reported that KRAS wild-type status is associated with decreased metastasis, better clinicopathological features, and longer survival of patients with CRC[63-66,70-71].

FINDING SUMMARY
KRAS encodes a member of the RAS family of small GTPases. Activating mutations in RAS genes can cause uncontrolled cell proliferation and tumor formation[72-73]. No alterations in KRAS were identified in this case.

GENE
NRAS

ALTERATION
wildtype

POTENTIAL TREATMENT STRATEGIES
Lack of mutations in KRAS or NRAS is associated with clinical benefit of treatment with EGFR-targeting antibodies cetuximab[54-57] or panitumumab[58-60] in patients with CRC. Therefore, these agents are indicated for treatment of patients with CRC lacking such mutations (NCCN Guidelines v2.2019).

FREQUENCY & PROGNOSIS
The majority of colorectal cancers (CRCs) (91-98%) have been reported to lack NRAS mutations[21,69,74-79]. NRAS wild-type status has been reported to be associated with decreased frequency of metastasis[69] and longer survival[79-80] of patients with CRC.

FINDING SUMMARY
NRAS encodes a member of the RAS family of small GTPases that mediate transduction of growth signals. Activation of RAS signaling causes cell growth, differentiation, and survival by activating the RAF-MAPK-ERK, PI3K, and other pathways[72]. No alterations in NRAS were identified in this case.

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