FOUNDATIONONE®CDx

PATIENT	TUMOR TYPE	REPORT DATE
Liu, Yuan-Kai	Colon adenocarcinoma (CRC)	28 Feb 2023
	COUNTRY CODE	ORDERED TEST #
	TW	ORD-1570858-01

ABOUT THE TEST FoundationOne®CDx is a next-generation sequencing (NGS) based assay that identifies genomic findings within hundreds of cancer-related genes.

PATIENT
DISEASE Colon adenocarcinoma (CRC)
NAME Liu, Yuan-Kai
DATE OF BIRTH 26 May 1980
SEX Male
MEDICAL RECORD # 49277409

PHYSICIAN
ORDERING PHYSICIAN Yeh, Yi-Chen
MEDICAL FACILITY Taipei Veterans General Hospital
ADDITIONAL RECIPIENT None
MEDICAL FACILITY ID 205872
PATHOLOGIST Not Provided

SPECIMEN
SPECIMEN SITE Colon
SPECIMEN ID S112-05465A (PF23016)
SPECIMEN TYPE Slide Deck
DATE OF COLLECTION 13 February 2023
SPECIMEN RECEIVED 20 February 2023

Biomarker Findings

Microsatellite status - MS-Stable
Tumor Mutational Burden - 5 Muts/Mb

Genomic Findings

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

BRAF V600E
KRAS wildtype
NRAS wildtype
APC T1556fs*3
PIK3CA E545K
RB1 K95fs*16
SMAD4 R361C
TP53 K132R

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

Report Highlights

- Targeted therapies with **NCCN categories of evidence** in this tumor type: **Encorafenib + Cetuximab** (p. 11)
- Targeted therapies with **potential resistance** based on this patient's genomic findings: ⊗ **Cetuximab** (p. 12), **Panitumumab** (p. 13)
- Evidence-matched **clinical trial options** based on this patient's genomic findings: (p. 16)
- Variants with **prognostic implications** for this tumor type that may impact treatment decisions: ***BRAF*** **V600E** (p. 5)

BIOMARKER FINDINGS	THERAPY AND CLINICAL TRIAL IMPLICATIONS	
Microsatellite status - MS-Stable	**No therapies or clinical trials.** See Biomarker Findings section	
Tumor Mutational Burden - 5 Muts/Mb	**No therapies or clinical trials.** See Biomarker Findings section	
GENOMIC FINDINGS	**THERAPIES WITH CLINICAL RELEVANCE (IN PATIENT'S TUMOR TYPE)**	**THERAPIES WITH CLINICAL RELEVANCE (IN OTHER TUMOR TYPE)**
BRAF **-** V600E	Encorafenib + Cetuximab [2A]	Dabrafenib + Trametinib
	⊗ ***Cetuximab***	Encorafenib + Binimetinib
10 Trials see p. 17	⊗ ***Panitumumab***	Vemurafenib + Cobimetinib
KRAS **-** wildtype	⊗ ***Cetuximab***	none
0 Trials	⊗ ***Panitumumab***	
NRAS **-** wildtype	⊗ ***Cetuximab***	none
0 Trials	⊗ ***Panitumumab***	

⊗ *Extensive evidence showing variant(s) in this sample may confer resistance to this therapy*

[ ] *NCCN category*

Electronically signed by Douglas A. Mata, MD, MPH | 28 February 2023
Julia Elvin, M.D., Ph.D., Laboratory Director CLIA: 22D2027531
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Sample Preparation: 150 Second St., 1st Floor, Cambridge, MA 02141 · CLIA: 22D2027531
Sample Analysis: 150 Second St., 1st Floor, Cambridge, MA 02141 · CLIA: 22D2027531
Post-Sequencing Analysis: 150 Second St., 1st Floor. Cambridge, MA 02141 · CLIA: 22D2027531

GENOMIC FINDINGS	THERAPIES WITH CLINICAL RELEVANCE (IN PATIENT'S TUMOR TYPE)	THERAPIES WITH CLINICAL RELEVANCE (IN OTHER TUMOR TYPE)
APC - T1556fs*3	none	none
3 Trials see p. 16		
PIK3CA - E545K	none	none
10 Trials see p. 19		

✕ *Extensive evidence showing variant(s) in this sample may confer resistance to this therapy*

☐ *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 approved therapies; however, the agents listed in this report may have varied clinical evidence in the patient's tumor type. Therapies and the clinical trials listed in this report may not be complete and exhaustive. 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. This report should be regarded and used as a supplementary source of information and not as the single basis for the making of a therapy decision. All treatment decisions remain the full and final responsibility of the treating physician and physicians should refer to approved prescribing information for all therapies.

Therapies contained in this report may have been approved by the US FDA.

BIOMARKER

Microsatellite status

RESULT
MS-Stable

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

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 microsatellite-stable (MSS) metastatic colorectal cancer (CRC), 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]. For patients with MSS CRC, a Phase 2 study combining ipilimumab and nivolumab reported an overall DCR of 25% (10/40)[7]. Two Phase 1 studies for patients with MSS CRC treated with regorafenib and nivolumab reported PFSs of 7.9 months[8] and 5.7 months[9], and a patient with MSS CRC refractory to chemotherapy treated with the PD-1 inhibitor sintilimab and regorafenib reported a CR[10].

— Nontargeted Approaches —

MSI has not been found to be a predictive biomarker for combination chemotherapy regimens, including FOLFOX[11-12] and FOLFIRI[13-14]. Patients with MSS CRC are more likely to benefit from postsurgical fluorouracil (FU)-based adjuvant therapy[15-16] but less likely to benefit from irinotecan chemotherapy[17].

FREQUENCY & PROGNOSIS

MSS colorectal cancers (CRCs) make up 70-85% of CRC cases[3,18-22]. MSS colorectal cancers are molecularly heterogeneous, driven by diverse mechanisms such as extensive DNA methylation, oncogenic mutations in KRAS or BRAF, or chromosomal instability[22]. Multiple studies have shown that MSS CRCs have a worse prognosis than MSI-high tumors[18,23-29].

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 is microsatellite-stable (MSS), equivalent to the clinical definition of an MSS tumor: one with mutations in none of the tested microsatellite markers[19,32-33]. MSS status indicates MMR proficiency and typically correlates with intact expression of all MMR family proteins[19-20,31,33].

BIOMARKER

Tumor Mutational Burden

RESULT
5 Muts/Mb

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

On the basis of clinical evidence in solid tumors, increased TMB may be associated with greater sensitivity to immunotherapeutic agents, including anti-PD-L1[34-36], anti-PD-1 therapies[34-37], and combination nivolumab and ipilimumab[38-43]. In multiple pan-tumor studies, increased tissue tumor mutational burden (TMB) was associated with sensitivity to immune checkpoint inhibitors[34-37,44-48]. In the KEYNOTE 158 trial of pembrolizumab monotherapy for patients with solid tumors, significant improvement in ORR was observed for patients with TMB ≥10 Muts/Mb (as measured by this assay) compared with those with TMB <10 Muts/Mb in a large cohort that included multiple tumor types[44]; similar findings were observed in the KEYNOTE 028 and 012 trials[37]. At the same TMB cutpoint, retrospective analysis of patients with solid tumors treated with any checkpoint inhibitor identified that tissue TMB scores ≥ 10 Muts/Mb were associated with prolonged time to treatment failure compared with scores <10 muts/Mb (HR=0.68)[48]. For patients with solid tumors treated with nivolumab plus ipilimumab in the CheckMate 848 trial, improved responses were observed in patients with a tissue TMB ≥ 10 Muts/Mb independent of blood TMB at any cutpoint in matched samples[49]. However, support for higher TMB thresholds and efficacy was observed in the prospective Phase 2 MyPathway trial of atezolizumab for patients with pan-solid tumors, where improved ORR and DCR was seen in patients with TMB ≥ 16 Muts/Mb than those with TMB ≥ 10 and <16 Muts/Mb[47]. Similarly, 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[50] or those with lower TMB treated with PD-1 or PD-L1-targeting agents[35]. 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)[34]. 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[51].

FREQUENCY & PROGNOSIS

Elevated tumor mutational burden (TMB) has been reported in 8-25% of colorectal cancer (CRC) samples[21,52-53]. Multiple studies have reported that up to 90% of hypermutated CRC cases exhibit high levels of microsatellite instability (MSI-H) and mismatch repair deficiency (MMR-D)[21,52]. 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[52]. A subset of CRCs that harbor increased TMB but not MSI-H are driven by mutations in POLE, which leads to an "ultramutated" phenotype with especially high TMB[21,52]. Tumors with increased TMB harbor BRAF V600E mutations more frequently than those with low TMB[21,52], whereas TMB-low tumors more frequently harbor mutations in TP53 and APC[21]. The prognostic value of tumor mutational burden (TMB) in colorectal cancer (CRC) is context- and therapy-dependent. A study of tissue TMB (tTMB) in 145 CRC samples showed longer OS in TMB-high samples compared with TMB-low ones[54]. Similarly, for patients with metastatic CRC treated with first-line chemotherapy combined with bevacizumab or cetuximab, high tissue TMB (tTMB-H) was associated with longer OS[55]. For patients treated with adjuvant chemotherapy, tTMB-H was associated with better 5-year relapse-free survival[56]. However, for patients with EGFR/BRAF-inhibitor-treated, BRAF-mutated microsatellite stable (MSS) metastatic CRC, intermediate tTMB was associated with significantly poorer PFS and OS compared with TMB-low status; patients with primary resistance to EGFR/BRAF blockage had higher TMB than those sensitive to these therapies[57]. In a study for 61 patients with metastatic, MSS CRC treated with best standard of care, plasma TMB scores ≥28 Muts/Mb (approximately 14 Muts/Mb as measured by this assay) were associated with reduced OS compared with plasma TMB scores <28 Muts/Mb (3.0 vs. 5.3 months, HR=0.76, p=0.007), whereas tTMB was not found to be prognostic in this population[58].

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[59-60] and cigarette smoke in lung cancer[61-62], treatment with temozolomide-based chemotherapy in glioma[63-64], mutations in the proofreading domains of DNA polymerases encoded by the POLE and POLD1 genes[21,65-68], and microsatellite instability (MSI)[21,65,68]. 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[34,44,51].

GENE
BRAF

ALTERATION
V600E

TRANSCRIPT ID
NM_004333.4

CODING SEQUENCE EFFECT
1799T>A

VARIANT CHROMOSOMAL POSITION
chr7:140453136

VARIANT ALLELE FREQUENCY (% VAF)
40.4%

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

Significant benefit for patients with BRAF V600-mutated colorectal cancers (CRC) has been achieved with combinatorial approaches involving BRAF inhibitors, EGFR-targeting antibodies, and MEK inhibitors[69-72]. In a Phase 3 study for patients with metastatic CRC on second- or third-line treatments, doublet therapy with the RAF inhibitor encorafenib and the EGFR antibody cetuximab showed superior mOS to cetuximab plus chemotherapy (9.3 vs. 5.9 months, HR=0.61, n=220 and n=221), and similar benefit was seen for a triplet therapy cohort adding the MEK inhibitor binimetinib (OS of 9.3 months, n=224)[73]. Combinations of other RAF inhibitors such as dabrafenib or vemurafenib with EGFR antibodies such as panitumumab have also resulted in clinical benefit for similar patient populations in Phase 1 and 2 studies. A trial of dabrafenib and panitumumab with or without the MEK inhibitor trametinib reported a 21% ORR and 86% DCR (n=91) for the triplet combination and a 10% ORR and 90% DCR (n=20) for the doublet therapy[69]. Multiple similar studies of vemurafenib with panitumumab or cetuximab doublet therapy have also reported a benefit[70-71]. In a randomized Phase 2 study for patients with 0-4 previous lines of therapy, the addition of vemurafenib to cetuximab and irinotecan significantly improved ORR (17% vs. 4.2%, n=50 and n=50) and DCR (65% vs. 21%)[72]. A Phase 2 trial evaluating the investigational agent spartalizumab, an anti-PD-1 antibody, with dabrafenib and trametinib reported an ORR of 35% (n=20) and DCR of 75%[74]. Extensive clinical evidence supports a significant benefit in BRAF-inhibitor and MEK-inhibitor doublet therapy for patients with BRAF V600E-mutated metastatic CRC. A Phase 2 study of vemurafenib plus cobimetinib for patients with advanced BRAF V600E-mutated CRC reported an ORR of 29% (n=28) and DCR of 57%[75], and a similar trial of dabrafenib and trametinib reported a 12% ORR (n=43) and 67% DCR[76]. A basket trial of the combination of encorafenib and binimetinib for patients with BRAF V600-mutated solid cancers elicited 1 PR and 1 SD for 3 patients with CRC[77]. In 2 Phase 1 studies evaluating the MEK-pan-RAF dual inhibitor CH5126766, 3 patients harboring BRAF V600E mutations experienced PRs, including 2 patients with melanoma[78] and 1 patient with low-grade serous ovarian carcinoma[79]. Based on clinical data in solid tumors, patients with tumors harboring BRAF V600 mutations may benefit from treatment with type-II RAF inhibitors such as tovorafenib, lifirafenib, and belvarafenib[80-81].

— Potential Resistance —

On the basis of extensive clinical data, BRAF V600 mutation does not generally associate with significant clinical benefit from addition of cetuximab or panitumumab to chemotherapy (NCCN Colon Cancer Guidelines, v1.2022)[82-91]. Low response rates to cetuximab or panitumumab monotherapy or combination with chemotherapy have been frequently observed among patients with BRAF V600-mutated CRC, although similarly low response rates in this patient population were also often observed to chemotherapy alone; additionally, response rates were generally lower for patients with BRAF-mutated tumors than for those whose tumors were BRAF-wildtype[84,87-88,91-94]. For a limited number of patients with CRC treated with cetuximab- or panitumumab-containing chemotherapy regimens, BRAF V600E was found to be present at the time of progression[95-100], to be a mechanism of acquired[101-102] or primary[103] resistance, or to be enriched in tumors of non-responders versus responders[98].

FREQUENCY & PROGNOSIS

BRAF mutations have been reported in approximately 5-19% of colorectal cancer samples[92,104-107]. BRAF V600E is a strong adverse prognostic marker in colorectal cancer[108]. BRAF mutations have been associated with poor prognosis and shorter survival for patients with colorectal cancer, particularly those with metastatic disease, as well as with smoking history[12,84,86,109-115]. Analysis of individual BRAF mutations in 2127 patients with advanced colorectal cancer treated with chemotherapy with or without cetuximab revealed that BRAF V600E associated with poor prognosis (HR 2.60, P=1.0e-15, with median reduction of survival being 320 days) and distinct clinicopathological features, including correlation with increased peritoneal metastases compared to BRAF wild-type tumors (24% vs. 12%, P=0.0015), while BRAF D594G inactivating mutation was not prognostic (HR 1.30, P=0.37) and had similar clinicopathologic features as BRAF wild-type tumors[116].

FINDING SUMMARY

BRAF encodes a member of the RAF family of protein kinases, which includes ARAF, BRAF, and CRAF. These kinases function downstream of RAS as part of the MAPK (RAF-MEK-ERK) signaling cascade that facilitates cell proliferation, survival and transformation[117-118]. BRAF mutations have been reported in up to 20% of all cancers, with the majority of mutations occurring at the V600 position[119-120]. Among the V600 mutations, V600E accounts for 70-80% of observations, V600K for 10-30%, and V600R for 5-7%, with V600D comprising the majority of the rest[119,121-122]. Mutations at V600 are Class 1 BRAF alterations that have been shown to constitutively activate BRAF kinase and hyperactivate the downstream MEK-ERK signaling, promoting oncogenic transformation[119,123]. In multiple cancer types, multiple mutations at V600, including V600E, V600K, V600R, V600D, and V600M, exhibited sensitivity to V600-targeted therapies[122,124-134]; other mutations at this position are predicted to behave similarly.

GENE
KRAS

ALTERATION
wildtype

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

Lack of mutations in KRAS or NRAS is associated with clinical benefit of treatment with EGFR-targeting antibodies cetuximab[84,135-137] or panitumumab[86,138-139] for patients with CRC. Therefore, these agents are indicated to treat patients with CRC lacking such mutations (NCCN Guidelines v1.2022).

— Potential Resistance —

Based on clinical data, it is unclear whether the presence of PIK3CA mutations is associated with reduced sensitivity to cetuximab in patients with colorectal cancer (CRC). Detection of PIK3CA mutations at progression was observed for patients with CRC following treatment with cetuximab[97,140-141]. A study comparing PIK3CA exon 9 and 20 mutations reported that for patients with chemotherapy-refractory CRC, PIK3CA exon 20 mutations were associated with less benefit from cetuximab compared with PIK3CA wildtype; this effect was not observed with PIK3CA exon 9 mutations[142]. In contrast, multiple studies and case reports of cetuximab treatment of CRC have reported similar responses in patients with PIK3CA exon 9 or 20 mutations compared with PIK3CA wildtype[87,143-148].

FREQUENCY & PROGNOSIS

Approximately 50-65% of colorectal cancers (CRCs) have been reported to lack KRAS mutations[104,149-156]. Numerous studies have reported that KRAS wild-type status is associated with decreased metastasis, better clinicopathological features, and longer survival of patients with CRC[150-153,157-158].

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[159-160]. No alterations in KRAS were identified in this case.

GENE
NRAS

ALTERATION
wildtype

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

Lack of mutations in KRAS or NRAS is associated with clinical benefit of treatment with EGFR-targeting antibodies cetuximab[84,135-137] or panitumumab[86,138-139] for patients with CRC. Therefore, these agents are indicated to treat patients with CRC lacking such mutations (NCCN Guidelines v1.2022).

— Potential Resistance —

Based on clinical data, it is unclear whether the presence of PIK3CA mutations is associated with reduced sensitivity to cetuximab in patients with colorectal cancer (CRC). Detection of PIK3CA mutations at progression was observed for patients with CRC following treatment with cetuximab[97,140-141]. A study comparing PIK3CA exon 9 and 20 mutations reported that for patients with chemotherapy-refractory CRC, PIK3CA exon 20 mutations were associated with less benefit from cetuximab compared with PIK3CA wildtype; this effect was not observed with PIK3CA exon 9 mutations[142]. In contrast, multiple studies and case reports of cetuximab treatment of CRC have reported similar responses in patients with PIK3CA exon 9 or 20 mutations compared with PIK3CA wildtype[87,143-148].

FREQUENCY & PROGNOSIS

The majority of colorectal cancers (CRCs) (91-98%) have been reported to lack NRAS mutations[21,142,156,161-165]. NRAS wild-type status has been reported to be associated with decreased frequency of metastasis[156] and longer survival[165-166] 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[159]. No alterations in NRAS were identified in this case.

GENE
APC

ALTERATION
T1556fs*3

TRANSCRIPT ID
NM_000038.4

CODING SEQUENCE EFFECT
4666_4667insA

VARIANT CHROMOSOMAL POSITION
chr5:112175951

VARIANT ALLELE FREQUENCY (% VAF)
29.5%

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

There are no approved drugs targeting APC inactivation in cancer. Loss of APC function leads to accumulation of beta-catenin and upregulation of WNT pathway transcription programs[167], and potential therapeutic approaches to target this pathway include CBP/beta-catenin antagonists, which interfere with the ability of beta-catenin to interact with transcriptional co-activator CBP[168-169]. In a Phase 1 trial of the CBP/beta-catenin antagonist E7386, 1 patient with APC-mutated small bowel adenocarcinoma achieved a PR with tumor shrinkage of -69% and response duration of 165 days[170]; preclinical data support sensitivity of APC-deficient gastric or colorectal cancer models to E7386[171-172].

FREQUENCY & PROGNOSIS

APC mutations have been found in 73% of tumors in the colorectal adenocarcinoma TCGA dataset[21]. In 1 study, loss of heterozygosity (LOH) of APC was observed in 32% of colorectal cancer (CRC) samples[173]. The prognostic significance of APC mutations in sporadic CRC remains unclear[174]. Solid tumors with WNT/beta-catenin pathway alterations, as seen here, were observed to have significantly less T-cell inflammation in one study[175].

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[176]. Alterations such as seen here may disrupt APC function or expression[177-181].

POTENTIAL GERMLINE IMPLICATIONS

One or more of the APC 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) associated with familial adenomatous polyposis (ClinVar, Sep 2022)[182]. Follow-up germline testing would be needed to distinguish whether the finding in this patient is somatic or germline. Germline mutations in APC are found in more than 90% of patients with familial adenomatous polyposis (FAP)[183-185]. The prevalence for FAP in the general population is estimated to be 1:8,300 from birth[186], and in the appropriate clinical context germline testing of APC is recommended.

GENE
PIK3CA

ALTERATION
E545K

TRANSCRIPT ID
NM_006218.2

CODING SEQUENCE EFFECT
1633G>A

VARIANT CHROMOSOMAL POSITION
chr3:178936091

VARIANT ALLELE FREQUENCY (% VAF)
39.0%

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

Clinical and preclinical data in various tumor types indicate that PIK3CA activating alterations may predict sensitivity to therapies targeting PI3K[187-194], AKT[195-196], or mTOR[197-204]. The Phase 2 NCI-MATCH study of copanlisib for patients with refractory solid tumors harboring PIK3CA mutations with or without PTEN loss met its primary endpoint with an ORR of 16% (4/25 PRs); responses (PR or SD >6 months) were seen in patients with ameloblastoma, liposarcoma, and carcinomas of the endometrium, ovary, esophagus, lung, and prostate[194]. However, the Phase 2 study of copanlisib for patients with endometrial carcinoma harboring PIK3CA hotspot mutations failed to report any objective responses (n=11)[193]. Two other studies of copanlisib for patients with genomically unselected tumors reported 1 CR and 2 PRs (1 unconfirmed) among 16 total patients with PIK3CA-mutated solid tumors with or without PTEN alterations[191-192]. Emerging evidence suggests that the glutaminase inhibitor telaglenastat has clinical activity in PIK3CA-mutated colorectal cancer (CRC). A Phase 1 trial of telaglenastat and capecitabine for patients with CRC who progressed on fluoropyrimidine chemotherapy observed numerically increased median PFS for patients with PIK3CA mutation compared with patients with wildtype PIK3CA status (24.8 vs. 16 weeks, n=7 vs. n=4), including SD >30 weeks for 3 patients with PIK3CA mutation[205].

— Potential Resistance —

Multiple clinical studies report that inhibitors of the PI3K-AKT-mTOR pathway have not produced significant clinical benefit as monotherapies to treat CRC, even for tumors that harbor alterations in PIK3CA or PTEN; data are more limited for alterations in other genes in this pathway[206-208]. Based on clinical data, it is unclear whether the presence of PIK3CA mutations is associated with reduced sensitivity to cetuximab in patients with colorectal cancer (CRC). Detection of PIK3CA mutations at progression was observed for patients with CRC following treatment with cetuximab[97,140-141]. A study comparing PIK3CA exon 9 and 20 mutations reported that for patients with chemotherapy-refractory CRC, PIK3CA exon 20 mutations were associated with less benefit from cetuximab compared with PIK3CA wildtype; this effect was not observed with PIK3CA exon 9 mutations[142]. In contrast, multiple studies and case reports of cetuximab treatment of CRC have reported similar responses in patients with PIK3CA exon 9 or 20 mutations compared with PIK3CA wildtype[87,143-148].

FREQUENCY & PROGNOSIS

PIK3CA mutations have been reported in up to 19% of colorectal cancers (CRCs)[21,209]. A meta-analysis of 864 patients with colorectal cancer (CRC) treated with cetuximab- or panitumumab-based therapy showed that PIK3CA mutations, particularly in exon 20 (H1047R), are significantly associated with worse response[210] and shorter PFS and OS[142]. A study of 354 patients with metastatic CRC observed no difference in OS between patients with PIK3CA mutations versus those without (21.7 months vs. 22.4 months, respectively); however, the study did not include treatment information for the patients[211].

FINDING SUMMARY

PIK3CA encodes p110-alpha, which is the catalytic subunit of phosphatidylinositol 3-kinase (PI3K). The PI3K pathway is involved in cell signaling that regulates a number of critical cellular functions, including cell growth, proliferation, differentiation, motility, and survival[212-213]. PIK3CA alterations that have been characterized as activating, such as observed here, are predicted to be oncogenic[214-235].

GENE
RB1

ALTERATION
K95fs*16

TRANSCRIPT ID
NM_000321.2

CODING SEQUENCE EFFECT
284_287AAAA>TAT

VARIANT CHROMOSOMAL POSITION
chr13:48916754-48916757

VARIANT ALLELE FREQUENCY (% VAF)
63.9%

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

On the basis of limited clinical data[236] and strong preclinical data[237-240], RB1 inactivation may be associated with sensitivity to inhibitors of Aurora kinase A, particularly in small cell lung cancer (SCLC). A clinical study evaluating the Aurora kinase A inhibitor alisertib for patients with prostate cancer did not find an association between RB1 deletion and clinical benefit[241]. Other approaches to target RB1 inactivation under investigation in preclinical studies include inhibitors of BCL-2 family members[242] and activation of the NOTCH pathway[243].

FREQUENCY & PROGNOSIS

RB1 mutations have been reported in <1% of colorectal adenocarcinoma cases[21,244]. Published data investigating the prognostic implications of RB1 alterations in colorectal carcinoma are limited (PubMed, Sep 2022).

FINDING SUMMARY

RB1 encodes the retinoblastoma protein (Rb), a tumor suppressor and negative regulator of the cell cycle[245-246]. Alterations such as seen here may disrupt RB1 function or expression[247-253].

POTENTIAL GERMLINE IMPLICATIONS

Mutations in RB1 underlie the development of retinoblastoma (RB), a rare tumor that arises at a rate of approximately 1:20,000 live births, with nearly 5,000 new cases worldwide per year[254]. Germline mutations in RB1 account for approximately 40% of RB tumors[255] and are associated with an increased risk of developing secondary malignancies that include soft tissue and bone sarcoma and malignant melanoma[256-257]. In the appropriate clinical context, germline testing of RB1 is recommended.

GENE
SMAD4

ALTERATION
R361C

TRANSCRIPT ID
NM_005359.5

CODING SEQUENCE EFFECT
1081C>T

VARIANT CHROMOSOMAL POSITION
chr18:48591918

VARIANT ALLELE FREQUENCY (% VAF)
59.3%

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

There are no targeted therapies available to address genomic alterations in SMAD4. Preclinical studies in colorectal cancer have reported associations of SMAD4 inactivation or loss with sensitivity to inhibitors of Aurora kinase A[258] and the Wnt/beta-catenin pathway[259].

— Nontargeted Approaches —

Clinical studies have reported associations of SMAD4 loss or low SMAD4 expression with improved responses to chemotherapeutic agents in patients with pancreatic cancer[260-262] and non-small cell lung cancer (NSCLC)[263]. Other clinical studies in pancreatic cancer have reported an association of high SMAD4 expression with better responses to neoadjuvant chemotherapy[264] and adjuvant chemoradiotherapy[265].

FREQUENCY & PROGNOSIS

SMAD4 mutation or homozygous deletion is most frequently observed in pancreatic adenocarcinoma (43%)[266], pancreatic acinar cell carcinoma (26%)[267], cholangiocarcinoma (25%)[268], small intestine cancer (20%)[269], appendiceal adenocarcinoma (14-20% mutation; 57% deletion)[270-271], colorectal adenocarcinoma (CRC; 14%)[21], esophageal adenocarcinoma (14%)[272], and stomach adenocarcinoma (13%)[273]. In preclinical studies, SMAD4 loss of function has been implicated in the development of mucinous neoplasms of the pancreas, including mucinous cystic neoplasms (MCN)[274] and intraductal papillary mucinous neoplasms (IPMN)[275]; in clinical samples, SMAD4 homozygous deletion has been observed in 10% of IPMNs and 8% of MCNs, and mutation was also observed in 5% of IPMNs[276]. SMAD4 gene alterations have been associated with reduced OS for patients with pancreatic adenocarcinoma[277]. Reduced SMAD4 expression has been associated with worse prognosis in various cancer types, including CRC[278-280], appendiceal mucinous neoplasm[281], gastric adenocarcinoma[282-283], esophageal adenocarcinoma[284], esophageal squamous cell carcinoma[285], breast cancer[286], and prostate cancer[287].

FINDING SUMMARY

SMAD4, also known as DPC4, encodes a tumor suppressor that regulates transcriptional activity downstream of TGF-beta receptor signaling[288-289]. SMAD4 alterations that result in loss or disruption of the MH1 domain (aa 18-142), MH2 domain (aa 323-552), or SAD domain (aa 275-320) are predicted to be inactivating[290-303].

POTENTIAL GERMLINE IMPLICATIONS

One or more of the SMAD4 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) associated with juvenile polyposis syndrome (ClinVar, Sep 2022)[182]. Follow-up germline testing would be needed to distinguish whether the finding in this patient is somatic or germline. Germline SMAD4 mutations, including those at the R361 hotspot, have been observed in patients with juvenile polyposis syndrome[304-306], which is associated with an increased risk of gastrointestinal cancers[307]. The penetrance of deleterious SMAD4 mutations in patients with colon cancer is estimated at 20% by age 35 and 70% by age 65[308]. In the appropriate clinical context, germline testing of SMAD4 is recommended.

GENE
TP53

ALTERATION
K132R

TRANSCRIPT ID
NM_000546.4

CODING SEQUENCE EFFECT
395A>G

VARIANT CHROMOSOMAL POSITION
chr17:7578535

VARIANT ALLELE FREQUENCY (% VAF)
53.0%

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

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[309-312] or p53 gene therapy such as SGT53[313-317]. In a Phase 1 study, adavosertib in combination with gemcitabine, cisplatin, or carboplatin elicited PRs in 9.7% and SDs in 53% of patients with solid tumors; the response rate was 21% (4/19) for patients with TP53 mutations versus 12% (4/33) for patients who were TP53 wildtype[318]. A Phase 2 trial of adavosertib in combination with chemotherapy (gemcitabine, carboplatin, paclitaxel, or doxorubicin) reported a 32% (30/94, 3 CR) ORR and a 73% (69/94) DCR for patients with platinum-refractory TP53-mutated ovarian, Fallopian tube, or peritoneal cancer[319]. A smaller Phase 2 trial of adavosertib in combination with carboplatin achieved a 43% (9/21, 1 CR) ORR and a 76% (16/21) DCR for patients with platinum-refractory TP53-mutated ovarian cancer[320]. The combination of adavosertib with paclitaxel and carboplatin for patients with TP53-mutated ovarian cancer also significantly increased PFS compared with paclitaxel and carboplatin alone[321]. In the Phase 2 VIKTORY trial, patients with TP53-mutated metastatic and/or recurrent gastric cancer experienced a 24% (6/25) ORR with adavosertib combined with paclitaxel[322]. A Phase 1 trial of neoadjuvant adavosertib in combination with cisplatin and docetaxel for head and neck squamous cell carcinoma (HNSCC) elicited a 71% (5/7) response rate for patients with TP53 alterations[323]. The Phase 2 FOCUS4-C trial for patients with TP53- and RAS-mutated colorectal cancer reported improvement in PFS (3.61 vs. 1.87 months, HR=0.35, p=0.0022), but not OS (14.0 vs 12.8 months, p=0.93), following adavosertib treatment compared with active monitoring[324]. In a Phase 1b clinical trial of SGT-53 in combination with docetaxel for patients with solid tumors, 75% (9/12) of evaluable patients experienced clinical benefit, including 2 confirmed and 1 unconfirmed PRs and 2 instances of SD with significant tumor shrinkage[317]. Missense mutations leading to TP53 inactivation may be sensitive to therapies that reactivate mutated p53 such as eprenetapopt. In a Phase 1b trial for patients with p53-positive high-grade serous ovarian cancer, eprenetapopt combined with carboplatin and pegylated liposomal doxorubicin achieved a 52% (11/21) response rate and 100% DCR[325]. A Phase 1 trial of eprenetapopt with pembrolizumab for patients with solid tumors reported an ORR of 10% (3/29)[326].

FREQUENCY & PROGNOSIS

TP53 mutations have been reported in up to 75% of colorectal cancer cases[21,88,327-331]. A study reported p53 expression in 49% of analyzed colorectal cancer cases[332]. TP53 mutation has not been consistently demonstrated to be a significant independent prognostic marker in the context of CRC[333].

FINDING SUMMARY

Functional loss of the tumor suppressor p53, which is encoded by the TP53 gene, is common in aggressive advanced cancers[334]. Alterations such as seen here may disrupt TP53 function or expression[335-339].

POTENTIAL GERMLINE IMPLICATIONS

Germline mutations in TP53 are associated with the very rare autosomal dominant disorder Li-Fraumeni syndrome and the early onset of many cancers[340-342], including sarcomas[343-344]. Estimates for the prevalence of germline TP53 mutations in the general population range from 1:5,000[345] to 1:20,000[344]. 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[346]. In the appropriate clinical context, germline testing of TP53 is recommended.

POTENTIAL CLONAL HEMATOPOIESIS IMPLICATIONS

Variants seen in this gene have been reported to occur in clonal hematopoiesis (CH), an age-related process in which hematopoietic stem cells acquire somatic mutations that allow for clonal expansion[347-352]. CH in this gene has been associated with increased mortality, risk of coronary heart disease, risk of ischemic stroke, and risk of secondary hematologic malignancy[347-348]. Clinical management of patients with CH in this gene may include monitoring for hematologic changes and reduction of controllable risk factors for cardiovascular disease[353]. Comprehensive genomic profiling of solid tumors detects nontumor alterations that are due to CH[351,354-355]. Patient-matched peripheral blood mononuclear cell sequencing is required to conclusively determine if this alteration is present in tumor or is secondary to CH.

Encorafenib + Cetuximab

Assay findings association

BRAF
V600E

AREAS OF THERAPEUTIC USE

Encorafenib is an inhibitor of BRAF, and cetuximab is a monoclonal antibody that targets EGFR. The combination is FDA approved to treat patients with BRAF V600E-mutated colorectal cancer (CRC). Please see the drug label for full prescribing information.

GENE ASSOCIATION

Patients with BRAF V600-mutated CRC are considered unlikely to benefit from cetuximab, alone or in combination with chemotherapy, unless combined with BRAF inhibitors (NCCN Colon Cancer Guidelines, v1.2022). Response rates to cetuximab, both as monotherapy and in combination with chemotherapy, have generally been found to be low for patients with BRAF V600-mutated CRC, independent of treatment line and chemotherapy backbone[84,87,92-94,96,100,115,142,356-360] . However, significant clinical responses have been reported for patients with BRAF V600-mutated CRC treated with cetuximab in combination with the BRAF inhibitor vemurafenib[70], the 2 in combination with irinotecan[361], or cetuximab in combination with BRAF inhibitor encorafenib[362-363] .

SUPPORTING DATA

For patients with BRAF V600E-mutated metastatic colorectal cancer (CRC), the Phase 3 BREAKWATER study evaluating encorafenib and cetuximab with mFOLFOX6 and/or FOLFIRI reported an ORR of 67-68% ([8/12]-[13/19]) for untreated patients and an ORR of 50-61% ([4/8]-[11/18]) for previously treated patients[364]. The Phase 3 BEACON study for previously treated patients with BRAF V600E-mutated metastatic CRC demonstrated significantly improved efficacy of encorafenib and cetuximab doublet therapy over standard irinotecan and cetuximab therapy (median OS [mOS] of 9.3 vs. 5.9 months, HR=0.61; median PFS [mPFS] of 4.3 vs. 1.5 months, HR=0.44; ORR of 20% vs. 1.8%)[362,365] . In the same study, triplet therapy of encorafenib and cetuximab combined with the MEK inhibitor binimetinib resulted in similar efficacy as the doublet therapy, compared with standard therapy (mOS of 9.3 vs. 5.9 months, HR=0.60; mPFS of 4.5 vs. 1.5 months, HR=0.42; ORR of 27% vs. 1.8%)[365]. In a Phase 1/2 trial for patients with microsatellite stable and BRAF V600E-positive metastatic CRC, treatment with triplet encorafenib, cetuximab, and nivolumab resulted in an ORR of 50% (11/22 PRs), a DCR of 95% (21/22), an mPFS of 7.4 months, and an mOS of 15.1 months[366].

Cetuximab

Resistance of variant(s) to associated therapy is likely

Assay findings association

BRAF
V600E

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). Please see the drug label for full prescribing information.

GENE ASSOCIATION

Therapies targeting EGFR, including cetuximab, have been shown to have significant clinical activity for patients with CRC[84,135-137,367-368] ; wild-type KRAS and NRAS are predictive biomarkers for the efficacy of cetuximab in metastatic CRC (NCCN Colon Cancer Guidelines v1.2022). A study comparing PIK3CA exon 9 and 20 mutations reported that for patients with chemotherapy-refractory CRC, PIK3CA exon 20 mutations were associated with less benefit from cetuximab compared with PIK3CA wildtype (ORR of 0.0% [0/9] vs. 37% [121/329], PFS of 11.5 vs. 24 weeks, and OS of 34 vs. 51 weeks); this effect was not observed with PIK3CA exon 9 mutations (ORR of 29% [6/21] vs. 36% [115/317], PFS of 23.5 vs. 24 weeks, and OS of 46 vs. 51 weeks)[142]. The presence of PIK3CA exon 9 and 20 mutations showed no correlation with objective tumor response to cetuximab but was associated with reduced PFS in response to cetuximab salvage therapy (HR=2.1)[369]. In contrast, multiple studies and case reports of cetuximab treatment of CRC have reported similar responses in patients with PIK3CA exon 9 or 20 mutations compared with PIK3CA wildtype[87,143-148,370] . Patients with BRAF V600-mutated CRC are considered unlikely to benefit from cetuximab, alone or in combination with chemotherapy, unless combined with BRAF inhibitors (NCCN Colon Cancer Guidelines, v1.2022). Response rates to cetuximab, both as monotherapy and in combination with chemotherapy, have generally been found to be low for patients with BRAF V600-mutated CRC, independent of treatment line and chemotherapy backbone[84,87,92-94,96,100,115,142,356-360] . However, significant clinical responses have been reported for patients with BRAF V600-mutated CRC treated with cetuximab in combination with the BRAF inhibitor vemurafenib[70], the 2 in combination with irinotecan[361], or cetuximab in combination with BRAF inhibitor encorafenib[362-363] .

SUPPORTING DATA

Cetuximab has been shown to improve OS, PFS, and response rate for patients with KRAS-wildtype colorectal cancer (CRC), both in combination with FOLFIRI, FOLFOX4, or irinotecan[84,135,367-368,371] and as monotherapy for chemotherapy-refractory patients[87,137] . The Phase 3 study STRATEGIC-1 reported a similar duration of disease control (DDC) for patients with unresectable metastatic CRC (mCRC) and KRAS-, NRAS-, and BRAF-wildtype status treated with mFOLFOX-bevacizumab alternated with a cetuximab regimen in first or second line, respectively (overall DDC 22.5 vs. 23.5 months); in addition, the study reported similar OS (37.8 vs. 34.4 months) and higher numerical ORR for patients treated with cetuximab in the first line followed by mFOLFOX-bevacizumab compared with those receiving EGFR-directed antibodies in the second or third line[372]. A prospective study of cetuximab monotherapy for patients with KRAS-, NRAS-, and BRAF-wildtype mCRC reported 11% (2/19) PRs and 58% (11/19) SDs[147]. The Phase 2 AVETUX trial of cetuximab combined with avelumab and mFOLFOX6 for patients with RAS- and BRAF-wildtype mCRC resulted in an ORR of 81% (4 CR and 27 PRs, n=37) and a DCR of 89%[373]. In the Phase 3 ASPECCT study, panitumumab was found to be non-inferior to cetuximab with respect to median OS (10.4 vs. 10.0 months, HR=0.97) for patients with previously treated KRAS exon 2 wildtype metastatic colorectal cancer; median PFS was also similar between the two treatment groups (4.4 vs. 4.1 months, HR=1.00)[374]. In a similar patient population, a Phase 2 study of combination panitumumab and irinotecan versus combination cetuximab and irinotecan also demonstrated non-inferiority with respect to median PFS (5.4 vs. 4.3 months, HR = 0.64) and median OS (14.9 vs. 11.5 months, HR=0.66)[375].

Panitumumab

Resistance of variant(s) to associated therapy is likely

Assay findings association

BRAF
V600E

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 for patients with CRC[138,374,376] ; wild-type KRAS and NRAS are predictive biomarkers for the efficacy of panitumumab in metastatic CRC (NCCN Colon Cancer Guidelines v1.2022). Patients with BRAF V600-mutated CRC are considered unlikely to benefit from panitumumab, alone or in combination with chemotherapy (NCCN Colon Cancer Guidelines, v1.2022). Response rates to panitumumab, both as monotherapy and in combination with chemotherapy, have generally been found to be low for patients with BRAF V600-mutated CRC, independent of line of treatment and chemotherapy backbone[88,91,95,99-100,142,358] . However, significant clinical responses have been reported for patients with BRAF V600E-mutated CRC upon treatment with panitumumab in combination with the BRAF inhibitor dabrafenib and the MEK inhibitor trametinib[377].

SUPPORTING DATA

Panitumumab has been shown to improve OS, PFS, and ORR for patients with KRAS-wildtype colorectal cancer (CRC), both in combination with FOLFOX4, FOLFIRI, irinotecan, or best supportive care[89,138,378-380] , and as monotherapy for chemotherapy-refractory patients[88,374,376] . The Phase 3 PARADIGM trial comparing panitumumab plus mFOLFOX6 versus bevacizumab plus mFOLFOX6 as first-line treatment for patients with RAS-wildtype left-sided metastatic CRC demonstrated that treatment with panitumumab significantly improved median OS (mOS; 36.2 months vs. 31.3 months) compared with bevacizumab[381]. A Phase 2 trial reported that, for patients with unresectable RAS-wildtype colorectal adenocarcinoma treated with panitumumab plus FOLFOX4, maintenance with a combination of panitumumab plus fluorouracil and leucovorin was superior to panitumumab monotherapy (10-month PFS OF 59% vs. 49%)[382]. In the Phase 3 ASPECCT study, panitumumab was found to be non-inferior to cetuximab with respect to median OS (10.4 vs. 10.0 months, HR=0.97) for patients with previously treated KRAS exon 2 wildtype metastatic colorectal cancer; median PFS was also similar between the two treatment groups (4.4 vs. 4.1 months, HR=1.00)[374]. In a similar patient population, a Phase 2 study of combination panitumumab and irinotecan versus combination cetuximab and irinotecan also demonstrated non-inferiority with respect to median PFS (5.4 vs. 4.3 months, HR = 0.64) and median OS (14.9 vs. 11.5 months, HR=0.66)[375].

Dabrafenib + Trametinib

Assay findings association

BRAF
V600E

AREAS OF THERAPEUTIC USE

Dabrafenib is a BRAF V600 selective inhibitor and trametinib is a MEK inhibitor. These 2 therapies are FDA approved in combination to treat metastatic non-small cell lung cancer (NSCLC) with BRAF V600E mutation, advanced anaplastic thyroid cancer (ATC) with BRAF V600E mutation, and advanced solid tumors with BRAF V600E mutation in adult and pediatric patients 6 years of age and older. This combination is also approved to treat patients with melanoma with BRAF V600E/K mutations. Please see the drug label for full prescribing information.

GENE ASSOCIATION

On the basis of clinical evidence in various solid tumors and hematologic malignancies, BRAF activating alterations may confer sensitivity to the combination of BRAF V600-targeted therapies and MEK inhibitors such as dabrafenib and trametinib[76,383-393] .

SUPPORTING DATA

The combination of BRAF inhibitors with MEK inhibitors has shown clinical activity for patients with BRAF V600-mutated metastatic colorectal carcinoma (mCRC). A Phase 1/2 open-label trial combining dabrafenib and trametinib for BRAF V600-mutated mCRC reported an ORR of 12% (5/43, including 1 CR with a response duration >36 months)[76]. For patients with BRAF V600E-mutated mCRC, a combination of dabrafenib, trametinib, and the EGFR-targeting antibody panitumumab elicited a 21% (19/91) ORR, 86% (78/91) DCR, and 7.6-month estimated median duration of response (DoR)[69]. A Phase 2 trial evaluating dabrafenib and trametinib in combination with the anti-PD-1 immune checkpoint inhibitor spartalizumab reported a 33% (7/21) ORR for patients with BRAF V600-mutated mCRC, with 5.6 months median DoR; within microsatellite stable patients, the ORR was 42% (5/12)[74]. One case report describes a patient with colon adenocarcinoma who responded to a combination of dabrafenib, trametinib, and oxaliplatin[394]. Dabrafenib can induce adverse effects such as the development of cutaneous squamous cell carcinomas and keratoacanthomas caused by inactivation of wildtype BRAF that leads to paradoxical activation of the MAPK pathway, but it has been reported to be well tolerated in patients with BRAF V600E-mutated thyroid cancer[124,395-396] . Patients with melanoma harboring BRAF V600E or V600K mutation treated with a combination of dabrafenib and trametinib experienced significantly lower rates of cutaneous squamous cell carcinoma and regression of established BRAF inhibitor-induced skin lesions[384-385,397-399] .

Encorafenib + Binimetinib

Assay findings association

BRAF
V600E

AREAS OF THERAPEUTIC USE

The combination of the BRAF inhibitor encorafenib and MEK inhibitor binimetinib is FDA approved to treat patients with melanoma with BRAF V600E or BRAF V600K mutations. Please see the drug label for full prescribing information.

GENE ASSOCIATION

On the basis of clinical efficacy in the treatment of patients with BRAF V600-mutated melanoma[400-403] , and activity in colorectal, thyroid, and lung cancer[403-405] , activating alterations affecting BRAF predict sensitivity to the combination of encorafenib and binimetinib.

SUPPORTING DATA

A Phase 1/2 trial of encorafenib combined with binimetinib for patients with BRAF V600E- or BRAF V600K-mutated solid tumors reported an ORR of 18.2% (2/11) for the subset of patients with metastatic colorectal cancer[403]. The combination of encorafenib and binimetinib has been reported to provide clinical benefit for patients with various solid tumors harboring BRAF V600 activating alterations[400,403-405] , and has been studied primarily in the context of BRAF V600-mutated melanoma where patients treated with this combination achieved greater PFS and OS compared with encorafenib or vemurafenib monotherapy[400-401,406] . A combination of encorafenib, binimetinib, and the CDK4/6 inhibitor ribociclib in a Phase 1b trial for patients with BRAF V600-mutant cancers elicited responses in melanoma, astrocytoma, unknown carcinoma, and in 1 of 3 patients with colorectal cancer; a Phase 2 study of this combination in V600-mutant melanoma reported an ORR of 52.4% (22/42), including 5 CRs, median PFS of 9.2 months, and median OS of 19.4 months[77].

Vemurafenib + Cobimetinib

Assay findings association

BRAF
V600E

AREAS OF THERAPEUTIC USE

Vemurafenib is a selective inhibitor of BRAF with V600 mutations and cobimetinib is a MEK inhibitor. The combination is FDA approved to treat patients with melanoma with BRAF V600E or V600K mutations. Please see the drug label for full prescribing information.

GENE ASSOCIATION

On the basis of clinical evidence in melanoma and colorectal carcinoma, BRAF activating alterations may confer sensitivity to the combination of BRAF V600-targeted therapies and MEK inhibitors such as vemurafenib and cobimetinib[75,407-408] .

SUPPORTING DATA

The Phase 2 TAPUR study of vemurafenib plus cobimetinib for patients with advanced BRAF V600E-mutated CRC reported an ORR of 29% (8/28), median PFS of 15.8 weeks, and median OS of 38.9 weeks[75].
Vemurafenib can induce adverse effects, such as the development of cutaneous squamous cell carcinomas, keratoacanthomas, and new primary melanomas caused by inactivation of wildtype BRAF and leading to paradoxical activation of the MAPK pathway[125,395] . In a Phase 1b trial, patients with BRAF V600E-mutated melanoma treated with a combination of vemurafenib and cobimetinib had increased RR (87%) and PFS (13.7 months) compared to the RR and PFS values previously reported for vemurafenib or MEK inhibitor monotherapy; this combination also resulted in lower rates of cutaneous SCC[409].

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.

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
APC

ALTERATION
T1556fs*3

RATIONALE
Based on preclinical and limited clinical data, APC inactivation may be associated with sensitivity to CBP/beta-catenin interaction inhibitors.

NCT05091346

PHASE 1/2

A Study of E7386 in Combination With Pembrolizumab in Previously Treated Participants With Selected Solid Tumors

TARGETS
CBP, Beta-catenin, PD-1

LOCATIONS: Fukuoka (Japan), Osaka (Japan), Shizouka (Japan), Tokyo (Japan), Chiba-shi (Japan), Kashiwa (Japan), Sapporo shi (Japan), Glasgow (United Kingdom), Manchester (United Kingdom), London (United Kingdom)

NCT04008797

PHASE 1

A Study of E7386 in Combination With Other Anticancer Drug in Participants With Solid Tumor

TARGETS
CBP, Beta-catenin, FGFRs, RET, PDGFRA, VEGFRs, KIT

LOCATIONS: Kurume (Japan), Matsuyama (Japan), Seodaemun (Korea, Republic of), Osakasayama (Japan), Nagoya (Japan), Kawasaki (Japan), Chuo-Ku (Japan), Koto-ku (Japan), Chiba (Japan), Kashiwa (Japan)

NCT03264664

PHASE 1

Study of E7386 in Participants With Selected Advanced Neoplasms

TARGETS
CBP, Beta-catenin

LOCATIONS: Glasgow (United Kingdom), Manchester (United Kingdom), London (United Kingdom), Sutton (United Kingdom)

GENE
BRAF

ALTERATION
V600E

RATIONALE
BRAF activating alterations may predict sensitivity to inhibitors of BRAF, MEK, or ERK. Limited clinical and preclinical studies indicate BRAF mutations may predict sensitivity to MEK-pan-RAF dual inhibitors.

NCT04607421

PHASE 3

BRAF V600E-mutant Colorectal Cancer Study of Encorafenib Taken With Cetuximab Plus or Minus Chemotherapy (BREAKWATER)

TARGETS
VEGFA, MEK, BRAF, EGFR

LOCATIONS: Taipei (Taiwan), Taoyuan (Taiwan), Taichung (Taiwan), Fuzhou (China), Tainan (Taiwan), Kaohsiung (Taiwan), Hangzhou (China), Shanghai (China), Busan (Korea, Republic of), Nanjing (China)

NCT04913285

PHASE 1

A Study to Evaluate KIN-2787 in Subjects With BRAF Mutation Positive Solid Tumors

TARGETS
BRAF, MEK

LOCATIONS: Taipei (Taiwan), Shanghai (China), Gyeonggi-do (Korea, Republic of), Cheongju-si (Korea, Republic of), Incheon (Korea, Republic of), Seoul (Korea, Republic of), Perth (Australia), Wollstonecraft (Australia), Amsterdam (Netherlands), Villejuif (France)

NCT05004350

PHASE 2

A Study Evaluating the Combination of Encorafenib and Cetuximab Versus Irinotecan/Cetuximab or Infusional 5-fluorouracil (5-FU)/Folinic Acid (FA)/Irinotecan (FOLFIRI)/Cetuximab in Chinese Patients With BRAF V600E Mutant Metastatic Colorectal Cancer.

TARGETS
MEK, BRAF, EGFR

LOCATIONS: Fuzhou (China), Xiamen (China), Shantou (China), Hangzhou (China), Ganzhou (China), Shanghai (China), Nanchang (China), Changzhou (China), Shenzhen (China), Guangzhou (China)

NCT04984369

PHASE 2

The Efficacy of HLX208 (BRAF V600E Inhibitor) With Cetuximab for Metastatic Colorectal Cancer (mCRC) With BRAF V600E Mutation After First-line Treatment

TARGETS
EGFR, BRAF

LOCATIONS: Shanghai (China)

NCT03727763

PHASE 2

FIVC in Advanced Colorectal Cancer Patients With BRAF V600E Mutation.

TARGETS
EGFR, BRAF

LOCATIONS: Shanghai (China)

NCT03781219

PHASE 1

A PhaseI Study of HL-085 Plus Vemurafenib in Solid Tumor With BRAF V600 Mutation

TARGETS
MEK, BRAF

LOCATIONS: Hangzhou (China), Zhengzhou (China), Beijing (China)

NCT04803318

PHASE 2

Trametinib Combined With Everolimus and Lenvatinib for Recurrent/Refractory Advanced Solid Tumors

TARGETS
mTOR, FGFRs, RET, PDGFRA, VEGFRs, KIT, MEK

LOCATIONS: Guangzhou (China)

NCT04985604

PHASE 1/2

DAY101 Monotherapy or in Combination With Other Therapies for Patients With Solid Tumors

TARGETS
BRAF, MEK

LOCATIONS: Busan (Korea, Republic of), Seoul (Korea, Republic of), Clayton (Australia), Edegem (Belgium), Oregon, Barcelona (Spain), Madrid (Spain), California, Colorado

NCT04999761

PHASE 1

AB122 Platform Study

TARGETS
PD-1, HSP90, FGFRs

LOCATIONS: Ehime (Japan), Wakayama (Japan), Osaka (Japan), Aichi (Japan), Shizuoka (Japan), Kanagawa (Japan), Tokyo (Japan), Chiba (Japan), Hokkaido (Japan)

NCT03284502

PHASE 1

Cobimetinib and HM95573 in Patients With Locally Advanced or Metastatic Solid Tumors

TARGETS
MEK, RAFs, NRAS

LOCATIONS: Hwasun (Korea, Republic of), Pusan (Korea, Republic of), Seongnam (Korea, Republic of), Seoul (Korea, Republic of), Goyang-si (Korea, Republic of)

GENE
PIK3CA

ALTERATION
E545K

RATIONALE
PIK3CA activating mutations may lead to activation of the PI3K-AKT-mTOR pathway and may therefore indicate sensitivity to inhibitors of this pathway. Strong clinical data support sensitivity of PIK3CA-mutated solid tumors to the PI3K-alpha inhibitor alpelisib. Several clinical studies have shown that inhibitors of the PI3K-AKT-mTOR pathway have not produced significant clinical benefit when used as a monotherapy in patients with colorectal cancer; combination therapies may be required to overcome this lack of response. On the basis of preclinical and limited clinical data, PIK3CA activating mutations may predict sensitivity to glutaminase inhibitors.

NCT04589845

PHASE 2

Tumor-Agnostic Precision Immuno-Oncology and Somatic Targeting Rational for You (TAPISTRY) Platform Study

TARGETS
TRKB, ALK, TRKC, ROS1, TRKA, RET, PD-L1, AKTs, ERBB2, MDM2, PI3K-alpha, RAFs, NRAS

LOCATIONS: Taipei City (Taiwan), Taoyuan County (Taiwan), Tainan (Taiwan), Shanghai City (China), Shanghai (China), Shatin (Hong Kong), Hong Kong (Hong Kong), Seoul (Korea, Republic of), Xi'an (China), Tianjin (China)

NCT04337463

PHASE NULL

ATG-008 Combined With Toripalimab in Advanced Solid Tumors

TARGETS
mTORC1, mTORC2, PD-1

LOCATIONS: Chongqing (China), Chengdu (China)

NCT04803318

PHASE 2

Trametinib Combined With Everolimus and Lenvatinib for Recurrent/Refractory Advanced Solid Tumors

TARGETS
mTOR, FGFRs, RET, PDGFRA, VEGFRs, KIT, MEK

LOCATIONS: Guangzhou (China)

NCT04526470

PHASE 1/2

Alpelisib and Paclitaxel in PIK3CA-altered Gastric Cancer

TARGETS
PI3K-alpha

LOCATIONS: Seongnam-si (Korea, Republic of), Seoul (Korea, Republic of)

NCT03772561

PHASE 1

Phase I Study of AZD5363 + Olaparib + Durvalumab in Patients With Advanced or Metastatic Solid Tumor Malignancies

TARGETS
PARP, AKTs, PD-L1

LOCATIONS: Singapore (Singapore)

NCT04801966

PHASE NULL

Safety and Oversight of the Individually Tailored Treatment Approach: A Novel Pilot Study

TARGETS
CDK4, CDK6, PI3K-alpha, PD-L1, MEK, PARP, PD-1, BRAF

LOCATIONS: Melbourne (Australia)

NCT04551521

PHASE 2

CRAFT: The NCT-PMO-1602 Phase II Trial

TARGETS
PD-L1, AKTs, MEK, BRAF, ALK, RET, ERBB2

LOCATIONS: Würzburg (Germany), Mainz (Germany), Heidelberg (Germany), Tübingen (Germany)

NCT04317105

PHASE 1/2

Testing the Addition of an Anti-cancer Drug, Copanlisib, to the Usual Immunotherapy (Nivolumab With or Without Ipilimumab) in Patients With Advanced Solid Cancers That Have Changes in the Following Genes: PIK3CA and PTEN

TARGETS
PD-1, CTLA-4, PI3K

LOCATIONS: Toronto (Canada), Texas, Virginia

NCT04817956

PHASE 2

Improving Public Cancer Care by Implementing Precision Medicine in Norway

TARGETS
PD-L1, VEGFA, ERBB2, ALK, RET, PARP, SMO, TRKB, TRKC, ROS1, TRKA, MEK, BRAF, PI3K-alpha, FGFR1, FGFR2, FGFR3, MET, KIT, ABL

LOCATIONS: Tromsø (Norway), Bodø (Norway), Hamar (Norway), Oslo (Norway), Fredrikstad (Norway), Drammen (Norway), Trondheim (Norway), Skien (Norway), Førde (Norway), Bergen (Norway)

NCT03006172

PHASE 1

To Evaluate the Safety, Tolerability, and Pharmacokinetics of GDC-0077 Single Agent in Participants With Solid Tumors and in Combination With Endocrine and Targeted Therapies in Participants With Breast Cancer

TARGETS
PI3K-alpha, Aromatase, ER, CDK6, CDK4

LOCATIONS: London (United Kingdom), Surrey (United Kingdom), Bordeaux (France), Barcelona (Spain), Valencia (Spain), Toronto (Canada), Massachusetts, New York, Tennessee

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.

CD22
R511W

CTNNA1
R383C

JAK3
Y399C

MST1R
V670G

MTOR
T1834_T1837del

NBN
amplification

NTRK1
E275A

PALB2
E352Q

PARK2
P159L

PDGFRA
A401D

PTEN
P30L

RAD21
amplification

ROS1
Y469_N471del

Electronically signed by Douglas A. Mata, MD, MPH | 28 February 2023
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Sample Preparation: 150 Second St., 1st Floor, Cambridge, MA 02141 · CLIA: 22D2027531
Sample Analysis: 150 Second St., 1st Floor, Cambridge, MA 02141 · CLIA: 22D2027531
Post-Sequencing Analysis: 150 Second St., 1st Floor. Cambridge, MA 02141 · CLIA: 22D2027531

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 or WTX)*	
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*	*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*	*EMSY (C11orf30)*	*EP300*	*EPHA3*	*EPHB1*
EPHB4	*ERBB2*	*ERBB3*	*ERBB4*	*ERCC4*	*ERG*	*ERRFI1*	*ESR1*	*EZH2*
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	*GID4 (C17orf39)*	*GNA11*	*GNA13*	*GNAQ*	*GNAS*	*GRM3*	*GSK3B*	*H3-3A (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*	*MRE11 (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*	*NSD2 (WHSC1 or MMSET)*		*NSD3 (WHSC1L1)*	*NT5C2*	*NTRK1*	*NTRK2*	*NTRK3*
P2RY8	*PALB2*	*PARP1*	*PARP2*	*PARP3*	*PAX5*	*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*
PRKN (PARK2)	*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*	*TENT5C (FAM46C)*		*TET2*	*TGFBR2*
TIPARP	*TNFAIP3*	*TNFRSF14*	*TP53*	*TSC1*	*TSC2*	*TYRO3*	*U2AF1*	*VEGFA*
VHL	*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

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

FoundationOne CDx fulfills the requirements of the European Directive 98/79 EC for in vitro diagnostic medical devices and is registered as a CE-IVD product by Foundation Medicine's EU Authorized Representative, Qarad b.v.b.a, Cipalstraat 3, 2440 Geel, Belgium. CE

ABOUT FOUNDATIONONE CDX

FoundationOne CDx was developed and its performance characteristics determined by Foundation Medicine, Inc. (Foundation Medicine). FoundationOne CDx may be used for clinical purposes and should not be regarded as purely investigational or for research only. Foundation Medicine's clinical reference laboratories are qualified to perform high-complexity clinical testing.

Please refer to technical information for performance specification details: www.rochefoundationmedicine.com/f1cdxtech.

INTENDED USE

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), tumor mutational burden (TMB), and for selected forms of ovarian cancer, loss of heterozygosity (LOH) score, 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 therapies in accordance with 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.

TEST PRINCIPLE

FoundationOne CDx will be performed exclusively as a laboratory service using DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tumor samples. The proposed assay will employ 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. The assay therefore includes detection of alterations in a total of 324 genes.

Using an Illumina® HiSeq platform, hybrid capture–selected libraries will be sequenced to high uniform depth (targeting >500X median coverage with >99% of exons at coverage >100X). Sequence data will be processed using a customized analysis pipeline designed to accurately detect all classes of genomic alterations, including base substitutions, indels, focal copy number amplifications, homozygous gene deletions, and selected genomic rearrangements (e.g.,gene fusions). Additionally, genomic signatures including loss of heterozygosity (LOH), microsatellite instability (MSI) and tumor mutational burden (TMB) will be reported.

THE REPORT

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. The F1CDx report may be used as an aid to inform molecular eligibility for clinical trials. 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.

Diagnostic Significance

FoundationOne CDx identifies alterations to select cancer-associated genes or portions of genes (biomarkers). In some cases, the Report also highlights selected negative test results regarding biomarkers of clinical significance.

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.

Ranking of Therapies and Clinical Trials

Ranking of Therapies in Summary Table

Therapies are ranked based on the following criteria: Therapies with clinical benefit (ranked alphabetically within each evidence category), followed by therapies associated with resistance (when applicable).

Ranking of 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®. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®). © National Comprehensive Cancer Network, Inc. 2023. All rights reserved. To view the most recent and complete version of the guidelines, go online to NCCN.org. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way.

Limitations

1. In the fraction-based MSI algorithm, a tumor specimen will be categorized as MSI-H, MSS, or MS-Equivocal according to the fraction of microsatellite loci determined to be altered or unstable (i.e., the fraction unstable loci score). In the F1CDx assay, MSI is evaluated based on a genome-wide analysis across >2000 microsatellite loci. For a given microsatellite locus, non-somatic alleles are discarded, and the microsatellite is categorized as unstable if remaining alleles differ from the reference genome. The final fraction unstable loci score is calculated as the number of unstable microsatellite loci divided by the number of evaluable microsatellite loci. The MSI-H and MSS cut-off thresholds were determined by

analytical concordance to a PCR comparator assay using a pan-tumor FFPE tissue sample set. Patients with results categorized as "MS-Stable" with median exon coverage <300X, "MS-Equivocal," or "Cannot Be Determined" should receive confirmatory testing using a validated orthogonal (alternative) method.

2. 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.
3. Homologous Recombination status may be reported for epithelial ovarian, peritoneal, or Fallopian tube carcinomas (Coleman et al., 2017; 28916367). Samples with deleterious *BRCA1/2* alteration and/or Loss of Heterozygosity (LOH) score ≥ 16% will be reported as "HRD Positive" and samples with absence of these findings will be reported as "HRD Not Detected," agnostic of potential secondary *BRCA1/2* reversion alterations. Certain potentially deleterious missense or small in-frame deletions in *BRCA1/2* may not be classified as deleterious and, in the absence of an elevated LOH profile, samples with such mutations may be classified as "HRD Not Detected." A result of "HRD Not Detected" does not rule out the presence of a *BRCA1/2* alteration or an elevated LOH profile outside the assay performance characteristic limitations.
4. 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. Detection of LOH has been verified only for ovarian cancer patients, and 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. Performance of the LOH classification has not been established for samples below 35% tumor content. 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.
5. Alterations reported may include somatic (not inherited) or germline (inherited) alterations; however, the test does not distinguish between germline and somatic alterations. The test does not provide information about susceptibility.
6. Biopsy may pose a risk to the patient when archival tissue is not available for use with the assay. The patient's physician should determine whether the patient is a candidate for biopsy.
7. Reflex testing to an alternative FDA approved companion diagnostic should be performed for patients who have an *ERBB2* amplification result detected with copy number equal to 4 (baseline ploidy of tumor +2) for confirmatory testing. While this result is considered negative by FoundationOne®CDx (F1CDx), in a clinical concordance study with an FDA approved FISH test, 70% (7 out of 10 samples) were positive, and 30% (3 out of 10 samples) were negative by the FISH test with an average ratio of 2.3. The frequency of *ERBB2* copy number 4 in breast cancer is estimated to be approximately 2%. Multiple references listed in https://www.mycancergenome.org/content/disease/breast-cancer/ERBB2/238/ report the frequency of HER2 overexpression as 20% in breast cancer. Based on the F1CDx HER2 CDx concordance study, approximately 10% of HER2 amplified samples had copy number 4. Thus, total frequency is conservatively estimated to be approximately 2%.

REPORT HIGHLIGHTS

The Report Highlights includes select genomic and therapeutic information with potential impact on patient care and treatment that is specific to the genomics and tumor type of the sample analyzed. This section may highlight information including targeted therapies with potential sensitivity or resistance; evidence-matched clinical trials; and variants with potential diagnostic, prognostic, nontargeted treatment, germline, or clonal hematopoiesis implications. Information included in the Report Highlights is expected to evolve with advances in scientific and clinical research. Findings included in the Report Highlights should be considered in the context of all other information in this report and other relevant patient information. Decisions on patient care and treatment are the responsibility of the treating physician.

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*

VARIANTS TO CONSIDER FOR FOLLOW-UP GERMLINE TESTING

The variants indicated for consideration of follow-up germline testing are 1) limited to reportable short variants with a protein effect listed in the ClinVar genomic database (Landrum et al., 2018; 29165669) as Pathogenic, Pathogenic/Likely Pathogenic, or Likely Pathogenic (by an expert panel or multiple submitters), 2) associated with hereditary cancer-predisposing disorder(s), 3) detected at an allele frequency of >10%, and 4) in select genes reported by the ESMO Precision Medicine Working Group (Mandelker et al., 2019; 31050713) to have a greater than 10% probability of germline origin if identified during tumor sequencing. The selected genes are *ATM, BAP1, BRCA1, BRCA2, BRIP1, CHEK2, FH, FLCN, MLH1, MSH2, MSH6, MUTYH, PALB2, PMS2, POLE, RAD51C, RAD51D, RET, SDHA, SDHB, SDHC, SDHD, TSC2,* and *VHL*, and are not inclusive of all cancer susceptibility genes. The content in this report should not substitute for genetic counseling or follow-up germline testing, which is needed to distinguish whether a finding in this patient's

tumor sequencing is germline or somatic. Interpretation should be based on clinical context.

VARIANTS THAT MAY REPRESENT CLONAL HEMATOPOIESIS

Variants that may represent clonal hematopoiesis (CH) are limited to select reportable short variants in defined genes identified in solid tumors only. Variant selection was determined based on gene tumor-suppressor or oncogene status, known role in solid tumors versus hematological malignancies, and literature prevalence. The defined genes are *ASXL1, CBL, DNMT3A, IDH2, JAK2, KMT2D (MLL2), MPL, MYD88, SF3B1, TET2*, and *U2AF1* and are not inclusive of all CH genes. The content in this report should not substitute for dedicated hematological workup. Comprehensive genomic profiling of solid tumors detects nontumor alterations that are due to CH. Patient-matched peripheral blood mononuclear cell sequencing is required to conclusively determine if this alteration is present in tumor or is secondary to CH. Interpretation should be based on clinical context.

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

This Report makes no promises or guarantees that a particular drug will be effective in the treatment of disease in 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 in this Report 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 entirely within the discretion of the treating 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. Certain sample or variant characteristics may result in reduced sensitivity. FoundationOne CDx is performed using DNA derived from tumor, and as such germline events may not be reported.

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

REFERENCE SEQUENCE INFORMATION

Sequence data is mapped to the human genome, Genome Reference Consortium Human Build 37 (GRCh37), also known as hg19.

MR Suite Version (RG) 7.6.0

The median exon coverage for this sample is 893x

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