PATIENT

DISEASE Colon adenocarcinoma (CRC)
NAME Galloway, David
DATE OF BIRTH 08 July 1970
SEX Male
MEDICAL RECORD # 237351

PHYSICIAN

ORDERING PHYSICIAN Blitman, Maury
MEDICAL FACILITY Cancer Care Northwest - Spokane Valley
ADDITIONAL RECIPIENT None
MEDICAL FACILITY ID 312271
PATHOLOGIST Provided, Not

SPECIMEN

SPECIMEN SITE Colon
SPECIMEN ID SH-19-14386-A5
SPECIMEN TYPE Block
DATE OF COLLECTION 19 July 2019
SPECIMEN RECEIVED 21 October 2020

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 MS-Stable §
Tumor Mutational Burden 3 Muts/Mb §
APC S1344*
CCND1 amplification §
FGF19 amplification §
FGF4 amplification §
TP53 splice site 376-1G>A

§ 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).

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

Electronically signed by Tyler Janovitz, M.D., PhD | 28 October 2020
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Sample Preparation: 7010 Kit Creek Road, Morrisville, NC 27560 - CLIA: 34D2044309
Sample Analysis: 7010 Kit Creek Road, Morrisville, NC 27560 - CLIA: 34D2044309
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FoundationOne®CDx (F1CDx) is a qualitative next generation sequencing based in vitro diagnostic test that uses targeted high throughput hybridization-based capture technology 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 formalin-fixed, paraffin-embedded (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 is 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)
	MET single nucleotide variants (SNVs) and indels that lead to *MET* exon 14 skipping	Tabrecta™ (Capmatinib)
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 alteration	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)
Cholangiocarcinoma	*FGFR2* fusions and select rearrangements	Pemazyre™ (Pemigatinib)
Prostate cancer	Homologous Recombination Repair (HRR) gene (*BRCA1, BRCA2, ATM, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D* and *RAD54L*) alterations	Lynparza® (Olaparib)
Solid Tumors	*TMB* ≥ 10 mutations per megabase	Keytruda® (Pembrolizumab)

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FOUNDATIONONE®CDx

PATIENT	TUMOR TYPE	REPORT DATE
Galloway, David	Colon adenocarcinoma (CRC)	28 Oct 2020
	COUNTRY CODE US	ORDERED TEST # ORD-0927864-01

ABOUT THE TEST FoundationOne®CDx is the first and only FDA-Approved comprehensive companion diagnostic for all solid tumors.

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

PATIENT

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Biomarker Findings

Microsatellite status - MS-Stable
Tumor Mutational Burden - 3 Muts/Mb

Genomic Findings

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

KRAS wildtype
NRAS wildtype
CCND1 amplification
APC S1344*
FGF19 amplification
FGF4 amplification
TP53 splice site 376-1G>A

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

5 Therapies with Clinical Benefit
0 Therapies with Lack of Response
10 Clinical Trials

BIOMARKER FINDINGS	ACTIONABILITY	
Microsatellite status - MS-Stable	No therapies or clinical trials. see Biomarker Findings section	
Tumor Mutational Burden - 3 Muts/Mb	No therapies or clinical trials. see Biomarker Findings section	
GENOMIC FINDINGS	**THERAPIES WITH CLINICAL BENEFIT (IN PATIENT'S TUMOR TYPE)**	**THERAPIES WITH CLINICAL BENEFIT (IN OTHER TUMOR TYPE)**
KRAS - wildtype	Cetuximab 2A Panitumumab 2A	none
0 Trials		
NRAS - wildtype	Cetuximab 2A Panitumumab 2A	none
0 Trials		
CCND1 - amplification	none	Abemaciclib Palbociclib Ribociclib
10 Trials *see p. 10*		

☐ *NCCN category*

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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
MS-Stable

POTENTIAL TREATMENT STRATEGIES

On the basis of clinical evidence, MSS tumors are significantly less likely than MSI-H tumors to respond to anti-PD-1 immune checkpoint inhibitors[1-3], including approved therapies nivolumab and pembrolizumab[4]. In a retrospective analysis of 361 patients with solid tumors treated with pembrolizumab, 3% were MSI-H and experienced a significantly higher ORR compared with non-MSI-H cases (70% vs. 12%, p=0.001)[5]. For patients with chemotherapy-refractory metastatic colorectal cancer, 92% of which were MSS or MSI-Intermediate, a Phase 3 trial reported no OS advantage from the combination of the PD-L1 inhibitor atezolizumab plus cobimetinib relative to regorafenib (8.9 vs. 8.5 months, HR=1.00); atezolizumab monotherapy similarly did not prolong OS (7.1 vs. 8.5 months, HR=1.19)[6]. MSI has not been found to be a predictive biomarker for combination chemotherapy regimens, including FOLFOX[7-8] and FOLFIRI[9-10]. Patients with MSS CRC are more likely to benefit from postsurgical fluorouracil (FU)-based adjuvant therapy[11-12] but less likely to benefit from irinotecan chemotherapy[13].

FREQUENCY & PROGNOSIS

MSS colorectal cancers (CRCs) make up 70-85% of CRC cases[3,14-18]. MSS colorectal cancers are molecularly heterogeneous, driven by diverse mechanisms such as extensive DNA methylation, oncogenic mutations in KRAS or BRAF, or chromosomal instability[18]. Multiple studies have shown that MSS CRCs have a worse prognosis than MSI-high tumors[14,19-25].

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[16]. 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[16,26-27]. This sample is microsatellite-stable (MSS), equivalent to the clinical definition of an MSS tumor: one with mutations in none of the tested microsatellite markers[15,28-29]. MSS status indicates MMR proficiency and typically correlates with intact expression of all MMR family proteins[15-16,27,29].

BIOMARKER

Tumor Mutational Burden

RESULT
3 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[30-32] and anti-PD-1 therapies[30-34]. In multiple pan-tumor studies, higher TMB has been reported to be associated with increased ORR and OS from treatment with immune checkpoint inhibitors[30-34]. Higher TMB was found to be significantly associated with improved OS upon immune checkpoint inhibitor treatment of patients with 9 types of advanced tumors[30]. Analyses across several solid tumor types reported that patients with higher TMB (defined as ≥16-20 Muts/Mb) achieved greater clinical benefit from PD-1 or PD-L1-targeting monotherapy, compared with patients with higher TMB treated with chemotherapy[35] or those with lower TMB treated with PD-1 or PD-L1-targeting agents[31]. However, the KEYNOTE 158 trial of pembrolizumab monotherapy in patients with solid tumors found significant improvement in ORR in patients with TMB ≥10 Muts/Mb (based on this assay or others) compared to those with TMB <10 Muts/Mb, in a large cohort that included multiple tumor types; similar findings were observed in the KEYNOTE 028 and 012 trials[33-34]. Together, these studies suggest that patients with TMB ≥10 Muts/Mb may derive clinical benefit from PD-1 or PD-L1 inhibitors. In CRC specifically, a retrospective analysis of immune checkpoint inhibitor efficacy reported significantly improved OS for patients with tumors harboring TMB ≥9.8 Muts/MB compared with those with tumors with TMB <9.8 Muts/Mb (– equivalency <12 Muts/Mb as measured by this assay)[30]. Another retrospective 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[36].

FREQUENCY & PROGNOSIS

Elevated TMB has been reported in 8-25% of colorectal cancer (CRC) samples[17,37-39]. 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)[17,39]. 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[37-39]. 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[17,39]. Tumors with increased TMB harbor BRAF V600E mutations more frequently than those with low TMB[17,39], whereas TMB-low tumors more frequently harbor mutations in TP53 and APC[17]. 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[14,19-25].

FINDING SUMMARY

Tumor mutation 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[40-41] and cigarette smoke in lung cancer[42-43], treatment with temozolomide-based chemotherapy in glioma[44-45], mutations in the proofreading domains of DNA polymerases encoded by the POLE and POLD1 genes[17,46-48], and microsatellite instability (MSI)[17,46,49]. This sample harbors a TMB below levels that would be predicted to be associated with sensitivity to PD-1- or PD-L1-targeting immune checkpoint inhibitors, alone or in combination with other agents[30,34,36].

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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[50-53] or panitumumab[54-56] 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[57-65]. Numerous studies have reported that KRAS wild-type status is associated with decreased metastasis, better clinicopathological features, and longer survival of patients with CRC[59-62,66-67].

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[68-69]. 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[50-53] or panitumumab[54-56] 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[17,65,70-75]. NRAS wild-type status has been reported to be associated with decreased frequency of metastasis[65] and longer survival[75-76] 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[68]. No alterations in NRAS were identified in this case.

GENE
CCND1

ALTERATION
amplification

POTENTIAL TREATMENT STRATEGIES
Amplification or overexpression of CCND1 may predict sensitivity to CDK4/6 inhibitors, such as abemaciclib, palbociclib, and ribociclib[77-81], although as monotherapy these agents have shown limited activity in tumor types other than breast cancer[80,82]. In refractory advanced solid tumors with CCND1 (n=39) or CCND3 (n=1) amplification and retinoblastoma protein expression, palbociclib resulted in SD for 39% (14/36) of patients and a median PFS of 1.8 months in the NCI-MATCH trial[83]; 4 patients (13%, 4/36 overall) with squamous cell carcinomas (lung, esophageal, or laryngeal) or adenoid cystic carcinoma experienced prolonged SD in this study[83]. Among 9 patients with CCND1-amplified advanced solid tumors, 1 patient with bladder cancer responded to ribociclib in a Phase 2 trial[84].

FREQUENCY & PROGNOSIS
While CCND1 amplification was detected in <1% of cases in the Colorectal Adenocarcinoma TCGA dataset[17], another study reported CCND1 copy number gain in 36% (22/60) of cases[85]. CCND1 copy number gain was more prevalent in stage 4 tumors (67%; 10/15) than in stage 1-3 tumors (27%; 12/45)[85]. High expression of cyclin D1 has been reported in 55-68% of CRC cases and has been found to occur more frequently in tumors exhibiting microsatellite instability (MSI)[85-91]. The role of cyclin D1 expression in prognosis for patients with CRC is unclear, with several studies correlating high cyclin D1 expression with improved survival and better outcome and other studies associating cyclin D1 with lymph node metastasis, tumor invasiveness, and poor overall survival[85-87,92-94].

FINDING SUMMARY
CCND1 encodes cyclin D1, a binding partner of the kinases CDK4 and CDK6, that regulates RB activity and cell cycle progression. Amplification of CCND1 has been positively correlated with cyclin D1 overexpression[95] and may lead to excessive proliferation[96-97].

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GENE

APC

ALTERATION
S1344*

TRANSCRIPT ID
NM_000038

CODING SEQUENCE EFFECT
4031C>A

VARIANT ALLELE FREQUENCY (% VAF)
62.47%

POTENTIAL TREATMENT STRATEGIES

There are no approved drugs targeted to APC defects or WNT upregulation in solid tumors. Preclinical studies have reported that APC inactivation or beta-catenin activation confer synthetic lethality when TRAIL receptors are upregulated and the TRAIL death receptor program is activated[98]. In addition, the COX-2 inhibitor celecoxib was shown to reduce WNT signaling in cancer cell lines[99-100]. A preclinical study has found that a small-molecule tankyrase inhibitor shows some activity in APC-mutant CRC models[101].

FREQUENCY & PROGNOSIS

APC alterations have been found in 77% of tumors in the Colorectal Adenocarcinoma TCGA dataset[17]. Inactivation of APC leads to activation of the Wnt/beta-catenin pathway, which is thought to play a role in the adenoma-carcinoma transition in some cancers, including colorectal cancer (CRC)[102]. The prognostic significance of APC mutations in sporadic CRC remains unclear[103].

FINDING SUMMARY

APC (adenomatous polyposis coli) encodes a tumor suppressor with critical roles in regulating cell division and adhesion. APC interacts with beta-catenin and controls signaling in the WNT pathway, which regulates embryonic development and cell differentiation[104]. APC alterations that disrupt the beta-catenin binding domain (amino acids 1020-2035), such as observed here, are likely to impair APC binding to beta-catenin and may upregulate Wnt signaling[105-109] and are therefore predicted to be inactivating. Germline mutations in APC are found in more than 90% of patients with familial adenomatous polyposis (FAP)[110-112]. The prevalence for FAP in the general population is estimated to be 1:8,300 from birth[113], and in the appropriate clinical context germline testing of APC is recommended.

GENE

FGF19

ALTERATION
Amplification

POTENTIAL TREATMENT STRATEGIES

There are no targeted therapies that directly address genomic alterations in FGF19. However, FGF19 amplification predicts sensitivity to FGFR4 inhibitors in liver cancer cell lines[114-115]; selective FGFR4 inhibition reduced tumor burden in an FGF19-amplified HCC xenograft model[116]. A Phase 1 study of the FGFR4 inhibitor fisogatinib (BLU-554) for patients with previously treated hepatocellular carcinoma (HCC), most of whom had received prior sorafenib treatment, reported a 16.7% ORR (11/66, 1 CR, ongoing for >1.5 years) and a median PFS of 3.3 months for FGF19-IHC-positive patients; poorer outcomes (0% ORR, PFS of 2.3 months) were observed for patients with negative or unknown FGF19 IHC scores[117]. Acquisition of FGFR4 mutations may represent a mechanism of resistance for patients with FGF19 overexpression who initially responded but then progressed on fisogatinib[115]. Preliminary results from the dose escalation part of a Phase 1/2 study evaluating another FGFR4 inhibitor, FGF401, showed an ORR of 7.6% (4/53), SD rate of 52.8% (28/53), and a median time to progression of 4.1 months; responses were observed in both FGF19-positive and FGF19-negative cases[118]. In a retrospective analysis, a trend toward response to sorafenib treatment and FGF19 copy number gain was observed in patients with HCC, and 2 patients harboring FGF19 copy number gain experienced a CR[119]. A case study reported activity of pan-FGFR inhibitors in FGF-amplified cancers; following treatment with a selective pan-FGFR inhibitor, a patient with head and neck squamous cell carcinoma (HNSCC) and amplification of 11q13 (FGF3, FGF4, FGF19) and 12p13 (FGF6 and FGF23) experienced a radiologic CR[120]. Other therapies targeting FGF19 or FGFR4 signaling are in development[121].

FREQUENCY & PROGNOSIS

In the TCGA datasets, FGF19 amplification has been reported with highest incidence in esophageal carcinoma (34%), head and neck squamous cell carcinoma (23%), breast carcinoma (14%), lung squamous cell carcinoma (13%), and bladder urothelial carcinoma (10%) (cBioPortal, 2020). In HCC, FGF19 is an important driver gene[116,122-123], and FGF19 protein expression correlates with tumor progression and poorer prognosis[124]. Exogenous FGF19 has been shown to promote prostate cancer tumorigenesis in a preclinical study[125], and the presence of FGF19-positive tissues is an independent factor for worse prognosis following radical prostatectomy[126].

FINDING SUMMARY

FGF19 encodes fibroblast growth factor 19, an FGFR4 ligand involved with bile acid synthesis and hepatocyte proliferation in the liver[116,127]. FGF19 lies in a region of chromosome 11q13 frequently amplified in a diverse range of malignancies that also contains FGF3, FGF4, and CCND1[128]. Correlation between FGF19 amplification and protein expression has been demonstrated in hepatocellular carcinoma (HCC)[129] but was not observed in several other tumor types[123].

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GENE
FGF4

ALTERATION
amplification

POTENTIAL TREATMENT STRATEGIES

FGF_4 amplification and overexpression was associated with cell sensitivity to the multikinase inhibitor sorafenib in preclinical studies[130-131] and amplification of FGF_4/FGF_3 in HCC significantly correlated with patient response to sorafenib ($p=0.006$)[130]. Limited data suggest that pan-FGFR inhibitors show activity in FGF amplified cancers; following treatment with a selective pan-FGFR inhibitor, a patient with head and neck squamous cell carcinoma (HNSCC) and amplification of 11q13 (FGF_3, FGF_4, FGF_{19}) and 12p13 (FGF6 and FGF_{23}) experienced a radiologic CR[132].

FREQUENCY & PROGNOSIS

FGF_4 lies in a region of chromosome 11q13 that also contains FGF_{19}, FGF_3, and $CCND_1$, the latter gene encoding cyclin D_1, a key regulator of cell cycle progression. This chromosomal region is frequently amplified in a diverse range of malignancies[96] including esophageal carcinoma (35%), head and neck squamous cell carcinoma (HNSCC; 23%), breast invasive carcinoma (14%), lung squamous cell carcinoma (13%), bladder urothelial carcinoma (10%), ovarian serous cystadenocarcinoma (5%), stomach adenocarcinoma (7%), skin melanoma (4%), and hepatocellular carcinoma (HCC; 5%), however FGF_4 amplification is rare in hematopoietic and lymphoid malignancies, reported in less than 1% of samples analyzed (cBioPortal, 2020).

FINDING SUMMARY

FGF_4 encodes fibroblast growth factor 4, which plays a central role in development of the teeth[133] and acts synergistically with other FGFs and SHH (sonic hedgehog) to regulate limb outgrowth in vertebrate development[134]. FGF_4 lies in a region of chromosome 11q13 that also contains FGF_{19}, FGF_3, and $CCND_1$, the latter gene encoding cyclin D_1, a key regulator of cell cycle progression. Amplification of FGF_4, along with that of FGF_3, FGF_{19}, and $CCND_1$, has been reported in a variety of cancers[96,130,135-138] and may confer sensitivity to the multi-kinase inhibitor sorafenib[130].

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GENE
TP53

ALTERATION
splice site 376-1G>A

TRANSCRIPT ID
NM_000546

CODING SEQUENCE EFFECT
376-1G>A

VARIANT ALLELE FREQUENCY (% VAF)
47.85%

POTENTIAL TREATMENT STRATEGIES

There are no approved therapies to address TP53 mutation or loss. However, tumors with TP53 loss of function alterations may be sensitive to the WEE1 inhibitor adavosertib[139-142], or p53 gene therapy and immunotherapeutics such as SGT-53[143-147] and ALT-801[148]. In a Phase 1 study, adavosertib in combination with gemcitabine, cisplatin, or carboplatin elicited PRs in 9.7% (17/176) and SDs in 53.4% (94/176) of patients with solid tumors; the response rate was 21.1% (4/19) in patients with TP53 mutations versus 12.1% (4/33) in patients who were TP53 wild-type[149]. A Phase 2 trial of adavosertib in combination with chemotherapy (gemcitabine, carboplatin, paclitaxel, or doxorubicin) reported a 31.9% (30/94, 3 CR) ORR and a 73.4% (69/94) DCR in patients with platinum-refractory TP53-mutated ovarian, Fallopian tube, or peritoneal cancer[150]. A smaller Phase 2 trial of adavosertib in combination with carboplatin achieved a 42.9% (9/21, 1 CR) ORR and a 76.2% (16/21) DCR in patients with platinum-refractory TP53-mutated ovarian cancer[151]. The combination of adavosertib with paclitaxel and carboplatin in patients with TP53-mutated ovarian cancer also significantly increased PFS compared with paclitaxel and carboplatin alone[152]. In the Phase 2 VIKTORY trial, patients with TP53-mutated metastatic and/or recurrent gastric cancer experienced a 24.0% (6/25) ORR with adavosertib combined with paclitaxel[153]. A Phase 1 trial of neoadjuvant adavosertib in combination with cisplatin and docetaxel for head and neck squamous cell carcinoma (HNSCC) elicited a 71.4% (5/7) response rate for patients with TP53 alterations[154]. In a Phase 1b clinical trial of SGT-53 in combination with docetaxel in patients with solid tumors, 75.0% (9/12) of evaluable patients experienced clinical benefit, including 2 confirmed and 1 unconfirmed PRs and 2 instances of SD with significant tumor shrinkage[147]. Additionally, the combination of a CHK1 inhibitor and irinotecan reportedly reduced tumor growth and prolonged survival in a TP53-mutated, but not TP53-wild-type, breast cancer xenotransplant mouse model[155]. ATR inhibitor treatment of chronic lymphocytic leukemia (CLL) cells with biallelic inactivation of TP53 suppressed cell viability, promoted DNA damage, and attenuated xenograft growth in preclinical studies[156-157]; however, ATR inhibitors as monotherapy had little effect on these parameters in solid tumor models in other preclinical studies[158-159]. Therefore, it is unclear whether TP53 inactivation predicts sensitivity to ATR inhibition.

FREQUENCY & PROGNOSIS

TP53 mutations have been reported in up to 60% of colorectal cancer cases[17,160-165]. A study reported p53 expression in 49% of analyzed colorectal cancer cases[90]. TP53 mutation has not been consistently demonstrated to be a significant independent prognostic marker in the context of CRC[166]. Variants seen in this gene have been reported to occur in clonal hematopoiesis of indeterminate potential (CHIP), an age-related process in which hematopoietic stem cells acquire somatic mutations that allow for clonal expansion[167-172]. CHIP is associated with increased mortality, risk of coronary heart disease, risk of ischemic stroke, and risk of secondary hematologic malignancy[167-169]. Clinical management of patients with CHIP may include monitoring for hematologic changes and reduction of controllable risk factors for cardiovascular disease[173]. Comprehensive genomic profiling of solid tumors detects nontumor alterations that are due to CHIP[171,174-175]. Patient-matched peripheral blood mononuclear cell sequencing is required to conclusively determine if this alteration is present in tumor or is secondary to CHIP.

FINDING SUMMARY

Functional loss of the tumor suppressor p53, which is encoded by the TP53 gene, is common in aggressive advanced cancers[176]. Alterations that have been functionally characterized as inactivating and/or result in the disruption or partial or complete loss of the region encoding the TP53 DNA-binding domain (DBD, aa 100-292) or the tetramerization domain (aa 325-356), such as observed here, are thought to dysregulate the transactivation of p53-dependent genes and are predicted to promote tumorigenesis[177-181]. One or more of the TP53 variants observed here has been described in the ClinVar database as a likely pathogenic or pathogenic germline mutation (by an expert panel or multiple submitters with no conflicts) associated with Li-Fraumeni syndrome (ClinVar, Mar 2020)[182]. Follow-up germline testing would be needed to distinguish whether the finding in this patient is somatic or germline. Germline mutations in TP53 are associated with the very rare autosomal dominant disorder Li-Fraumeni syndrome and the early onset of many cancers[183-185], including sarcomas[186-187]. Estimates for the prevalence of germline TP53 mutations in the general population range from 1:5,000[188] to 1:20,000[187]. For pathogenic TP53 mutations identified during tumor sequencing, the rate of germline mutations was 1% in the overall population and 6% in tumors arising before age 30[189]. In the appropriate clinical context, germline testing of TP53 is recommended.

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Cetuximab

Assay findings association

KRAS
wildtype

NRAS
wildtype

AREAS OF THERAPEUTIC USE

Cetuximab is a monoclonal antibody that targets EGFR. It is FDA approved for the treatment of head and neck squamous cell carcinoma (HNSCC) and KRAS-wild-type, EGFR-expressing metastatic colorectal cancer (CRC). Cetuximab is also approved for BRAF V600E-mutated CRC in combination with the BRAF inhibitor encorafenib. Please see the drug label for full prescribing information.

GENE ASSOCIATION

Therapies targeting EGFR, including cetuximab, have been shown to have significant clinical activity in patients with CRC[50-53,190-191] ; wild-type KRAS and NRAS are predictive biomarkers for the efficacy of cetuximab in metastatic CRC (NCCN Guidelines v2.2019).

SUPPORTING DATA

Cetuximab has been shown to improve OS, PFS, and response rate in patients with KRAS wild-type CRC, both as first-line combination therapy with FOLFIRI or FOLFOX4[50-51,191] and as monotherapy or combination therapy with irinotecan for chemotherapy-refractory patients[52-53,190] . A study of first line cetuximab in patients with KRAS/NRAS/BRAF mutation-negative metastatic CRC resulted in limited efficacy, with 10.5% (2/19) of participants experiencing PRs and 57.9% (11/19) experiencing SDs[192]. The Phase 2 AVETUX trial of cetuximab combined with avelumab and mFOLFOX6 for patients with RAS/BRAF wild-type metastatic CRC resulted in an ORR of 79.5% (6 CR and 25 PRs, n=39) and a DCR of 92.3%[193].

Panitumumab

Assay findings association

KRAS
wildtype

NRAS
wildtype

AREAS OF THERAPEUTIC USE

Panitumumab is a monoclonal antibody that targets EGFR. It is FDA approved to treat KRAS wild-type and NRAS wild-type metastatic colorectal cancer (CRC) combined with chemotherapy or as monotherapy for patients who have progressed on prior chemotherapy. Please see the drug label for full prescribing information.

GENE ASSOCIATION

Therapies targeting EGFR, including panitumumab, have been shown to have significant clinical activity in patients with CRC[54,194-195] ; wild-type KRAS and NRAS are predictive biomarkers for the efficacy of panitumumab in metastatic CRC (NCCN Guidelines v2.2019).

SUPPORTING DATA

Panitumumab has been shown to improve OS, PFS, and ORR in patients with KRAS wild-type CRC, both as first-line combination therapy with FOLFOX4[54] and as monotherapy for chemotherapy-refractory patients[194-195] . An open-label, randomized Phase 2 trial reported that in patients with unresectable RAS-wild-type colorectal adenocarcinoma treated with first-line panitumumab plus FOLFOX4, maintenance with a combination of panitumumab plus fluorouracil and leucovorin was superior to panitumumab monotherapy (10-month PFS 59% vs. 49%)[196].

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Abemaciclib

Assay findings association

CCND1
amplification

AREAS OF THERAPEUTIC USE

Abemaciclib inhibits the cyclin-dependent kinases 4 and 6 (CDK4/6) and is FDA approved to treat hormone receptor-positive (HR+), HER2-negative (HER2–) advanced or metastatic breast cancer in combination with an aromatase inhibitor as initial endocrine-based therapy for postmenopausal women, in combination with fulvestrant for women who have progressed on endocrine therapy, or as monotherapy for adults who have progressed on endocrine therapy and chemotherapy in the metastatic setting. Please see the drug label for full prescribing information.

GENE ASSOCIATION

On the basis of clinical data in breast cancer and mantle cell lymphoma[80,197], CCND1 amplification or activation may be associated with response to abemaciclib. In a Phase 1 study, 4/10 patients with CCND1-amplified breast cancer responded to single-agent abemaciclib, with all of the responders having HR+ tumors[80].

SUPPORTING DATA

Abemaciclib has been investigated primarily in the context of breast cancer[80,82,198]. In a Phase 1 study evaluating abemaciclib as monotherapy, 13% (2/15) of patients with colorectal carcinoma experienced stable disease[80].

Palbociclib

Assay findings association

CCND1
amplification

AREAS OF THERAPEUTIC USE

Palbociclib inhibits the cyclin-dependent kinases 4 and 6 (CDK4/6) and is FDA approved to treat hormone receptor (HR)-positive/HER2-negative advanced or metastatic breast cancer in combination with an aromatase inhibitor as first-line therapy for postmenopausal women or in combination with fulvestrant following progression on endocrine therapy. Please see the drug label for full prescribing information.

GENE ASSOCIATION

Clinical studies in liposarcoma and mantle cell lymphoma as well as responses in patients with breast cancer or melanoma indicate that activation of cyclin D-CDK4/6 may predict sensitivity to therapies such as palbociclib[81,84,199-200].

SUPPORTING DATA

Palbociclib has been studied primarily for the treatment of ER+ breast cancer[78,201-202]. Single-agent palbociclib has shown limited activity against solid tumors, with a Phase 1 study reporting no partial responses (PR) and a 16% (6/37) stable disease (SD) rate (>9 months)[77]. A Phase 2 trial of palbociclib in patients with KRAS-mutant colorectal cancer (CRC) also reported no responses, although SD was observed for 33% (5/15) of patients[203]. Palbociclib combined with the MEK inhibitor trametinib achieved ongoing partial responses for 2/28 (7%) of patients with solid tumors, including one patient with CRC and a NRAS Q61K mutation[204]. For various tumor types, preclinical studies suggest that palbociclib may be useful in combination with other therapies targeting oncogenic drivers such as MEK, BRAF, PI3K, or IGF1R[205-209].

Ribociclib

Assay findings association

CCND1
amplification

AREAS OF THERAPEUTIC USE

Ribociclib inhibits the cyclin-dependent kinases 4 and 6 (CDK4/6) and is FDA approved in combination with an aromatase inhibitor as first-line therapy to treat women with hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2–) advanced or metastatic breast cancer. Ribociclib is also approved in combination with fulvestrant to treat postmenopausal women with HR+, HER2– advanced or metastatic breast cancer, either as first-line therapy or following disease progression on endocrine therapy. Please see the drug label for full prescribing information.

GENE ASSOCIATION

On the basis of clinical responses for 3 patients with bladder cancer, BRAF/NRAS-wild-type melanoma, or ER-positive breast cancer[79,84], CCND1 amplification or expression may predict sensitivity to CDK4/6 inhibitors such as ribociclib. In a prospective trial, 1 out of 12 patients with CCND1-amplified solid tumors responded to ribociclib[84].

SUPPORTING DATA

Clinical data on the efficacy of ribociclib for the treatment of colorectal cancer are limited (PubMed, Aug 2020). The Phase 1 Signature study of ribociclib for the treatment of patients with CDK4/6 pathway activated tumors reported clinical benefit for 18.4% (19/103) of cases, 58% (11/19) of whom had p16INK4a mutation or loss[84]. Phase 1 studies of ribociclib for the treatment of patients with Rb+ advanced solid tumors reported 2.4% partial responses and 23.5-34.4% stable diseases (SD)[79,210]; the 3 responders had alterations in the CDK4/6 pathway[79].

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

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NOTE Clinical trials are ordered by gene and prioritized by: age range inclusion criteria for pediatric patients, proximity to ordering medical facility, later trial phase, and verification of trial information within the last two months. While every effort is made to ensure the accuracy of the information contained below, the information available in the public domain is continually updated and should be investigated by the physician or research staff. This is not a comprehensive list of all available clinical trials. Foundation Medicine displays a subset of trial options and ranks them in this order of descending priority: Qualification for pediatric trial → Geographical proximity → Later trial phase. Clinical trials listed here may have additional enrollment criteria that may require medical screening to determine final eligibility. For additional information about listed clinical trials or to conduct a search for additional trials, please see clinicaltrials.gov. Or, visit https://www.foundationmedicine.com/genomic-testing#support-services.

GENE
CCND1

ALTERATION
amplification

RATIONALE
CCND1 amplification or overexpression may activate CDK4/6 and may predict sensitivity to CDK4/6 inhibitors.

NCT03994796

PHASE 2

Genetic Testing in Guiding Treatment for Patients With Brain Metastases

TARGETS
ALK, ROS1, TRKA, TRKB, TRKC, CDK4, CDK6, PI3K, mTOR

LOCATIONS: Idaho, Washington, Montana, Oregon

NCT03297606

PHASE 2

Canadian Profiling and Targeted Agent Utilization Trial (CAPTUR)

TARGETS
VEGFRs, ABL, SRC, ALK, AXL, MET, ROS1, TRKA, TRKC, DDR2, KIT, EGFR, PD-1, CTLA-4, PARP, CDK4, CDK6, CSF1R, FLT3, RET, mTOR, ERBB2, ERBB3, BRAF, MEK, SMO

LOCATIONS: Vancouver (Canada), Edmonton (Canada), Saskatoon (Canada), Regina (Canada), London (Canada), Toronto (Canada), Kingston (Canada), Ottawa (Canada), Montreal (Canada)

NCT03099174

PHASE 1

This Study in Patients With Different Types of Cancer (Solid Tumours) Aims to Find a Safe Dose of Xentuzumab in Combination With Abemaciclib With or Without Hormonal Therapies. The Study Also Tests How Effective These Medicines Are in Patients With Lung and Breast Cancer.

TARGETS
CDK4, CDK6, IGF-1, IGF-2, Aromatase, ER

LOCATIONS: Nevada, California, Minnesota, North Carolina, Connecticut, Florida, Tampere (Finland), Turku (Finland), Helsinki (Finland), Plerin Sur Mer (France)

NCT02896335

PHASE 2

Palbociclib In Progressive Brain Metastases

TARGETS
CDK4, CDK6

LOCATIONS: Massachusetts

NCT03965845

PHASE 1/2

A Study of Telaglenastat (CB-839) in Combination With Palbociclib in Patients With Solid Tumors

TARGETS
CDK4, CDK6, GLS

LOCATIONS: Texas, Georgia

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ORDERED TEST # ORD-0927864-01

CLINICAL TRIALS

NCT03446157

PHASE 2

Palbociclib and Cetuximab in Metastatic Colorectal Cancer

TARGETS
EGFR, CDK4, CDK6

LOCATIONS: North Carolina

NCT03310879

PHASE 2

Study of the CDK4/6 Inhibitor Abemaciclib in Solid Tumors Harboring Genetic Alterations in Genes Encoding D-type Cyclins or Amplification of CDK4 or CDK6

TARGETS
CDK4, CDK6

LOCATIONS: Massachusetts

NCT02897375

PHASE 1

Palbociclib With Cisplatin or Carboplatin in Advanced Solid Tumors

TARGETS
CDK4, CDK6

LOCATIONS: Georgia

NCT03454035

PHASE 1

Ulixertinib/Palbociclib in Patients With Advanced Pancreatic and Other Solid Tumors

TARGETS
MAPK3, MAPK1, CDK4, CDK6

LOCATIONS: North Carolina

NCT03065062

PHASE 1

Study of the CDK4/6 Inhibitor Palbociclib (PD-0332991) in Combination With the PI3K/mTOR Inhibitor Gedatolisib (PF-05212384) for Patients With Advanced Squamous Cell Lung, Pancreatic, Head & Neck and Other Solid Tumors

TARGETS
PI3K-alpha, PI3K-gamma, mTORC1, mTORC2, CDK4, CDK6

LOCATIONS: Massachusetts

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NOTE One or more variants of unknown significance (VUS) were detected in this patient's tumor. These variants may not have been adequately characterized in the scientific literature at the time this report was issued, and/or the genomic context of these alterations makes their significance unclear. We choose to include them here in the event that they become clinically meaningful in the future.

DDR2	***ERG***	***KDR***	***MST1R***
I159T	G151A	T562N	G1385V
POLE	***RAD51***	***RET***	***ZNF217***
D490N	T76M	L56M	P368R

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INTENDED USE

FoundationOne®CDx (F1CDx) is a qualitative next generation sequencing based in vitro diagnostic test that uses targeted high throughput hybridization-based capture technology 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 formalin-fixed, paraffin-embedded (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 is 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)
	MET single nucleotide variants (SNVs) and indels that lead to *MET* exon 14 skipping	Tabrecta™ (Capmatinib)
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)
Cholangiocarcinoma	*FGFR2* fusions and select rearrangements	Pemazyre™ (Pemigatinib)
Prostate cancer	Homologous Recombination Repair (*HRR*) gene (*BRCA1, BRCA2, ATM, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D* and *RAD54L*) alterations	Lynparza® (Olaparib)
Solid Tumors	*TMB* ≥10 mutations per megabase	Keytruda® (Pembrolizumab)

The median exon coverage for this sample is N/A

TEST PRINCIPLE

FoundationOne®CDx (F1CDx) is performed exclusively as a laboratory service using DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tumor samples. The assay employs a single DNA extraction method from routine FFPE biopsy or surgical resection specimens, 50-1000 ng of which will undergo whole-genome shotgun library construction and hybridization-based capture of all coding exons from 309 cancer-related genes, one promoter region, one non-coding (ncRNA), and select intronic regions from 34 commonly rearranged genes, 21 of which also include the coding exons (refer to Table 2 and Table 3 for complete list of genes included in F1CDx). In total, the assay detects alterations in a total of 324 genes. Using the Illumina® HiSeq 4000 platform, hybrid capture-selected libraries are sequenced to high uniform depth (targeting >500X median coverage with >99% of exons at coverage >100X). Sequence data is then processed using a customized analysis pipeline designed to detect all classes of genomic alterations, including base substitutions, indels, copy number alterations (amplifications and homozygous gene deletions), and selected genomic rearrangements (e.g., gene fusions). Additionally, genomic signatures including microsatellite instability (MSI), tumor mutational burden (TMB), and positive homologous recombination deficiency (HRD) status (tBRCA-positive and/or LOH high) are reported.

PERFORMANCE CHARACTERISTICS

Please refer to product label:
foundationmedicine.com/f1cdx

LIMITATIONS

1. For *in vitro* diagnostic use.
2. For prescription use only. This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.
3. A negative result does not rule out the presence of a mutation below the limits of detection of the assay.
4. Samples with <25% tumor may have decreased sensitivity for the detection of CNAs including *ERBB2*.
5. Clinical performance of Tagrisso® (osimertinib) in patients with an *EGFR* exon 20 T790M mutation detected with an allele fraction <5% is ongoing and has not been established.
6. Concordance with other validated methods for CNA (with the exception of *ERBB2*) and gene rearrangement (with the exception of *ALK*) detection has not been demonstrated and will be provided in the post-market setting. Confirmatory testing using a clinically validated assay should be performed for all CNAs and rearrangements not associated with CDx claims noted in Table 1 of the Intended Use, but used for clinical decision making.
7. The MSI-H/MSS designation by FMI FoundationOne®CDx (F1CDx) test is based on genome wide analysis of 95 microsatellite loci and not based on the 5 or 7 MSI loci described in current clinical practice guidelines. Refer https://www.accessdata.fda.gov/cdrh_docs/pdf17/P170019B.pdf for additional details on methodology. The threshold for MSI-H/MSS was determined by analytical concordance to comparator assays (IHC and PCR) using uterine, cecum and colorectal cancer FFPE tissue. Patients with microsatellite status of "Cannot Be Determined" should be retested with an orthogonal (alternative) method. The clinical validity of the qualitative MSI designation has not been established.
8. TMB by F1CDx is determined by counting all synonymous and non-synonymous variants present at 5% allele frequency or greater (after filtering) and the total number is reported as mutations per megabase (mut/Mb) unit. Observed TMB is dependent on characteristics of the specific tumor focus tested for a patient (e.g., primary vs. metastatic, tumor content) and the testing platform used for the detection; therefore, observed TMB results may vary between different specimens for the same patient and between detection methodologies employed on the same sample. The TMB calculation may differ from TMB calculations used by other assays depending on variables such as the amount of genome interrogated, percentage of tumor, assay limit of detection (LoD), filtering of alterations included in the score, and the read depth and other bioinformatic test specifications. Refer to the SSED for a detailed description of these variables in FMI's TMB calculation https://www.accessdata.fda.gov/cdrh_docs/pdf17/P170019B.pdf. The clinical validity of TMB defined by this panel has been established for TMB as a qualitative output for a cut-off of 10 mutations per megabase but has not been established for TMB as a quantitative score.
9. Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care in a given community.
10. The test is intended to be performed on specific serial number-controlled instruments by Foundation Medicine, Inc.
11. Alterations in polyT homopolymer runs may not be reliably detected in BRCA1/2.
12. Certain large rearrangements in BRCA1/2 including large scale genomic deletions (affecting at least one whole exon), insertions or other deleterious genomic rearrangements including inversions or transversion events, may not be detected in an estimated 5% of ovarian cancer patients with BRCA1/2 mutations by F1CDx.
13. Certain potentially deleterious missense or small in-frame deletions in BRCA1/2 may not be reported under the "CDx associated findings" but may be reported in the "Other alterations and biomarkers identified" section in the patient report.
14. Alterations at allele frequencies below the established limit of detection may not be detected consistently.
15. Detection of LOH has been verified only for ovarian cancer patients.
16. Performance of the LOH classification has not been established for samples below 35% tumor content and with LOH scores near the cutoff of 16.
17. There may be potential interference of ethanol with LOH detection. The interfering effects of xylene, hemoglobin, and triglycerides on the LOH score have not been demonstrated.

MR Suite Version 1.0.0

FoundationOne CDx is designed to include genes known to be somatically altered in human solid tumors that are validated targets for therapy, either approved or in clinical trials, and/or that are unambiguous drivers of oncogenesis based on current knowledge. The current assay interrogates 324 genes as well as introns of 36 genes involved in rearrangements. The assay will be updated periodically to reflect new knowledge about cancer biology.

DNA GENE LIST: ENTIRE CODING SEQUENCE FOR THE DETECTION OF BASE SUBSTITUTIONS, INSERTION/DELETIONS, AND COPY NUMBER ALTERATIONS

ABL1	*ACVR1B*	*AKT1*	*AKT2*	*AKT3*	*ALK*	*ALOX12B*	*AMER1 (FAM123B)*	*APC*
AR	*ARAF*	*ARFRP1*	*ARID1A*	*ASXL1*	*ATM*	*ATR*	*ATRX*	*AURKA*
AURKB	*AXIN1*	*AXL*	*BAP1*	*BARD1*	*BCL2*	*BCL2L1*	*BCL2L2*	*BCL6*
BCOR	*BCORL1*	*BRAF*	*BRCA1*	*BRCA2*	*BRD4*	*BRIP1*	*BTG1*	*BTG2*
BTK	*C11orf30 (EMSY)*	*C17orf39 (GID4)*	*CALR*	*CARD11*	*CASP8*	*CBFB*	*CBL*	*CCND1*
CCND2	*CCND3*	*CCNE1*	*CD22*	*CD274 (PD-L1)*	*CD70*	*CD79A*	*CD79B*	*CDC73*
CDH1	*CDK12*	*CDK4*	*CDK6*	*CDK8*	*CDKN1A*	*CDKN1B*	*CDKN2A*	*CDKN2B*
CDKN2C	*CEBPA*	*CHEK1*	*CHEK2*	*CIC*	*CREBBP*	*CRKL*	*CSF1R*	*CSF3R*
CTCF	*CTNNA1*	*CTNNB1*	*CUL3*	*CUL4A*	*CXCR4*	*CYP17A1*	*DAXX*	*DDR1*
DDR2	*DIS3*	*DNMT3A*	*DOT1L*	*EED*	*EGFR*	*EP300*	*EPHA3*	*EPHB1*
EPHB4	*ERBB2*	*ERBB3*	*ERBB4*	*ERCC4*	*ERG*	*ERRFI1*	*ESR1*	*EZH2*
FAM46C	*FANCA*	*FANCC*	*FANCG*	*FANCL*	*FAS*	*FBXW7*	*FGF10*	*FGF12*
FGF14	*FGF19*	*FGF23*	*FGF3*	*FGF4*	*FGF6*	*FGFR1*	*FGFR2*	*FGFR3*
FGFR4	*FH*	*FLCN*	*FLT1*	*FLT3*	*FOXL2*	*FUBP1*	*GABRA6*	*GATA3*
GATA4	*GATA6*	*GNA11*	*GNA13*	*GNAQ*	*GNAS*	*GRM3*	*GSK3B*	*H3F3A*
HDAC1	*HGF*	*HNF1A*	*HRAS*	*HSD3B1*	*ID3*	*IDH1*	*IDH2*	*IGF1R*
IKBKE	*IKZF1*	*INPP4B*	*IRF2*	*IRF4*	*IRS2*	*JAK1*	*JAK2*	*JAK3*
JUN	*KDM5A*	*KDM5C*	*KDM6A*	*KDR*	*KEAP1*	*KEL*	*KIT*	*KLHL6*
KMT2A (MLL)	*KMT2D (MLL2)*	*KRAS*	*LTK*	*LYN*	*MAF*	*MAP2K1 (MEK1)*	*MAP2K2 (MEK2)*	*MAP2K4*
MAP3K1	*MAP3K13*	*MAPK1*	*MCL1*	*MDM2*	*MDM4*	*MED12*	*MEF2B*	*MEN1*
MERTK	*MET*	*MITF*	*MKNK1*	*MLH1*	*MPL*	*MRE11A*	*MSH2*	*MSH3*
MSH6	*MST1R*	*MTAP*	*MTOR*	*MUTYH*	*MYC*	*MYCL (MYCL1)*	*MYCN*	*MYD88*
NBN	*NF1*	*NF2*	*NFE2L2*	*NFKBIA*	*NKX2-1*	*NOTCH1*	*NOTCH2*	*NOTCH3*
NPM1	*NRAS*	*NSD3 (WHSC1L1)*	*NTSC2*	*NTRK1*	*NTRK2*	*NTRK3*	*P2RY8*	*PALB2*
PARK2	*PARP1*	*PARP2*	*PARP3*	*PAXS*	*PBRM1*	*PDCD1 (PD-1)*	*PDCD1LG2 (PD-L2)*	*PDGFRA*
PDGFRB	*PDK1*	*PIK3C2B*	*PIK3C2G*	*PIK3CA*	*PIK3CB*	*PIK3R1*	*PIM1*	*PMS2*
POLD1	*POLE*	*PPARG*	*PPP2R1A*	*PPP2R2A*	*PRDM1*	*PRKAR1A*	*PRKCI*	*PTCH1*
PTEN	*PTPN11*	*PTPRO*	*QKI*	*RAC1*	*RAD21*	*RAD51*	*RAD51B*	*RAD51C*
RAD51D	*RAD52*	*RAD54L*	*RAF1*	*RARA*	*RB1*	*RBM10*	*REL*	*RET*
RICTOR	*RNF43*	*ROS1*	*RPTOR*	*SDHA*	*SDHB*	*SDHC*	*SDHD*	*SETD2*
SF3B1	*SGK1*	*SMAD2*	*SMAD4*	*SMARCA4*	*SMARCB1*	*SMO*	*SNCAIP*	*SOCS1*
SOX2	*SOX9*	*SPEN*	*SPOP*	*SRC*	*STAG2*	*STAT3*	*STK11*	*SUFU*
SYK	*TBX3*	*TEK*	*TET2*	*TGFBR2*	*TIPARP*	*TNFAIP3*	*TNFRSF14*	*TP53*
TSC1	*TSC2*	*TYRO3*	*U2AF1*	*VEGFA*	*VHL*	*WHSC1*	*WT1*	*XPO1*
XRCC2	*ZNF217*	*ZNF703*						

DNA GENE LIST: FOR THE DETECTION OF SELECT REARRANGEMENTS

ALK	*BCL2*	*BCR*	*BRAF*	*BRCA1*	*BRCA2*	*CD74*	*EGFR*	*ETV4*
ETV5	*ETV6*	*EWSR1*	*EZR*	*FGFR1*	*FGFR2*	*FGFR3*	*KIT*	*KMT2A (MLL)*
MSH2	*MYB*	*MYC*	*NOTCH2*	*NTRK1*	*NTRK2*	*NUTM1*	*PDGFRA*	*RAF1*
RARA	*RET*	*ROS1*	*RSPO2*	*SDC4*	*SLC34A2*	*TERC**	*TERT***	*TMPRSS2*

*TERC is an NCRNA

**Promoter region of TERT is interrogated

ADDITIONAL ASSAYS: FOR THE DETECTION OF SELECT CANCER BIOMARKERS

Loss of Heterozygosity (LOH) score
Microsatellite (MS) status
Tumor Mutational Burden (TMB)

QUALIFIED ALTERATION CALLS (EQUIVOCAL AND SUBCLONAL)

An alteration denoted as "amplification –equivocal" implies that the FoundationOne®CDx assay data provide some, but not unambiguous, evidence that the copy number of a gene exceeds the threshold for identifying copy number amplification. The threshold used in FoundationOne CDx for identifying a copy number amplification is four (4) for *ERBB2* and six (6) for all other genes. Conversely, an alteration denoted as "loss – equivocal" implies that the FoundationOne CDx assay data provide some, but not unambiguous, evidence for homozygous deletion of the gene in question. An alteration denoted as "subclonal" is one that the FoundationOne CDx analytical methodology has identified as being present in <10% of the assayed tumor DNA.

PROFESSIONAL SERVICES FINDINGS

Incorporates analyses of peer-reviewed studies and other publicly available information identified by Foundation Medicine; these analyses and information may include associations between a molecular alteration (or lack of alteration) and one or more drugs with potential clinical benefit (or potential lack of clinical benefit), including drug candidates that are being studied in clinical research. Note: A finding of biomarker alteration does not necessarily indicate pharmacologic effectiveness (or lack thereof) of any drug or treatment regimen; a finding of no biomarker alteration does not necessarily indicate lack of pharmacologic effectiveness (or effectiveness) of any drug or treatment regimen.

RANKING OF ALTERATIONS AND THERAPIES

Biomarker and Genomic Findings

Therapies are ranked based on the following criteria: Therapies with clinical benefit in patient's tumor type (ranked alphabetically within each NCCN category) followed by therapies with clinical benefit in other tumor type (ranked alphabetically within each NCCN category).

Clinical Trials

Pediatric trial qualification → Geographical proximity → Later trial phase.

NATIONAL COMPREHENSIVE CANCER NETWORK® (NCCN®) CATEGORIZATION

Biomarker and genomic findings detected may be associated with certain entries within the NCCN Drugs & Biologics Compendium® (NCCN Compendium®) (www.nccn.org). The NCCN Categories of Evidence and Consensus indicated reflect the highest possible category for a given therapy in association with each biomarker or genomic finding. Please note, however, that the accuracy and applicability of these NCCN categories within a report may be impacted by the patient's clinical history, additional biomarker information, age, and/or co-occurring alterations. For additional information on the NCCN categories please refer to the NCCN Compendium®.

LEVEL OF EVIDENCE NOT PROVIDED

Drugs with potential clinical benefit (or potential lack of clinical benefit) are not evaluated for source or level of published evidence.

NO GUARANTEE OF CLINICAL BENEFIT

Foundation Medicine makes no promises or guarantees that a particular drug will be effective in the treatment of disease of any patient. This report also makes no promises or guarantees that a drug with potential lack of clinical benefit will in fact provide no clinical benefit.

NO GUARANTEE OF REIMBURSEMENT

Foundation Medicine makes no promises or guarantees that a healthcare provider, insurer or other third party payor, whether private or governmental, will reimburse a patient for the cost of FoundationOne CDx.

TREATMENT DECISIONS ARE RESPONSIBILITY OF PHYSICIAN

Drugs referenced may not be suitable for a particular patient. The selection of any, all or none of the drugs associated with potential clinical benefit (or potential lack of clinical benefit) resides with the physician. Indeed, the information in this Report must be considered in conjunction with all other relevant information regarding a particular patient, before the patient's treating physician recommends a course of treatment. Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care in a given community. A treating physician's decisions should not be based on a single test, such as this Test, or the information contained in this Report.

LOSS OF HETEROZYGOSITY SCORE

The LOH score is determined by analyzing SNPs spaced at 1Mb intervals across the genome on the FoundationOne CDx test and extrapolating an LOH profile, excluding arm- and chromosome-wide LOH segments. The LOH score result may be reported for epithelial ovarian, peritoneal, or Fallopian tube carcinomas. The LOH score will be reported as "Cannot Be Determined" if the sample is not of sufficient quality to confidently determine LOH.

MICROSATELLITE STATUS

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

TUMOR MUTATIONAL BURDEN

Tumor Mutational Burden (TMB) is determined by measuring the number of somatic mutations in sequenced genes on the FoundationOne CDx test and extrapolating to the genome as a whole. TMB is assayed for all FoundationOne CDx samples and is reported in Professional Services as the number of mutations per megabase (Muts/Mb) rounded to the nearest integer. Tumor Mutational Burden is reported as "Cannot Be Determined" if the sample is not of sufficient quality to confidently determine Tumor Mutational Burden.

VARIANT ALLELE FREQUENCY

Variant Allele Frequency (VAF) represents the fraction of sequencing reads in which the variant is observed. This attribute is not taken into account for therapy inclusion, clinical trial matching, or interpretive content. Caution is recommended in interpreting VAF to indicate the potential germline or somatic origin of an alteration, recognizing that tumor fraction and tumor ploidy of samples may vary.

Precision of VAF for base substitutions and indels

BASE SUBSTITUTIONS	%CV*
Repeatability	5.11 - 10.40
Reproducibility	5.95 - 12.31
INDELS	**%CV***
Repeatability	6.29 - 10.00
Reproducibility	7.33 - 11.71

**Interquartile Range = 1st Quartile to 3rd Quartile*

Electronically signed by Tyler Janovitz, M.D., PhD | 28 October 2020
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Genomic Findings with Evidence of Clinical Significance
Genomic findings listed at Level 2 are associated with clinical significance. Clinical significance may be indicated by evidence of therapeutic sensitivity or resistance and/or diagnostic, prognostic or other clinically relevant implications. Included in this category will be findings associated with clinical validity as supported by professional guidelines and/or peer-reviewed publications.

Genomic Findings with Potential Clinical Significance
Genomic findings listed at Level 3 are cancer-related mutations and biomarkers with potential clinical significance. These include findings in genes known to be associated with cancer and are supported by evidence from publicly available databases, and/or peer-reviewed publications.

A Fluid Approach to Reporting Levels
As additional information becomes available, as recognized by the clinical community (professional guidelines and/or peer-reviewed publications), findings may move between Levels 2 and 3 in accordance with the above descriptions.

SELECT ABBREVIATIONS

ABBREVIATION	DEFINITION
CR	Complete response
DCR	Disease control rate
DNMT	DNA methyltransferase
HR	Hazard ratio
ITD	Internal tandem duplication
MMR	Mismatch repair
muts/Mb	Mutations per megabase
NOS	Not otherwise specified
ORR	Objective response rate
OS	Overall survival
PD	Progressive disease
PFS	Progression-free survival
PR	Partial response
SD	Stable disease
TKI	Tyrosine kinase inhibitor

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1. Gatalica Z, et al. Cancer Epidemiol. Biomarkers Prev. (2014) pmid: 25392179
2. Kroemer G, et al. Oncoimmunology (2015) pmid: 26140250
3. Lal N, et al. Oncoimmunology (2015) pmid: 25949894
4. Le DT, et al. N. Engl. J. Med. (2015) pmid: 26028255
5. Ayers et al., 2016; ASCO-SITC Abstract P60
6. Ciardiello et al., 2018; ESMO Abstract LBA-004
7. Sinicrope FA, et al. J. Clin. Oncol. (2013) pmid: 24019539
8. Gavin PG, et al. Clin. Cancer Res. (2012) pmid: 23045248
9. Bertagnolli MM, et al. J. Clin. Oncol. (2009) pmid: 19273709
10. Van Cutsem E, et al. J. Clin. Oncol. (2009) pmid: 19451425
11. Ribic CM, et al. N. Engl. J. Med. (2003) pmid: 12867608
12. Sargent DJ, et al. J. Clin. Oncol. (2010) pmid: 20498393
13. Fallik D, et al. Cancer Res. (2003) pmid: 14522894
14. Guastadisegni C, et al. Eur. J. Cancer (2010) pmid: 20627535
15. Pawlik TM, et al. Dis. Markers (2004) pmid: 15528785
16. Kocarnik JM, et al. Gastroenterol Rep (Oxf) (2015) pmid: 26337942
17. Nature (2012) pmid: 22810696
18. Histopathology (2007) pmid: 17204026
19. Samowitz WS, et al. Cancer Epidemiol. Biomarkers Prev. (2001) pmid: 11535541
20. Elsaleh H, et al. Clin Colorectal Cancer (2001) pmid: 12445368
21. Brueckl WM, et al. Anticancer Res. () pmid: 12820457
22. Guldoboni M, et al. Am. J. Pathol. (2001) pmid: 11438476
23. Gryfe R, et al. N. Engl. J. Med. (2000) pmid: 10631274
24. Sinicrope FA, et al. Gastroenterology (2006) pmid: 16952542
25. Laghi L, et al. Dig Dis (2012) pmid: 22722556
26. You JF, et al. Br. J. Cancer (2010) pmid: 21081928
27. Balrwa NK, et al. Methods Mol. Biol. (2014) pmid: 24623249
28. Boland CR, et al. Cancer Res. (1998) pmid: 9823339
29. Boland CR, et al. Gastroenterology (2010) pmid: 20420947
30. Samstein RM, et al. Nat. Genet. (2019) pmid: 30643254
31. Goodman AM, et al. Mol. Cancer Ther. (2017) pmid: 28835386
32. Goodman AM, et al. Cancer Immunol Res (2019) pmid: 31405947
33. Cristescu R, et al. Science (2018) pmid: 30309915
34. Marabelle A, et al. Lancet Oncol. (2020) pmid: 32919526
35. Legrand et al., 2018; ASCO Abstract 12000
36. Fabrizio DA, et al. J Gastrointest Oncol (2018) pmid: 30151257
37. George et al., 2016; ASCO Abstract 3587
38. Nagahashi et al., 2016; ASCO Abstract e15103
39. Stadler ZK, et al. J. Clin. Oncol. (2016) pmid: 27022117
40. Pfeifer GP, et al. Mutat. Res. (2005) pmid: 15748635
41. Hill VK, et al. Annu Rev Genomics Hum Genet (2013) pmid: 23875803
42. Pfeifer GP, et al. Oncogene (2002) pmid: 12379884
43. Rizvi NA, et al. Science (2015) pmid: 25765070
44. Johnson BE, et al. Science (2014) pmid: 24336570
45. Choi S, et al. Neuro-oncology (2018) pmid: 29452419
46. Cancer Genome Atlas Research Network, et al. Nature (2013) pmid: 23636398
47. Briggs S, et al. J. Pathol. (2013) pmid: 23447401
48. Heitzer E, et al. Curr. Opin. Genet. Dev. (2014) pmid: 24583393
49. Roberts SA, et al. Nat. Rev. Cancer (2014) pmid: 25568919
50. Van Cutsem E, et al. J. Clin. Oncol. (2011) pmid: 21502544
51. Bokemeyer C, et al. Ann. Oncol. (2011) pmid: 21228335
52. Karapetis CS, et al. N. Engl. J. Med. (2008) pmid: 18946061
53. De Roock W, et al. Ann. Oncol. (2008) pmid: 17998284
54. Douillard JY, et al. Ann. Oncol. (2014) pmid: 24718886
55. Douillard JY, et al. N. Engl. J. Med. (2013) pmid: 24024839
56. Amado RG, et al. J. Clin. Oncol. (2008) pmid: 18316791
57. Lièvre A, et al. Cancer Res. (2006) pmid: 16618717
58. De Roock W, et al. Lancet Oncol. (2011) pmid: 21163703
59. Chen J, et al. BMC Cancer (2014) pmid: 25367198
60. Li W, et al. BMC Cancer (2015) pmid: 25929517
61. Hu J, et al. Medicine (Baltimore) (2016) pmid: 27977612
62. Zekri J, et al. Genet. Mol. Res. (2017) pmid: 28218784
63. Staudacher JJ, et al. Clin Transl Gastroenterol (2017) pmid: 29048416
64. Wang Y, et al. Virchows Arch. (2018) pmid: 29705968
65. Guo F, et al. Sci Rep (2018) pmid: 29666387
66. Mármol I, et al. Int J Mol Sci (2017) pmid: 28106826
67. Kwak MS, et al. Medicine (Baltimore) (2017) pmid: 28858102
68. Pylayeva-Gupta Y, et al. Nat. Rev. Cancer (2011) pmid: 21993244
69. Kahn S, et al. Anticancer Res. () pmid: 3310850
70. Pentheroudakis G, et al. BMC Cancer (2013) pmid: 23374602
71. Vaughn CP, et al. Genes Chromosomes Cancer (2011) pmid: 21305640
72. Janku F, et al. Target Oncol (2013) pmid: 23400451
73. De Roock W, et al. Lancet Oncol. (2010) pmid: 20619739
74. Irahara N, et al. Diagn. Mol. Pathol. (2010) pmid: 20736745
75. Schirripa M, et al. Int. J. Cancer (2015) pmid: 24806288
76. Cercek A, et al. Clin. Cancer Res. (2017) pmid: 28446505
77. Flaherty KT, et al. Clin. Cancer Res. (2012) pmid: 22090362
78. Finn RS, et al. Lancet Oncol. (2015) pmid: 25524798
79. Infante JR, et al. Clin. Cancer Res. (2016) pmid: 27542767
80. Patnaik A, et al. Cancer Discov (2016) pmid: 27217383
81. Leonard JP, et al. Blood (2012) pmid: 22383795
82. Dickler MN, et al. Clin. Cancer Res. (2017) pmid: 28533223
83. Clark et al., 2019; AACR Abstract LB-010/2
84. Peguero et al., 2016; ASCO Abstract 2528
85. Bahnassy AA, et al. BMC Gastroenterol (2004) pmid: 15385053
86. Jang KY, et al. Transl Oncol (2012) pmid: 23066445
87. Holland TA, et al. Int. J. Cancer (2001) pmid: 11494229
88. Ogino S, et al. Clin. Cancer Res. (2009) pmid: 19549773
89. Dekanić A, et al. Diagn Pathol (2011) pmid: 22050898
90. Wangefjord S, et al. Diagn Pathol (2013) pmid: 23337059
91. Nosho K, et al. Histopathology (2008) pmid: 18983468
92. McKay JA, et al. J. Pathol. (2002) pmid: 11920733
93. Hilska M, et al. Dis. Colon Rectum (2005) pmid: 16400510
94. Maeda K, et al. Int. J. Cancer (1997) pmid: 9221810
95. Elsheikh S, et al. Breast Cancer Res. Treat. (2008) pmid: 17653856
96. Fu M, et al. Endocrinology (2004) pmid: 15331580
97. Takahashi-Yanaga F, et al. Cell. Signal. (2008) pmid: 18023328
98. Zhang L, et al. Nature (2010) pmid: 20348907
99. Lu W, et al. Eur. J. Pharmacol. (2009) pmid: 19026633
100. Tuynman JB, et al. Cancer Res. (2008) pmid: 18281498
101. Lau T, et al. Cancer Res. (2013) pmid: 23539443
102. Fu Y, et al. Int. J. Cancer (2011) pmid: 21455986
103. Quyn AJ, et al. Surgeon (2008) pmid: 19110823
104. Logan CY, et al. Annu. Rev. Cell Dev. Biol. (2004) pmid: 15473860
105. Eklof Spink K, et al. EMBO J. (2001) pmid: 11707392
106. Liu J, et al. J. Mol. Biol. (2006) pmid: 16753179
107. Dikovskaya D, et al. J. Cell. Sci. (2010) pmid: 20144988
108. Murphy SJ, et al. Dig. Dis. Sci. (2007) pmid: 17410430
109. Aretz S, et al. Hum. Mutat. (2004) pmid: 15459959
110. Kerr SE, et al. J Mol Diagn (2013) pmid: 23159591
111. Annu Rev Pathol (2011) pmid: 21090969
112. Kastritis E, et al. Int. J. Cancer (2009) pmid: 18844223
113. Half E, et al. Orphanet J Rare Dis (2009) pmid: 19822006
114. Guagnano V, et al. Cancer Discov (2012) pmid: 23002168
115. Hatlen MA, et al. Cancer Discov (2019) pmid: 31575540
116. Hagel M, et al. Cancer Discov (2015) pmid: 25776529
117. Kim RD, et al. Cancer Discov (2019) pmid: 31575541
118. Chan et al., 2017; AACR Abstract CT106/24
119. Kaibori M, et al. Oncotarget (2016) pmid: 27384874
120. Dumbrava EI, et al. JCO Precis Oncol (2018) pmid: 31123723
121. Packer LM, et al. Cancer Discov (2015) pmid: 25847957
122. Desnoyers LR, et al. Oncogene (2008) pmid: 17599042
123. Sawey ET, et al. Cancer Cell (2011) pmid: 21397858
124. Miura S, et al. BMC Cancer (2012) pmid: 22309595
125. Feng S, et al. Cancer Res. (2013) pmid: 23440425
126. Nagamatsu H, et al. Prostate (2015) pmid: 25854696
127. Xie MH, et al. Cytokine (1999) pmid: 10525310
128. Int. J. Oncol. (2002) pmid: 12429977
129. Kan Z, et al. Genome Res. (2013) pmid: 23788652
130. Arao T, et al. Hepatology (2013) pmid: 22890726
131. Yamada T, et al. BMC Cancer (2015) pmid: 25885470
132. Dumbrava et al., 2018; doi/full/10.1200/PO.18.00100
133. Kratochwil K, et al. Genes Dev. (2002) pmid: 12502739
134. Scherz PJ, et al. Science (2004) pmid: 15256670
135. Zaharieva BM, et al. J. Pathol. (2003) pmid: 14646664
136. Arai H, et al. Cancer Genet. Cytogenet. (2003) pmid: 14499691
137. Ribeiro IP, et al. Tumour Biol. (2014) pmid: 24477574
138. Schulze K, et al. Nat. Genet. (2015) pmid: 25822088
139. Hiral H, et al. Cancer Biol. Ther. (2010) pmid: 20107315
140. Bridges KA, et al. Clin. Cancer Res. (2011) pmid: 21799033
141. Rajeshkumar NV, et al. Clin. Cancer Res. (2011) pmid: 21389100
142. Osman AA, et al. Mol. Cancer Ther. (2015) pmid: 25504633
143. Xu L, et al. Mol. Cancer Ther. (2002) pmid: 12489850
144. Xu L, et al. Mol. Med. (2001) pmid: 11713371
145. Camp ER, et al. Cancer Gene Ther. (2013) pmid: 23470564
146. Kim SS, et al. Nanomedicine (2015) pmid: 25240597
147. Pirollo KF, et al. Mol. Ther. (2016) pmid: 27357628
148. Hajdenberg et al., 2012; ASCO Abstract e15010
149. Leijen S, et al. J. Clin. Oncol. (2016) pmid: 27601554
150. Moore et al., 2019; ASCO Abstract 5513
151. Leijen S, et al. J. Clin. Oncol. (2016) pmid: 27998224
152. Oza et al., 2015; ASCO Abstract 5506
153. Lee J, et al. Cancer Discov (2019) pmid: 31315834
154. Méndez E, et al. Clin. Cancer Res. (2018) pmid: 29535125

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155. Ma CX, et al. J. Clin. Invest. (2012) pmid: 22446188
156. Kwok M, et al. Blood (2016) pmid: 26563132
157. Boudny M, et al. Haematologica (2019) pmid: 30975914
158. Dillon MT, et al. Mol. Cancer Ther. (2017) pmid: 28062704
159. Middleton FK, et al. Cancers (Basel) (2018) pmid: 30127241
160. Goh HS, et al. Cancer Res. (1995) pmid: 7585578
161. Berg M, et al. PLoS ONE (2010) pmid: 21103049
162. Han SW, et al. PLoS ONE (2013) pmid: 23700467
163. Peeters M, et al. Clin. Cancer Res. (2013) pmid: 23325582
164. Malhotra P, et al. Tumour Biol. (2013) pmid: 23526092
165. Di Bartolomeo M, et al. Target Oncol (2014) pmid: 23821376
166. Russo A, et al. J. Clin. Oncol. (2005) pmid: 16172461
167. Jaiswal S, et al. N. Engl. J. Med. (2014) pmid: 25426837
168. Genovese G, et al. N. Engl. J. Med. (2014) pmid: 25426838
169. Xie M, et al. Nat. Med. (2014) pmid: 25326804
170. Acuna-Hidalgo R, et al. Am. J. Hum. Genet. (2017) pmid: 28669404
171. Severson EA, et al. Blood (2018) pmid: 29678827
172. Fuster JJ, et al. Circ. Res. (2018) pmid: 29420212
173. Hematology Am Soc Hematol Educ Program (2018) pmid: 30504320
174. Chabon JJ, et al. Nature (2020) pmid: 32269342
175. Razavi P, et al. Nat. Med. (2019) pmid: 31768066
176. Brown CJ, et al. Nat. Rev. Cancer (2009) pmid: 19935675
177. Joerger AC, et al. Annu. Rev. Biochem. (2008) pmid: 18410249
178. Kato S, et al. Proc. Natl. Acad. Sci. U.S.A. (2003) pmid: 12826609
179. Kamada R, et al. J. Biol. Chem. (2011) pmid: 20978130
180. Zerdoumi Y, et al. Hum. Mol. Genet. (2017) pmid: 28472496
181. Yamada H, et al. Carcinogenesis (2007) pmid: 17690113
182. Landrum MJ, et al. Nucleic Acids Res. (2018) pmid: 29165669
183. Bougeard G, et al. J. Clin. Oncol. (2015) pmid: 26014290
184. Sorrell AD, et al. Mol Diagn Ther (2013) pmid: 23355100
185. Nichols KE, et al. Cancer Epidemiol. Biomarkers Prev. (2001) pmid: 11219776
186. Kleihues P, et al. Am. J. Pathol. (1997) pmid: 9006316
187. Gonzalez KD, et al. J. Clin. Oncol. (2009) pmid: 19204208
188. Lalloo F, et al. Lancet (2003) pmid: 12672316
189. Mandelker D, et al. Ann. Oncol. (2019) pmid: 31050713
190. Cunningham D, et al. N. Engl. J. Med. (2004) pmid: 15269313
191. Jonker DJ, et al. N. Engl. J. Med. (2007) pmid: 18003960
192. Molseyenko VM, et al. Clin Drug Investig (2018) pmid: 29470838
193. Stein et al., 2020; ASCO GI Abstract 96
194. Price TJ, et al. Lancet Oncol. (2014) pmid: 24739896
195. Van Cutsem E, et al. J. Clin. Oncol. (2007) pmid: 17470858
196. Pietrantonio F, et al. JAMA Oncol (2019) pmid: 31268481
197. Morschhauser et al., 2014; ASH Abstract 3067
198. Sledge GW, et al. J. Clin. Oncol. (2017) pmid: 28580882
199. Dickson MA, et al. JAMA Oncol (2016) pmid: 27124835
200. Dickson MA, et al. J. Clin. Oncol. (2013) pmid: 23569312
201. Turner NC, et al. N. Engl. J. Med. (2015) pmid: 26030518
202. DeMichele A, et al. Clin. Cancer Res. (2015) pmid: 25501126
203. O'Hara et al., 2015; ASCO GI Abstract 626
204. Sullivan et al., 2015; AACR-NCI-EORTC Abstract PR06
205. Kwong LN, et al. Nat. Med. (2012) pmid: 22983396
206. Huillard E, et al. Proc. Natl. Acad. Sci. U.S.A. (2012) pmid: 22586120
207. Vora SR, et al. Cancer Cell (2014) pmid: 25002028
208. Miller ML, et al. Sci Signal (2013) pmid: 24065146
209. Heilmann AM, et al. Cancer Res. (2014) pmid: 24986516
210. Yamada et al., 2015; AACR-NCI-EORTC Abstract B31

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