FOUNDATIONONE®CDx

PATIENT	TUMOR TYPE	REPORT DATE
Li, Tien-Shun	Stomach adenocarcinoma (NOS)	20 Sep 2022
	COUNTRY CODE: TW	ORDERED TEST #: ORD-1452967-01

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 Stomach adenocarcinoma (NOS)
NAME Li, Tien-Shun
DATE OF BIRTH 05 May 1953
SEX Male
MEDICAL RECORD # 43192832

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 Stomach
SPECIMEN ID S111-29028A (PF22105)
SPECIMEN TYPE Slide Deck
DATE OF COLLECTION 29 July 2022
SPECIMEN RECEIVED 10 September 2022

Biomarker Findings

Microsatellite status - MS-Stable
Tumor Mutational Burden - 4 Muts/Mb

Genomic Findings

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

KRAS amplification
MDM2 amplification
MTAP loss exons 3-8
CDKN2A/B CDKN2A loss, CDKN2B loss
SMAD4 loss
TP53 S261N
ZNF217 amplification

1 Disease relevant genes with no reportable alterations: ***ERBB2***

Report Highlights

- Evidence-matched **clinical trial options** based on this patient's genomic findings: (p. 11)

BIOMARKER FINDINGS	THERAPY AND CLINICAL TRIAL IMPLICATIONS	
Microsatellite status - MS-Stable	**No therapies or clinical trials.** See Biomarker Findings section	
Tumor Mutational Burden - 4 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)**
***KRAS* -** amplification	none	none
10 Trials see p. 11		
***MDM2* -** amplification	none	none
2 Trials see p. 13		
***MTAP* -** loss exons 3-8	none	none
3 Trials see p. 14		

Electronically signed by Erik Williams, M.D. | 20 September 2022
Julia Elvin, M.D., Ph.D., Laboratory Director CLIA: 22D2027531
Nimesh R. Patel, M.D., Laboratory Director CLIA: 34D2044309
Foundation Medicine, Inc. | 1.888.988.3639

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 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.

Electronically signed by Erik Williams, M.D. | 20 September 2022
Julia Elvin, M.D., Ph.D., Laboratory Director CLIA: 22D2027531
Nimesh R. Patel, M.D., Laboratory Director CLIA: 34D2044309
Foundation Medicine, Inc. | 1.888.988.3639

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

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].

FREQUENCY & PROGNOSIS

MSI-H tumors reportedly make up 12-35% of sporadic gastric cancers[6-10]. In the context of diffuse-type gastric cancer, a higher frequency of MSI-H tumors has been reported in familial (28%, 7/25) versus sporadic (7%, 7/107) tumors; no difference in frequency of MSI-H tumors was observed for intestinal-type gastric cancer[9]. MSI-H tumors have been frequently associated with hypermethylation and loss of MLH1 expression in cancers of the upper gastrointestinal tract, including esophageal, gastroesophageal junction, and gastric adenocarcinomas[9,11-15]. MSI-H gastric cancers are associated with certain clinicopathological and molecular features, including intestinal type differentiation, antral location, advanced age, reduced lymph node metastasis, and better prognosis[7-8,16-19]. A retrospective meta-analysis of the prognostic role of MSI in gastric cancers reported an increased DFS and OS in patients with MSI-H versus MSS/MSI-Low[20]. Conversely, in the same study, MSI-H was a negative predictor of response and MSS/MSI-Low correlated with increased benefit for patients treated with chemotherapy plus surgery as opposed to surgery alone[20]. In gastroesophageal cancer, MSI-H status was associated with shorter PFS compared to MSS patients for patients treated with chemotherapy (mPFS 4.8 months vs 6.9 months, HR=0.4)[21].

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[22]. 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[22-24]. 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[25-27]. MSS status indicates MMR proficiency and typically correlates with intact expression of all MMR family proteins[22,24,26-27].

Electronically signed by Erik Williams, M.D. | 20 September 2022
Julia Elvin, M.D., Ph.D., Laboratory Director CLIA: 22D2027531
Nimesh R. Patel, M.D., Laboratory Director CLIA: 34D2044309
Foundation Medicine, Inc. | 1.888.988.3639

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

BIOMARKER

Tumor Mutational Burden

RESULT
4 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[28-30], anti-PD-1 therapies[28-31], and combination nivolumab and ipilimumab[32-37]. In multiple pan-tumor studies, increased tissue tumor mutational burden (TMB) was associated with sensitivity to immune checkpoint inhibitors[28-31,38-42]. 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[38]; similar findings were observed in the KEYNOTE 028 and 012 trials[31]. 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)[42]. 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[43]. 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[41]. 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[44] or those with lower TMB treated with PD-1 or PD-L1-targeting agents[29].

FREQUENCY & PROGNOSIS

Gastric adenocarcinoma harbors a median TMB of 3.6 mutations per megabase (muts/Mb), and 5.5% of cases have high TMB (>20 muts/Mb)[45]. However, one study reported high TMB in 20% of intestinal type stomach adenocarcinomas specifically[46]. Another study of patients with gastric cancer reported hypermutation (10-200 muts/Mb) in 16.4% of cases, with significant overrepresentation of samples with microsatellite instability among the hypermutant cases[47]. For patients with gastric cancer, increased TMB is reported to be associated with prolonged OS[48-50]. One study observed that the OS and disease-free survival (DFS) benefits of postoperative chemotherapy were more pronounced in patients with TMB-low gastric cancer (stage Ib/II) compared to those with TMB-high; however, patients with stage III gastric cancer benefitted regardless of TMB level[51]. In esophageal cancer, patients with TMB-high who had not received radiotherapy had significantly reduced OS (p=0.038) compared to those with TMB-low[52].

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[53-54] and cigarette smoke in lung cancer[55-56], treatment with temozolomide-based chemotherapy in glioma[57-58], mutations in the proofreading domains of DNA polymerases encoded by the POLE and POLD1 genes[59-63], and microsatellite instability (MSI)[59,62-63]. This sample harbors a TMB level associated with lower rates of clinical benefit from treatment with PD-1- or PD-L1-targeting immune checkpoint inhibitors compared with patients with tumors harboring higher TMB levels, based on several studies in multiple solid tumor types[29-30,38].

GENE
KRAS

ALTERATION
amplification

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

Preclinical evidence suggests that KRAS activation may predict sensitivity to MEK inhibitors, such as trametinib, binimetinib, cobimetinib, and selumetinib[64-69]. Clinical evidence that KRAS amplification in the absence of a concurrent KRAS activating mutation is sensitive to MEK inhibitors is limited. A Phase 2 study of selumetinib plus docetaxel in patients with gastric cancer reported 1/2 patients with KRAS amplification experienced a PR[70]. A patient with cervical cancer harboring both KRAS and PIK3CA amplification treated with the combination of trametinib and the AKT inhibitor GSK2141795 achieved a SD[71].

FREQUENCY & PROGNOSIS

Amplification of KRAS has been found in up to 6% of stomach adenocarcinoma cases[19,72-75]. KRAS alterations, including mutations[76] and amplification[77-79] are associated with worse prognosis in patients with gastroesophageal cancer. One study reported that KRAS alteration did not significantly associate with OS in a cohort of patients with gastric, esophageal, or gastroesophageal adenocarcinoma[80]. Published data investigating the prognostic implications of KRAS alterations in esophageal squamous cell carcinoma are limited (PubMed, Sep 2022).

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[65,81]. In numerous cancer type-specific studies as well as a large-scale pan-cancer analysis, KRAS amplification was shown to correlate with increased expression[75,82-84]. Additionally, KRAS amplification correlated with sensitivity of cancer cell lines to KRAS knockdown, suggesting that amplified KRAS is an oncogenic driver[84].

GENE
MDM2

ALTERATION
amplification

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

MDM2 antagonists disrupt the MDM2-p53 interaction, thereby stabilizing p53[85]. Preclinical studies have suggested that the amplification of MDM2, in the absence of concurrent TP53 mutations, may increase sensitivity to these agents[86-87]. Preliminary Phase 1 studies of the MDM2-p53 antagonist alrizomadlin (APG-115) reported a PR in a patient with liposarcoma harboring an MDM2 amplification and wildtype for TP53 and SD in 21%–38% (6/28 and 5/13, respectively) of patients in genomically unselected solid tumors[88-89] . A Phase 2 trial of alrizomadlin in combination with pembrolizumab reported a PR in 1 of 3 patients with malignant peripheral nerve sheath tumor that had failed standard therapy, as well as PRs in patients with multiple types of solid tumors that had failed immunotherapy, including 1 out of 14 patients with non-small cell lung cancer; 1 out of 5 patients with urothelial carcinoma; and 2 out of5, 1 out of 5, and 1 out of 11 patients with mucosal, uveal, and cutaneous melanoma, respectively[90]. Phase 1b studies of the MDM2 inhibitor idasanutlin for refractory AML in combination with cytarabine or venetoclax reported anti-leukemic response rates of 33% (25/75) and 37% (11/30), respectively[91-92] ; clinical benefit (58% ORR, 7/12) with idasanutlin monotherapy has been reported for patients with polycythemia vera[93]. The dual MDM2/MDM4 inhibitor ALRN-6924 led to an ORR of 27% (4/15) for patients with TP53 wildtype peripheral T-cell lymphoma in a Phase 2 study[94]; responses have also been observed in TP53 wildtype AML, MDS, Merkel cell carcinoma, colorectal cancer, and liposarcoma[95-96] .

FREQUENCY & PROGNOSIS

MDM2 amplification has been reported in 4.2% of stomach adenocarcinomas[19], 3.7-5.2% of esophageal adenocarcinomas[10,21], and 12.3% (7/57) of gastroesophageal junction carcinomas[21]. MDM2 amplification has been reported in a small number of esophageal squamous cell carcinomas (SCCs); however, MDM2 overexpression has been reported more frequently[97-100]. MDM2 overexpression has been reported to be associated with tumor stage and lymph node metastasis in gastric cardia adenocarcinoma (GCA) tumors, and was associated with differentiation in distal gastric adenocarcinoma (DGA) tumors[101]. Additionally, high MDM2 expression has been associated with poor overall survival in patients with gastric cancer[102]. In separate studies, amplification and over-expression of MDM2 has been identified in up to 42% of gastric carcinomas and has also been associated with Helicobacter pylori-related gastric cancer[103-104].

FINDING SUMMARY

MDM2 encodes an E3 ubiquitin protein ligase, which mediates the ubiquitination and subsequent degradation of p53, Rb1, and other proteins[105-107]. MDM2 acts to prevent the activity of the tumor suppressor p53; therefore, overexpression or amplification of MDM2 may be oncogenic[108-109]. Overexpression or amplification of MDM2 is frequent in cancer[110]. Although two retrospective clinical studies suggest that MDM2 amplification may predict a short time-to-treatment failure on anti-PD-1/PD-L1 immune checkpoint inhibitors, with 4/5 patients with MDM2 amplification[111] and 2/3 patients with MDM2 or MDM4 amplification[112] experiencing tumor hyperprogression, amplification of MDM2 or MDM4 was not associated with shorter progression-free survival (PFS) in a retrospective analysis of non-small cell lung cancer (NSCLC) outcomes with immune checkpoint inhibitors (hazard ratio of 1.4, p=0.44)[113]. The latter study reported PFS of >2 months for 5/8 patients with MDM2/MDM4 amplification[113].

GENE
MTAP

ALTERATION
loss exons 3-8

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

MTAP inactivation produces specific metabolic vulnerabilities that may be sensitive to MAT2A[114-115] or PRMT5 inhibition[115-117]. A Phase 1 trial of MAT2A inhibitor AG-270 reported 1 PR and 2 SDs lasting longer than 6 months for patients with advanced solid tumors displaying MTAP loss[118]. Preclinical data suggest that MTAP loss sensitizes cells to S-adenosyl-L-methionine (SAM)-competitive PRMT5 inhibitors[119], dual PRMT1 and PRMT5 inhibitors[120-122], and PRMT5 inhibitors that selectively bind the PRMT5 when complexed with S-methyl-5'-thioadenosine (MTA), such as MRTX1719, TNG908, and AMG193[123]. In preclinical models, MTAP inactivation showed increased sensitivity to inhibitors of purine synthesis or purine analogs, especially upon addition of exogenous MTA[124-134]. A Phase 2 study of L-alanosine, an inhibitor of adenine synthesis, as a monotherapy for 65 patients with MTAP-deficient cancers reported no responses and SD for 24% (13/55) of patients[135]. Preclinical and limited clinical evidence suggest MTAP deficiency may confer sensitivity to pemetrexed[136].

FREQUENCY & PROGNOSIS

MTAP loss/homozygous deletion as well as loss of expression has been reported in a wide variety of solid tumors and hematologic cancers[137-138]; such events have been correlated with poor prognosis in a variety of cancer types, including hepatocellular carcinoma[139], gastrointestinal stromal tumors[140], mantle cell lymphoma (MCL)[141], melanoma[142-143], gastric cancer[144], myxofibrosarcoma[145], nasopharyngeal carcinoma[146], ovarian carcinoma[137] and non-small cell lung cancer[147]. MTAP loss was not prognostic in pediatric B-cell acute lymphocytic leukemia[148] or in astrocytoma[149]. However, MTAP has also been reported to be overexpressed in colorectal cancer (CRC) samples[150], and MTAP retention is thought to be important for prostate cancer growth due to continuous supply of SAM[151]. Germline SNPs in MTAP have been correlated with the development of cutaneous melanoma[152-153], esophageal cancer[154-155], osteosarcoma[156], and CRC[157].

FINDING SUMMARY

MTAP encodes S-methyl-5'-thioadenosine (MTA) phosphorylase, a tumor suppressor involved in polyamine metabolism and methionine synthesis, although its enzymatic function is dispensable for its tumor suppressor activity[158-159]. Decreased expression of MTAP leads to MTA accumulation within tumor cells and their microenvironment[139,160-161], thereby reducing intracellular arginine methylation[115-117] and altering cell signaling[161-162]. MTAP is located at 9p21, adjacent to CDKN2A and CDKN2B, with which it is frequently co-deleted in various cancers. Other alterations in MTAP are rare and have not been extensively characterized.

GENE
CDKN2A/B

ALTERATION
CDKN2A loss, CDKN2B loss

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

Preclinical data suggest that tumors with loss of p16INK4a function may be sensitive to CDK4/6 inhibitors, such as abemaciclib, ribociclib, and palbociclib[163-166]. Clinical data in mesothelioma, breast cancer, and uterine leiomyosarcoma indicate that CDKN2A loss may predict sensitivity to abemaciclib[167] and palbociclib treatment[168-169]. However, multiple other clinical studies have shown no significant correlation between p16INK4a loss or inactivation and therapeutic benefit of these agents[170-176]; it is not known whether CDK4/6 inhibitors would be beneficial in this case. Although preclinical studies have suggested that loss of p14ARF function may be associated with reduced sensitivity to MDM2 inhibitors[87,177], the clinical relevance of p14ARF as a predictive biomarker is not clear. Because the p15INK4b protein encoded by CDKN2B is known to inhibit CDK4, tumors with CDKN2B mutation or loss may predict sensitivity to CDK4/6 inhibitors, such as ribociclib, abemaciclib, and palbociclib[171,173-174,178-180].

FREQUENCY & PROGNOSIS

Loss of the 9p21 region that encompasses the CDKN2A/CDKN2B locus has been observed in 24-67% of esophageal squamous cell carcinoma (ESCC)[181-188], and 26-37% of esophageal adenocarcinoma samples, and is thought to be a critical step in the progression of Barrett esophagus to adenocarcinoma[189-192]. In the Stomach Adenocarcinoma TCGA dataset, concurrent putative homozygous deletion of both CDKN2A and CDKN2B has been reported in 10% of cases[19]. In gastric cancer, methylation of CDKN2A and CDKN2B has been reported in 67% of cases and hypermethylation or deletion of CDKN2A alone has been reported in 20-72% of cases[193-198]. Expression of p16INK4a has been shown to decrease during gastric cancer tumorigenesis, detected in 96% of normal gastric tissue, 92% of dysplastic gastric mucosa, and 48% of gastric carcinoma samples[197]. One study found that expression of p14ARF decreases during disease progression in esophageal adenocarcinoma, with 75% (57/76) of samples having no detectable p14ARF expression; moreover, expression of p14ARF strongly correlated with increased survival[199]. Loss or downregulation of p15INK4b and p16INK4a expression has been reported in ESCC tumor samples and correlated with downregulation of TGFBR2, increased inflammation, and increased DNA damage[200]. In addition, loss of p16INK4a, but not p15INK4b, has been associated with tumor progression and poor prognosis in patients with ESCC[181-182,187,201-202]. Inactivation of CDKN2A and CDKN2B by promoter methylation has been linked with poor survival in patients with gastric cancer[196].

FINDING SUMMARY

CDKN2A encodes two different, unrelated tumor suppressor proteins, p16INK4a and p14ARF, whereas CDKN2B encodes the tumor suppressor p15INK4b[203-204]. Both p15INK4b and p16INK4a bind to and inhibit CDK4 and CDK6, thereby maintaining the growth-suppressive activity of the Rb tumor suppressor; loss or inactivation of either p15INK4b or p16INK4a contributes to dysregulation of the CDK4/6-cyclin-Rb pathway and loss of cell cycle control[205-206]. The tumor suppressive functions of p14ARF involve stabilization and activation of p53, via a mechanism of MDM2 inhibition[207-208]. One or more alterations observed here are predicted to result in p16INK4a loss of function[209-230]. One or more alterations seen here are predicted to result in p14ARF loss of function[213,230-233]. CDKN2B alterations such as seen here are predicted to inactivate p15INK4b[234].

POTENTIAL GERMLINE IMPLICATIONS

Germline CDKN2A mutation is associated with melanoma-pancreatic cancer syndrome, a condition marked by increased risk of developing malignant melanoma and/or pancreatic cancer[235]. Mutation carriers within families may develop either or both types of cancer, and melanoma cases may be referred to as familial or hereditary melanoma[236-237]. CDKN2A is the most implicated gene in familial melanoma, with germline mutations present in 16% to 20% of familial melanoma cases[238-240]. CDKN2A alteration has also been implicated in familial melanoma-astrocytoma syndrome, an extremely rare tumor association characterized by dual predisposition to melanoma and nervous system tumors[241-243]. In the appropriate clinical context, germline testing of CDKN2A is recommended.

GENE
SMAD4

ALTERATION
loss

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[244] and the Wnt/beta-catenin pathway[245].

— 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[246-248] and non-small cell lung cancer (NSCLC)[249]. Other clinical studies in pancreatic cancer have reported an association of high SMAD4 expression with better responses to neoadjuvant chemotherapy[250] and adjuvant chemoradiotherapy[251].

FREQUENCY & PROGNOSIS

SMAD4 mutation or homozygous deletion is most frequently observed in pancreatic adenocarcinoma (43%)[252], pancreatic acinar cell carcinoma (26%)[253], cholangiocarcinoma (25%)[254], small intestine cancer (20%)[255], appendiceal adenocarcinoma (14-20% mutation; 57% deletion)[256-257], colorectal adenocarcinoma (CRC; 14%)[62], esophageal adenocarcinoma (14%)[258], and stomach adenocarcinoma (13%)[19]. In preclinical studies, SMAD4 loss of function has been implicated in the development of mucinous neoplasms of the pancreas, including mucinous cystic neoplasms (MCN)[259] and intraductal papillary mucinous neoplasms (IPMN)[260]; 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[261]. SMAD4 gene alterations have been associated with reduced overall survival for patients with pancreatic adenocarcinoma[262]. Reduced SMAD4 expression has been associated with worse prognosis in various cancer types, including CRC[263-265], appendiceal mucinous neoplasm[266], gastric adenocarcinoma[267-268], esophageal adenocarcinoma[269], esophageal squamous cell carcinoma[270], breast cancer[271], and prostate cancer[272].

FINDING SUMMARY

SMAD4, also known as DPC4, encodes a tumor suppressor that regulates transcriptional activity downstream of TGF-beta receptor signaling[273-274]. 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[275-288].

POTENTIAL GERMLINE IMPLICATIONS

Germline SMAD4 mutations, including those at the R361 hotspot, have been observed in patients with juvenile polyposis syndrome[289-291], which is associated with an increased risk of gastrointestinal cancers[292]. The penetrance of deleterious SMAD4 mutations in patients with colon cancer is estimated at 20% by age 35 and 70% by age 65[293]. In the appropriate clinical context, germline testing of SMAD4 is recommended.

GENE
TP53

ALTERATION
S261N

TRANSCRIPT ID
NM_000546

CODING SEQUENCE EFFECT
782G>A

VARIANT ALLELE FREQUENCY (% VAF)
13.3%

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[294-297], or p53 gene therapy and immunotherapeutics such as SGT-53[298-302] and ALT-801[303]. 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[304]. 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[305]. 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[306]. 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[307]. 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[308]. 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[309]. 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[310]. 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[302]. Missense mutations leading to TP53 inactivation may also be sensitive to therapies that reactivate mutated p53 such as APR-246[311-313]. In a Phase 1b trial for patients with p53-positive high-grade serous ovarian cancer, APR-246 combined with carboplatin and pegylated liposomal doxorubicin achieved a 52% (11/21) response rate and 100% DCR[314]. 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[315-316]; however, ATR inhibitors as monotherapy had little effect on these parameters in solid tumor models in other preclinical studies[317-318]. Therefore, it is unclear whether TP53 inactivation predicts sensitivity to ATR inhibition.

FREQUENCY & PROGNOSIS

TP53 is frequently mutated in cancers of the gastrointestinal tract, with alterations reported in 34–72% of esophageal, gastroesophageal junction, and gastric adenocarcinomas[19,190,319-320]. Overexpression of p53 protein, which may occur as a result of mutation, has been reported in approximately 36% of gastric cancers, with p53 expression reported to be more frequent in intestinal-type compared with diffuse-type gastric cancer[321-324]. While some studies have reported no association between TP53 mutation status and prognosis in patients with esophageal carcinoma or gastroesophageal junction adenocarcinoma[319-320] others have associated TP53 mutation and elevated p53 expression with poor prognosis for patients with esophageal squamous cell carcinoma[325-326] or stomach cancer[327-329].

FINDING SUMMARY

Functional loss of the tumor suppressor p53, which is encoded by the TP53 gene, is common in aggressive advanced cancers[108]. Alterations such as seen here may disrupt TP53 function or expression[330-334].

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[335-337], including sarcomas[338-339]. Estimates for the prevalence of germline TP53 mutations in the general population range from 1:5,000[340] to 1:20,000[339]. 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[341]. 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[342-347]. 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[342-343]. Clinical management of patients with CH in this gene may include monitoring for hematologic changes and reduction of controllable risk factors for cardiovascular disease[348]. Comprehensive genomic profiling of solid tumors detects nontumor alterations that are due to CH[346,349-350]. Patient-matched peripheral blood mononuclear cell sequencing is required to conclusively determine if this alteration is present in tumor or is secondary to CH.

GENE
ZNF217

ALTERATION
amplification

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

There are no available targeted therapies to address genomic alterations in ZNF217. Expression of ZNF217 may predict relapse of estrogen receptor (ER)-positive breast cancer under hormone therapy through its direct interaction with ER-alpha[351-352]. ZNF217 overexpression has also been associated with resistance to paclitaxel[353] and doxorubicin[354] in breast cancer cell lines. ZNF217 has been suggested as a potential biomarker for treatment with the DNA synthesis inhibitor and AKT inhibitor triciribine in breast cancer based on preclinical findings in cultured cells and xenografts expressing high levels of ZNF217; triciribine treatment also restored sensitivity to doxorubicin in these cells[355].

FREQUENCY & PROGNOSIS

Amplification and/or overexpression of ZNF217 has been reported in breast[356], ovarian[357-358], gastric[359-360], colon[361], prostate[362], esophageal[363], and urothelial carcinomas[364], glioblastoma[365], and ovarian carcinosarcomas[366]. Overexpression in these tumors has generally been linked with aggressive tumor behavior and poor clinical prognosis. High levels of ZNF217 expression result in dysregulation of a broad range of genes that may contribute to tumorigenesis[367-369], and increased expression or activation of ERBB3[356,370], FAK[356], Aurora kinase A[353], AKT[354], and TGF-beta/SMAD signaling[356] has been demonstrated in ZNF217-expressing tumors or cells.

FINDING SUMMARY

ZNF217 encodes a candidate oncogene that has likely roles in histone modification and transcriptional repression[354,371]. ZNF217 amplification has been correlated with protein overexpression in breast carcinoma tumors and cell lines[372]. The role of ZNF217 in promoting tumorigenesis was established in preclinical studies demonstrating that expression of ZNF217 results in the immortalization of both human mammary epithelial cells and ovarian surface epithelial cells in culture[373-374].

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
KRAS

ALTERATION
amplification

RATIONALE
KRAS activating mutations or amplification may predict sensitivity to inhibitors of MAPK pathway components, including MEK inhibitors.

NCT03281369

PHASE 1/2

A Study of Multiple Immunotherapy-Based Treatment Combinations in Patients With Locally Advanced Unresectable or Metastatic Gastric or Gastroesophageal Junction Cancer (G/GEJ) (Morpheus-Gastric Cancer)

TARGETS
MEK, CXCR4, VEGFRs, PD-L1

LOCATIONS: Taipei City (Taiwan), Zhongzheng Dist. (Taiwan), Tainan (Taiwan), Suwon-si, (Korea, Republic of), Seoul (Korea, Republic of), Seodaemun-Gu (Korea, Republic of), Songpa-gu (Korea, Republic of), Blacktown (Australia), Melbourne (Australia), Clayton (Australia)

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)

NCT03284502

PHASE 1

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

TARGETS
MEK, RAFs

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

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)

NCT05159245

PHASE 2

The Finnish National Study to Facilitate Patient Access to Targeted Anti-cancer Drugs

TARGETS
BRAF, VEGFRs, RET, KIT, ERBB2, TRKB, ALK, TRKC, ROS1, TRKA, SMO, PD-L1, MEK, CDK4, CDK6

LOCATIONS: Kuopio (Finland), Helsinki (Finland), Tampere (Finland), Turku (Finland)

NCT03905148

PHASE 1/2

Study of the Safety and Pharmacokinetics of BGB-283 and PD-0325901 in Patients With Advanced or Refractory Solid Tumors

TARGETS
RAFs, EGFR, MEK

LOCATIONS: Nedlands (Australia), Blacktown (Australia), Randwick (Australia), Melbourne (Australia), California, Texas

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)

NCT04720976

PHASE 1/2

JAB-3312 Activity in Adult Patients With Advanced Solid Tumors

TARGETS
MEK, SHP2, PD-1, EGFR, KRAS

LOCATIONS: Utah

NCT04965818

PHASE 1/2

Phase 1b/2 Study of Futibatinib in Combination With Binimetinib in Patients With Advanced KRAS Mutant Cancer

TARGETS
MEK, FGFRs

LOCATIONS: California, Indiana, Texas

NCT04214418

PHASE 1/2

Study of Combination Therapy With the MEK Inhibitor, Cobimetinib, Immune Checkpoint Blockade, Atezolizumab, and the AUTOphagy Inhibitor, Hydroxychloroquine in KRAS-mutated Advanced Malignancies

TARGETS
PD-L1, MEK

LOCATIONS: Rhode Island, New York

GENE
MDM2

ALTERATION
amplification

RATIONALE
Inhibitors of the MDM2-p53 interaction are being tested in clinical trials. Overexpression or amplification of MDM2 may increase sensitivity to these agents, but more data are required.

NCT04785196

PHASE 1/2

APG-115 in Combination With PD-1 Inhibitor in Patients With Advanced Liposarcoma or Advanced Solid Tumors

TARGETS
PD-1, MDM2

LOCATIONS: Shanghai (China), Guangzhou (China)

NCT03611868

PHASE 1/2

A Study of APG-115 in Combination With Pembrolizumab in Patients With Metastatic Melanomas or Advanced Solid Tumors

TARGETS
MDM2, PD-1

LOCATIONS: Brisbane (Australia), South Brisbane (Australia), Bedford Park (Australia), Heidelberg (Australia), California, Arizona, Missouri, Arkansas, Ohio, Pennsylvania

GENE
MTAP

ALTERATION
loss exons 3-8

RATIONALE
MTAP loss may predict sensitivity to MAT2A inhibitors, or to inhibitors that target PRMT5 when in complex with MTA.

NCT05094336

PHASE 1/2

AMG 193, Methylthioadenosine (MTA) Cooperative Protein Arginine Methyltransferase 5 (PRMT5) Inhibitor, Alone and in Combination With Docetaxel in Advanced Methylthioadenosine Phosphorylase (MTAP)-Null Solid Tumors

TARGETS
PRMT5-MTA

LOCATIONS: Nagoya-shi (Japan), Chuo-ku (Japan), Kashiwa-shi (Japan), Camperdown (Australia), Halle (Saale) (Germany), Salzburg (Austria), Würzburg (Germany), Ulm (Germany), Edegem (Belgium), Bruxelles (Belgium)

NCT05245500

PHASE 1/2

Phase 1/2 Study of MRTX1719 in Solid Tumors With MTAP Deletion

TARGETS
PRMT5-MTA

LOCATIONS: Colorado, Massachusetts, New York, Tennessee, Texas

NCT05275478

PHASE 1/2

Safety and Tolerability of TNG908 in Patients With MTAP-deleted Solid Tumors

TARGETS
PRMT5-MTA

LOCATIONS: Massachusetts, Tennessee, Texas, Virginia

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.

AKT3
rearrangement

ALK
D605N

CBL
R506Q

FANCC
P248R

NKX2-1
G322S

NTRK3
A380S

PARP3
R379Q

PIK3C2G
amplification

PTCH1
E44G and R1303C

SUFU
P482L

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. 2022. 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 fractional-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) 7.1.0

The median exon coverage for this sample is 621x

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. Hiyama T, et al. J. Gastroenterol. Hepatol. (2004) pmid: 15209621
7. Wu MS, et al. Cancer Res. (1998) pmid: 9537253
8. dos Santos NR, et al. Gastroenterology (1996) pmid: 8536886
9. Fang WL, et al. Biomed Res Int (2013) pmid: 23555086
10. Cancer Genome Atlas Research Network, et al. Nature (2017) pmid: 28052061
11. Gu M, et al. Hepatogastroenterology () pmid: 19621725
12. Sasaki S, et al. Oncol Lett (2011) pmid: 22866067
13. Fleisher AS, et al. Cancer Res. (1999) pmid: 10070967
14. Vasavi M, et al. Cancer Biomark (2010) pmid: 21045259
15. Zhang LH, et al. Pathol. Res. Pract. (2013) pmid: 24035280
16. Zhu L, et al. Mol Clin Oncol (2015) pmid: 26137290
17. Leung SY, et al. Cancer Res. (1999) pmid: 9892201
18. Seo JY, et al. World J. Gastroenterol. (2015) pmid: 26078567
19. Nature (2014) pmid: 25079317
20. Pietrantonio F, et al. J. Clin. Oncol. (2019) pmid: 31513484
21. Janjigian YY, et al. Cancer Discov (2018) pmid: 29122777
22. Kocarnik JM, et al. Gastroenterol Rep (Oxf) (2015) pmid: 26337942
23. You JF, et al. Br. J. Cancer (2010) pmid: 21081928
24. Bairwa NK, et al. Methods Mol. Biol. (2014) pmid: 24623249
25. Boland CR, et al. Cancer Res. (1998) pmid: 9823339
26. Pawlik TM, et al. Dis. Markers (2004) pmid: 15528785
27. Boland CR, et al. Gastroenterology (2010) pmid: 20420947
28. Samstein RM, et al. Nat. Genet. (2019) pmid: 30643254
29. Goodman AM, et al. Mol. Cancer Ther. (2017) pmid: 28835386
30. Goodman AM, et al. Cancer Immunol Res (2019) pmid: 31405947
31. Cristescu R, et al. Science (2018) pmid: 30309915
32. Ready N, et al. J. Clin. Oncol. (2019) pmid: 30785829
33. Hellmann MD, et al. N. Engl. J. Med. (2018) pmid: 29658845
34. Hellmann MD, et al. Cancer Cell (2018) pmid: 29657128
35. Hellmann MD, et al. Cancer Cell (2018) pmid: 29731394
36. Rozeman EA, et al. Nat Med (2021) pmid: 33558721
37. Sharma P, et al. Cancer Cell (2020) pmid: 32916128
38. Marabelle A, et al. Lancet Oncol. (2020) pmid: 32919526
39. Ott PA, et al. J. Clin. Oncol. (2019) pmid: 30557521
40. Cristescu R, et al. J Immunother Cancer (2022) pmid: 35101941
41. Friedman CF, et al. Cancer Discov (2022) pmid: 34876409
42. Sturgill EG, et al. Oncologist (2022) pmid: 35274716
43. Schenker at al., 2022; AACR Abstract 7845
44. Legrand et al., 2018; ASCO Abstract 12000
45. Chalmers ZR, et al. Genome Med (2017) pmid: 28420421
46. Frampton et al., 2016; ASCO Abstract 11558
47. Li X, et al. Cancer Res. (2016) pmid: 26857262
48. Cai L, et al. Cancer Commun (Lond) (2020) pmid: 32141230
49. Zhao DY, et al. World J Gastrointest Oncol (2021) pmid: 33510848
50. Wei XL, et al. Ther Adv Med Oncol (2021) pmid: 33613701
51. Wang D, et al. Gastric Cancer (2021) pmid: 33687617
52. Yuan C, et al. Aging (Albany NY) (2020) pmid: 32165590
53. Pfeifer GP, et al. Mutat. Res. (2005) pmid: 15748635
54. Hill VK, et al. Annu Rev Genomics Hum Genet (2013) pmid: 23875803
55. Pfeifer GP, et al. Oncogene (2002) pmid: 12379884
56. Rizvi NA, et al. Science (2015) pmid: 25765070
57. Johnson BE, et al. Science (2014) pmid: 24336570
58. Choi S, et al. Neuro-oncology (2018) pmid: 29452419
59. Cancer Genome Atlas Research Network, et al. Nature (2013) pmid: 23636398
60. Briggs S, et al. J. Pathol. (2013) pmid: 23447401
61. Heitzer E, et al. Curr. Opin. Genet. Dev. (2014) pmid: 24583393
62. Nature (2012) pmid: 22810696
63. Roberts SA, et al. Nat. Rev. Cancer (2014) pmid: 25568919
64. Nakano H, et al. Proc. Natl. Acad. Sci. U.S.A. (1984) pmid: 6320174
65. Pylayeva-Gupta Y, et al. Nat. Rev. Cancer (2011) pmid: 21993244
66. Yamaguchi T, et al. Int. J. Oncol. (2011) pmid: 21523318
67. Watanabe M, et al. Cancer Sci. (2013) pmid: 23438367
68. Gilmartin AG, et al. Clin. Cancer Res. (2011) pmid: 21245089
69. Yeh JJ, et al. Mol. Cancer Ther. (2009) pmid: 19372556
70. Lee et al., 2018; ASCO Abstract 4061
71. Liu JF, et al. Gynecol. Oncol. (2019) pmid: 31118140
72. Wang K, et al. Nat. Genet. (2014) pmid: 24816253
73. Kakiuchi M, et al. Nat. Genet. (2014) pmid: 24816255
74. Wang K, et al. Nat. Genet. (2011) pmid: 22037554
75. Mita H, et al. BMC Cancer (2009) pmid: 19545448
76. Warneke VS, et al. Diagn. Mol. Pathol. (2013) pmid: 23846438
77. Wong GS, et al. Nat. Med. (2018) pmid: 29808010
78. Qian Z, et al. Genes Chromosomes Cancer (2014) pmid: 24935174
79. Deng N, et al. Gut (2012) pmid: 22315472
80. Kato S, et al. Clin. Cancer Res. (2018) pmid: 30348637
81. Kahn S, et al. Anticancer Res. () pmid: 3310850
82. McIntyre A, et al. Neoplasia (2005) pmid: 16354586
83. Birkeland E, et al. Br. J. Cancer (2012) pmid: 23099803
84. Chen Y, et al. PLoS ONE (2014) pmid: 24874471
85. Cheok CF, et al. Nat Rev Clin Oncol (2011) pmid: 20975744
86. Ohnstad HO, et al. Cancer (2013) pmid: 23165797
87. Gamble LD, et al. Oncogene (2012) pmid: 21725357
88. Zhang et al., 2019; ASCO Abstract 3124
89. Rasco et al., 2019; ASCO Abstract 3126
90. Tolcher et al., 2021; ASCO Abstract 2506
91. Martinelli et al., 2016; EHA21 Abstract S504
92. Daver et al., 2018; ASH Abstract 767
93. Mascarenhas et al., 2019; ASH Abstract 134
94. Shustov et al., 2018; ASH Abstract 1623
95. Sallman et al., 2018; ASH Abstract 4066
96. Meric-Bernstam et al., 2017; ASCO Abstract 2505
97. Tanière P, et al. Int. J. Cancer (2000) pmid: 11004672
98. Hsu HS, et al. Ann. Surg. Oncol. (2011) pmid: 21080085
99. Taghavi N, et al. BMC Cancer (2010) pmid: 20388212
100. Cheng TH, et al. J. Cancer Res. Clin. Oncol. (2009) pmid: 19488782
101. Yao D, et al. Hum. Pathol. (2013) pmid: 23159155
102. Ye Y, et al. Cancer Sci. (2013) pmid: 23347235
103. Günther T, et al. Mod. Pathol. (2000) pmid: 10874665
104. Yang Z, et al. Clin Res Hepatol Gastroenterol (2012) pmid: 22306053
105. Sdek P, et al. Mol. Cell (2005) pmid: 16337594
106. Brady M, et al. Mol. Cell. Biol. (2005) pmid: 15632057
107. Li M, et al. Mol. Cell (2004) pmid: 15053880
108. Brown CJ, et al. Nat. Rev. Cancer (2009) pmid: 19935675
109. Cordon-Cardo C, et al. Cancer Res. (1994) pmid: 8306343
110. Beroukhim R, et al. Nature (2010) pmid: 20164920
111. Kato S, et al. Clin. Cancer Res. (2017) pmid: 28351930
112. Singavi et al., 2017; ESMO Abstract 1140PD
113. Rizvi H, et al. J. Clin. Oncol. (2018) pmid: 29337640
114. Kalev P, et al. Cancer Cell (2021) pmid: 33450196
115. Marjon K, et al. Cell Rep (2016) pmid: 27068473
116. Mavrakis KJ, et al. Science (2016) pmid: 26912361
117. Kryukov GV, et al. Science (2016) pmid: 26912360
118. Heist et al., 2019; AACR-NCI-EORTC Abstract B116
119. Guccione E, et al. Nat. Rev. Mol. Cell Biol. (2019) pmid: 31350521
120. Fedoriw A, et al. Cancer Cell (2019) pmid: 31257072
121. Srour N, et al. Cancer Cell (2019) pmid: 31287990
122. Gao G, et al. Nucleic Acids Res. (2019) pmid: 30916320
123. Smith CR, et al. J Med Chem (2022) pmid: 35041419
124. Hansen LJ, et al. Cancer Res. (2019) pmid: 31040154
125. Tang B, et al. Cancer Res. (2018) pmid: 29844120
126. Munshi PN, et al. Oncologist (2014) pmid: 24928612
127. de Oliveira SF, et al. PLoS ONE (2016) pmid: 26751376
128. Lubin M, et al. PLoS ONE (2009) pmid: 19478948
129. Tang B, et al. Cancer Biol. Ther. (2012) pmid: 22825330
130. Collins CC, et al. Mol. Cancer Ther. (2012) pmid: 22252602
131. Bertino JR, et al. Cancer Biol. Ther. (2011) pmid: 21301207
132. Coulthard SA, et al. Mol. Cancer Ther. (2011) pmid: 21282358
133. Miyazaki S, et al. Int. J. Oncol. (2007) pmid: 17912432
134. Efferth T, et al. Blood Cells Mol. Dis. () pmid: 11987241
135. Kindler HL, et al. Invest New Drugs (2009) pmid: 18618081
136. Alhalabi O, et al. Nat Commun (2022) pmid: 35379845
137. Wei R, et al. Sci Rep (2016) pmid: 27929028
138. Zhao M, et al. BMC Genomics (2016) pmid: 27556634
139. Kirovski G, et al. Am. J. Pathol. (2011) pmid: 21356366
140. Huang HY, et al. Clin. Cancer Res. (2009) pmid: 19887491
141. Marcé S, et al. Clin. Cancer Res. (2006) pmid: 16778103
142. Meyer S, et al. Exp. Dermatol. (2010) pmid: 20500769
143. Wild PJ, et al. Arch Dermatol (2006) pmid: 16618867
144. Kim J, et al. Genes Chromosomes Cancer (2011) pmid: 21412930
145. Li CF, et al. Oncotarget (2014) pmid: 25426549
146. He HL, et al. Medicine (Baltimore) (2015) pmid: 26656376
147. Su CY, et al. Eur J Surg Oncol (2014) pmid: 24969958
148. Mirebeau D, et al. Haematologica (2006) pmid: 16818274
149. Becker AP, et al. Pathobiology (2015) pmid: 26088413
150. Snezhkina AV, et al. Oxid Med Cell Longev (2016) pmid: 27433286
151. Bistulfi G, et al. Oncotarget (2016) pmid: 26910893

Electronically signed by Erik Williams, M.D. | 20 September 2022
Julia Elvin, M.D., Ph.D., Laboratory Director CLIA: 22D2027531
Nimesh R. Patel, M.D., Laboratory Director CLIA: 34D2044309
Foundation Medicine, Inc. | 1.888.988.3639

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

152. Antonopoulou K, et al. J. Invest. Dermatol. (2015) pmid: 25407435
153. Maccioni L, et al. BMC Cancer (2013) pmid: 23816148
154. Hyland PL, et al. Int J Epidemiol (2016) pmid: 26635288
155. Lin X, et al. Cancer Sci. (2017) pmid: 27960044
156. Zhi L, et al. J Cancer (2016) pmid: 27994653
157. Gu F, et al. Br. J. Cancer (2013) pmid: 23361049
158. Limm K, et al. PLoS ONE (2016) pmid: 27479139
159. Tang B, et al. G3 (Bethesda) (2014) pmid: 25387827
160. Limm K, et al. Eur. J. Cancer (2013) pmid: 23265702
161. Stevens AP, et al. J. Cell. Biochem. (2009) pmid: 19097084
162. Limm K, et al. Eur. J. Cancer (2014) pmid: 25087184
163. Konecny GE, et al. Clin. Cancer Res. (2011) pmid: 21278246
164. Katsumi Y, et al. Biochem. Biophys. Res. Commun. (2011) pmid: 21871868
165. Cen L, et al. Neuro-oncology (2012) pmid: 22711607
166. Logan JE, et al. Anticancer Res. (2013) pmid: 23898052
167. Fennell DA, et al. Lancet Oncol (2022) pmid: 35157829
168. Elvin JA, et al. Oncologist (2017) pmid: 28283584
169. Gao J, et al. Curr Oncol (2015) pmid: 26715889
170. Gopalan et al., 2014; ASCO Abstract 8077
171. Peguero et al., 2016; ASCO Abstract 2528
172. Konecny et al., 2016; ASCO Abstract 5557
173. DeMichele A, et al. Clin. Cancer Res. (2015) pmid: 25501126
174. Finn RS, et al. Lancet Oncol. (2015) pmid: 25524798
175. Infante JR, et al. Clin. Cancer Res. (2016) pmid: 27542767
176. Johnson DB, et al. Oncologist (2014) pmid: 24797823
177. Van Maerken T, et al. Mol. Cancer Ther. (2011) pmid: 21460101
178. Shapiro et al., 2013; ASCO Abstract 2500
179. Flaherty KT, et al. Clin. Cancer Res. (2012) pmid: 22090362
180. Dickson MA, et al. J. Clin. Oncol. (2013) pmid: 23569312
181. Xing EP, et al. Clin. Cancer Res. (1999) pmid: 10537333
182. Hao JJ, et al. BMC Cancer (2012) pmid: 22920630
183. Rumiato E, et al. Cancer Lett. (2011) pmid: 21757289
184. Kim SG, et al. Korean J Gastroenterol (2006) pmid: 17060721
185. Liu Q, et al. Oncogene (1995) pmid: 7845688
186. Muzeau F, et al. Int. J. Cancer (1997) pmid: 9212218
187. Chen J, et al. Int. J. Cancer (2008) pmid: 18241037
188. Hayashi K, et al. Oncogene (1997) pmid: 9333024
189. Koppert LB, et al. J Surg Oncol (2005) pmid: 16299787
190. Dulak AM, et al. Nat. Genet. (2013) pmid: 23525077
191. Bandla S, et al. Ann. Thorac. Surg. (2012) pmid: 22450065
192. Nancarrow DJ, et al. Cancer Res. (2008) pmid: 18519675
193. Xiong HL, et al. Asian Pac. J. Cancer Prev. (2013) pmid: 23803092
194. Lee TL, et al. Clin. Cancer Res. (2002) pmid: 12060614
195. Alves MK, et al. Pathobiology (2011) pmid: 21849808
196. Shi J, et al. Am J Cancer Res (2012) pmid: 22206050
197. He XS, et al. World J. Gastroenterol. (2001) pmid: 11819820
198. Tang S, et al. Chin. Med. J. (2003) pmid: 12890389
199. Huang Y, et al. J. Cell. Mol. Med. (2009) pmid: 18410530
200. Achyut BR, et al. PLoS Genet. (2013) pmid: 23408900
201. Tokugawa T, et al. Cancer Res. (2002) pmid: 12208744
202. Fujiwara S, et al. Dis. Esophagus (2008) pmid: 18269647
203. Quelle DE, et al. Cell (1995) pmid: 8521522
204. Mutat. Res. (2005) pmid: 15878778
205. Gazzeri S, et al. Oncogene (1998) pmid: 9484839
206. Oncogene (1999) pmid: 10498883
207. Sherr CJ, et al. Cold Spring Harb. Symp. Quant. Biol. (2005) pmid: 16869746
208. Ozenne P, et al. Int. J. Cancer (2010) pmid: 20549699
209. Ruas M, et al. Oncogene (1999) pmid: 10498896
210. Jones R, et al. Cancer Res. (2007) pmid: 17909018
211. Haferkamp S, et al. Aging Cell (2008) pmid: 18843795
212. Huot TJ, et al. Mol. Cell. Biol. (2002) pmid: 12417717
213. Rizos H, et al. J. Biol. Chem. (2001) pmid: 11518711
214. Gombart AF, et al. Leukemia (1997) pmid: 9324288
215. Yang R, et al. Cancer Res. (1995) pmid: 7780957
216. Parry D, et al. Mol. Cell. Biol. (1996) pmid: 8668202
217. Greenblatt MS, et al. Oncogene (2003) pmid: 12606942
218. Yarbrough WG, et al. J. Natl. Cancer Inst. (1999) pmid: 10491434
219. Poi MJ, et al. Mol. Carcinog. (2001) pmid: 11255261
220. Byeon IJ, et al. Mol. Cell (1998) pmid: 9660926
221. Kannengiesser C, et al. Hum. Mutat. (2009) pmid: 19260062
222. Lal G, et al. Genes Chromosomes Cancer (2000) pmid: 10719365
223. Koh J, et al. Nature (1995) pmid: 7777061
224. McKenzie HA, et al. Hum. Mutat. (2010) pmid: 20340136
225. Miller PJ, et al. Hum. Mutat. (2011) pmid: 21462282
226. Kutscher CL, et al. Physiol. Behav. (1977) pmid: 905385
227. Scaini MC, et al. Hum. Mutat. (2014) pmid: 24659262
228. Jenkins NC, et al. J. Invest. Dermatol. (2013) pmid: 23190892
229. Walker GJ, et al. Int. J. Cancer (1999) pmid: 10389768
230. Rutter JL, et al. Oncogene (2003) pmid: 12853981
231. Itahana K, et al. Cancer Cell (2008) pmid: 18538737
232. Zhang Y, et al. Mol. Cell (1999) pmid: 10360174
233. Zhang Y, et al. Cell (1998) pmid: 9529249
234. Jafri M, et al. Cancer Discov (2015) pmid: 25873077
235. Whelan AJ, et al. N Engl J Med (1995) pmid: 7666917
236. Adv Exp Med Biol (2010) pmid: 20687502
237. Hogg D, et al. J Cutan Med Surg (1998) pmid: 9479083
238. De Unamuno B, et al. Melanoma Res (2018) pmid: 29543703
239. Soura E, et al. J Am Acad Dermatol (2016) pmid: 26892650
240. Huerta C, et al. Acta Derm Venereol (2018) pmid: 29405243
241. Kaufman DK, et al. Neurology (1993) pmid: 8414022
242. Bahuau M, et al. Cancer Res (1998) pmid: 9622062
243. Chan AK, et al. Clin Neuropathol () pmid: 28699883
244. Shi C, et al. Oncogene (2022) pmid: 35393542
245. Park JW, et al. Cancer Med (2022) pmid: 35274815
246. Ormanns S, et al. Int J Mol Sci (2017) pmid: 28534865
247. Fei N, et al. Clin Transl Sci (2021) pmid: 34002944
248. Bachet JB, et al. Ann. Oncol. (2012) pmid: 22377565
249. Ziemke M, et al. Lung Cancer (2017) pmid: 28577946
250. Kassardjian A, et al. Pancreas (2020) pmid: 32897998
251. Pen SL, et al. Radiother Oncol (2021) pmid: 33667587
252. Witkiewicz AK, et al. Nat Commun (2015) pmid: 25855536
253. Jiao Y, et al. J. Pathol. (2014) pmid: 24293293
254. Churi CR, et al. PLoS ONE (2014) pmid: 25536104
255. Takeda et al., 2022; ASCO GI Abstract 642
256. Liu X, et al. Clin. Chem. (2014) pmid: 24821835
257. Maru D, et al. Oncogene (2004) pmid: 14647445
258. Wang K, et al. Oncologist (2015) pmid: 26336083
259. Izeradjene K, et al. Cancer Cell (2007) pmid: 17349581
260. Bardeesy N, et al. Genes Dev. (2006) pmid: 17114584
261. Springer S, et al. Gastroenterology (2015) pmid: 26253305
262. Blackford A, et al. Clin. Cancer Res. (2009) pmid: 19584151
263. Yan P, et al. Clin. Cancer Res. (2016) pmid: 26861460
264. Kozak MM, et al. J. Clin. Pathol. (2015) pmid: 25681512
265. Roth AD, et al. J. Natl. Cancer Inst. (2012) pmid: 23104212
266. Davison JM, et al. Am. J. Surg. Pathol. (2014) pmid: 24618609
267. Kim YH, et al. Ann. Oncol. (2004) pmid: 15033661
268. Xiangming C, et al. Clin. Cancer Res. (2001) pmid: 11234879
269. Singhi AD, et al. Am. J. Surg. Pathol. (2015) pmid: 25634752
270. Natsugoe S, et al. Clin. Cancer Res. (2002) pmid: 12060625
271. de Kruijf EM, et al. Ann. Oncol. (2013) pmid: 23022998
272. Shipitsin M, et al. Br. J. Cancer (2014) pmid: 25032733
273. Nat. Rev. Mol. Cell Biol. (2012) pmid: 22992590
274. Cell (2008) pmid: 18662538
275. Massagué J, et al. Genes Dev. (2005) pmid: 16322555
276. Morén A, et al. Oncogene (2000) pmid: 10980615
277. Xu J, et al. Proc. Natl. Acad. Sci. U.S.A. (2000) pmid: 10781087
278. Luo K, et al. Genes Dev. (1999) pmid: 10485843
279. Jones JB, et al. Nucleic Acids Res. (2000) pmid: 10871368
280. Fink SP, et al. Cancer Res. (2001) pmid: 11196171
281. De Bosscher K, et al. Biochem. J. (2004) pmid: 14715079
282. Shi Y, et al. Nature (1997) pmid: 9214508
283. Miyaki M, et al. Oncogene (1999) pmid: 10340381
284. Prokova V, et al. Biochemistry (2007) pmid: 17994767
285. Wu JW, et al. J. Biol. Chem. (2001) pmid: 11274206
286. Ding L, et al. J. Clin. Invest. (2009) pmid: 19139564
287. Kuang C, et al. Oncogene (2004) pmid: 14647410
288. Watanabe M, et al. EMBO Rep. (2000) pmid: 11265759
289. Houlston R, et al. Hum. Mol. Genet. (1998) pmid: 9811934
290. Woodford-Richens K, et al. Gut (2000) pmid: 10764709
291. Howe JR, et al. J. Med. Genet. (2004) pmid: 15235019
292. Brosens LA, et al. World J. Gastroenterol. (2011) pmid: 22171123
293. Kalia SS, et al. Genet. Med. (2017) pmid: 27854360
294. Hirai H, et al. Cancer Biol. Ther. (2010) pmid: 20107315
295. Bridges KA, et al. Clin. Cancer Res. (2011) pmid: 21799033
296. Rajeshkumar NV, et al. Clin. Cancer Res. (2011) pmid: 21389100
297. Osman AA, et al. Mol. Cancer Ther. (2015) pmid: 25504633
298. Xu L, et al. Mol. Cancer Ther. (2002) pmid: 12489850
299. Xu L, et al. Mol. Med. (2001) pmid: 11713371
300. Camp ER, et al. Cancer Gene Ther. (2013) pmid: 23470564
301. Kim SS, et al. Nanomedicine (2015) pmid: 25240597
302. Pirollo KF, et al. Mol. Ther. (2016) pmid: 27357628
303. Hajdenberg et al., 2012; ASCO Abstract e15010
304. Leijen S, et al. J. Clin. Oncol. (2016) pmid: 27601554
305. Moore et al., 2019; ASCO Abstract 5513
306. Leijen S, et al. J. Clin. Oncol. (2016) pmid: 27998224
307. Oza et al., 2015; ASCO Abstract 5506
308. Lee J, et al. Cancer Discov (2019) pmid: 31315834
309. Méndez E, et al. Clin. Cancer Res. (2018) pmid:

29535125
310. Seligmann JF, et al. J Clin Oncol (2021) pmid: 34538072
311. Lehmann S, et al. J. Clin. Oncol. (2012) pmid: 22965953
312. Mohell N, et al. Cell Death Dis (2015) pmid: 26086967
313. Fransson Å, et al. J Ovarian Res (2016) pmid: 27179933
314. Gourley et al., 2016; ASCO Abstract 5571
315. Kwok M, et al. Blood (2016) pmid: 26563132
316. Boudny M, et al. Haematologica (2019) pmid: 30975914
317. Dillon MT, et al. Mol. Cancer Ther. (2017) pmid: 28062704
318. Middleton FK, et al. Cancers (Basel) (2018) pmid: 30127241
319. Sengpiel C, et al. Cancer Invest. (2009) pmid: 19160092
320. Pühringer-Oppermann F, et al. J. Cancer Res. Clin. Oncol. (2006) pmid: 16538517
321. Lee HE, et al. Pathobiology (2014) pmid: 23969480
322. Sezer C, et al. J BUON () pmid: 23613399
323. Victorzon M, et al. Eur. J. Cancer (1996) pmid: 8664030
324. Gonçalves AR, et al. Pathol. Oncol. Res. (2011) pmid: 21116760
325. Han U, et al. Dis. Esophagus (2007) pmid: 17760650
326. Yamasaki M, et al. Ann. Surg. Oncol. (2010) pmid: 19941080
327. Liu X, et al. PLoS ONE (2012) pmid: 23285001
328. Wiksten JP, et al. Anticancer Res. () pmid: 18751407
329. Migliavacca M, et al. J. Cell. Physiol. (2004) pmid: 15254976
330. Joerger AC, et al. Annu. Rev. Biochem. (2008) pmid: 18410249
331. Kato S, et al. Proc. Natl. Acad. Sci. U.S.A. (2003) pmid: 12826609
332. Kamada R, et al. J. Biol. Chem. (2011) pmid: 20978130
333. Zerdoumi Y, et al. Hum. Mol. Genet. (2017) pmid: 28472496
334. Yamada H, et al. Carcinogenesis (2007) pmid: 17690113
335. Bougeard G, et al. J. Clin. Oncol. (2015) pmid: 26014290
336. Sorrell AD, et al. Mol Diagn Ther (2013) pmid: 23355100
337. Nichols KE, et al. Cancer Epidemiol. Biomarkers Prev. (2001) pmid: 11219776
338. Kleihues P, et al. Am. J. Pathol. (1997) pmid: 9006316
339. Gonzalez KD, et al. J. Clin. Oncol. (2009) pmid: 19204208
340. Lalloo F, et al. Lancet (2003) pmid: 12672316
341. Mandelker D, et al. Ann. Oncol. (2019) pmid: 31050713
342. Jaiswal S, et al. N. Engl. J. Med. (2014) pmid: 25426837
343. Genovese G, et al. N. Engl. J. Med. (2014) pmid: 25426838
344. Xie M, et al. Nat. Med. (2014) pmid: 25326804
345. Acuna-Hidalgo R, et al. Am. J. Hum. Genet. (2017) pmid: 28669404
346. Severson EA, et al. Blood (2018) pmid: 29678827
347. Fuster JJ, et al. Circ. Res. (2018) pmid: 29420212
348. Hematology Am Soc Hematol Educ Program (2018) pmid: 30504320
349. Chabon JJ, et al. Nature (2020) pmid: 32269342
350. Razavi P, et al. Nat. Med. (2019) pmid: 31768066
351. Nguyen NT, et al. Mol Oncol (2014) pmid: 24973012
352. Frietze S, et al. BMC Genomics (2014) pmid: 24962896
353. Thollet A, et al. Mol. Cancer (2010) pmid: 21059223
354. Huang G, et al. Hum. Mol. Genet. (2005) pmid: 16203743
355. Littlepage LE, et al. Cancer Discov (2012) pmid: 22728437
356. Vendrell JA, et al. Cancer Res. (2012) pmid: 22593193
357. Li J, et al. Int J Clin Exp Pathol (2014) pmid: 25031722
358. Rahman MT, et al. Anticancer Res. (2012) pmid: 22843878
359. Yang SH, et al. Clin. Cancer Res. (2005) pmid: 15701848
360. Shida A, et al. Anticancer Res. (2014) pmid: 25202062
361. Rooney PH, et al. J. Pathol. (2004) pmid: 15476264
362. Szczyrba J, et al. Int. J. Cancer (2013) pmid: 22815235
363. Geppert CI, et al. Br. J. Cancer (2014) pmid: 24853183
364. Toncheva D, et al. Tumour Biol. () pmid: 15897688
365. Mao XG, et al. Lab. Invest. (2011) pmid: 21483406
366. Schipf A, et al. Virchows Arch. (2008) pmid: 18193277
367. Quinlan KG, et al. Biochim. Biophys. Acta (2007) pmid: 17572303
368. Krig SR, et al. J. Biol. Chem. (2007) pmid: 17259635
369. Cowger JJ, et al. Oncogene (2007) pmid: 17130829
370. Krig SR, et al. Oncogene (2010) pmid: 20661224
371. Banck MS, et al. Epigenetics (2009) pmid: 19242095
372. Collins C, et al. Proc. Natl. Acad. Sci. U.S.A. (1998) pmid: 9671742
373. Nonet GH, et al. Cancer Res. (2001) pmid: 11245413
374. Li P, et al. Int. J. Cancer (2007) pmid: 17266044