FOUNDATIONONE®CDx

PATIENT
Chen, Ssu-Tung

TUMOR TYPE
Brain anaplastic astrocytoma
COUNTRY CODE
TW

REPORT DATE
28 Jul 2022
ORDERED TEST #
ORD-1416203-01

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 Brain anaplastic astrocytoma
NAME Chen, Ssu-Tung
DATE OF BIRTH 19 November 1988
SEX Male
MEDICAL RECORD # 48143197

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 Brain
SPECIMEN ID S111-24510 A (PF22084)
SPECIMEN TYPE Slide Deck
DATE OF COLLECTION 28 June 2022
SPECIMEN RECEIVED 20 July 2022

Biomarker Findings

Microsatellite status - MS-Stable
Tumor Mutational Burden - 1 Muts/Mb

Genomic Findings

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

NF1 R192*, G2397R, Y2331fs*4, rearrangement intron 25
PIK3CA H1047L - subclonal†
H3F3A K28M
TP53 P278A - subclonal, R337C, R267W, splice site 993+2T>C - subclonal, S94* - subclonal†

† See About the Test in appendix for details.

Report Highlights

- Variants with **diagnostic implications** that may indicate a specific cancer type: ***H3F3A*** **K28M** (p. 6)
- Targeted therapies with potential clinical benefit **approved in another tumor type**: **Everolimus** (p. 8), **Selumetinib** (p. 8), **Temsirolimus** (p. 9), **Trametinib** (p. 9)
- Variants that may inform **nontargeted treatment approaches** (e.g., chemotherapy) in this tumor type: ***H3F3A*** **K28M** (p. 6)
- Evidence-matched **clinical trial options** based on this patient's genomic findings: (p. 10)
- Variants with **prognostic implications** for this tumor type that may impact treatment decisions: ***H3F3A*** **K28M** (p. 6)

BIOMARKER FINDINGS	THERAPY AND CLINICAL TRIAL IMPLICATIONS	
Microsatellite status - MS-Stable	**No therapies or clinical trials.** see Biomarker Findings section	
Tumor Mutational Burden - 1 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)**
NF1 **-** R192*, G2397R, Y2331fs*4, rearrangement intron 25	none	Selumetinib
		Trametinib
10 Trials *see p. 10*		
PIK3CA **-** H1047L - subclonal	none	Everolimus
		Temsirolimus
10 Trials *see p. 12*		

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.

H3F3A - K28M ..... p. 6
TP53 - P278A - subclonal, R337C, R267W, splice site 993+2T>C - subclonal, S94* - subclonal ..... p. 7

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

Electronically signed by Erik Williams, M.D. | 28 July 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
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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.

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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-High has been reported in 3-8% of adult or pediatric astrocytomas and was generally not associated with Lynch syndrome[6-8]. Low-level MSI has been reported in 5-9% of glioblastoma (GBM) samples[9-11]. A large-scale study did not find high-level microsatellite instability (MSI-H) in any of 129 GBM samples[9], although a small-scale study reported MSI-H in 4 of 15 pediatric GBMs and 1 of 12 adult GBMs[12]. The frequency of MSI has been reported to be increased in relapsed compared to primary GBM[9], in GBMs with a previous lower grade astrocytoma[10], and in giant cell GBM compared to classic GBM[11].

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[13]. 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[13-15]. 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[16-18]. MSS status indicates MMR proficiency and typically correlates with intact expression of all MMR family proteins[13,15,17-18].

BIOMARKER

Tumor Mutational Burden

RESULT
1 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[19-21], anti-PD-1 therapies[19-22], and combination nivolumab and ipilimumab[23-28]. In glioma, a lack of association between TMB and clinical benefit from immune checkpoint inhibitors has been reported[19,29-30]. However, multiple case studies have reported that patients with ultramutated gliomas driven by POLE mutations have benefited from treatment with anti-PD-1[31-32] or anti-PD-L1[33] therapies. Therefore, although increased TMB alone may not be a strong biomarker for PD-1 or PD-L1 inhibitors in this cancer type, these agents may have efficacy for patients with glioma harboring both high TMB and POLE mutation.

FREQUENCY & PROGNOSIS

Anaplastic astrocytoma harbors a median TMB of 1.8 mutations per megabase (muts/Mb), and 2% of cases have high TMB (>20 muts/Mb)[34]. For pediatric patients, high TMB has been reported in a subset of high-grade gliomas, frequently in association with mutations in mismatch repair or proofreading genes and in TP53, whereas BRAF alterations or other oncogene fusions were observed more frequently in brain tumors harboring low TMB[35-36]. Increased TMB has been reported to correlate with higher tumor grade in glioma[37] and glioblastoma (GBM) tissue samples with biallelic mismatch repair deficiency (bMMRD)[31], as well as with shorter OS of patients with diffuse glioma[38].

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[39-40] and cigarette smoke in lung cancer[41-42], treatment with temozolomide-based chemotherapy in glioma[43-44], mutations in the proofreading domains of DNA polymerases encoded by the POLE and POLD1 genes[45-49], and microsatellite instability (MSI)[45,48-49]. This sample harbors a TMB below levels that would be predicted to be associated with sensitivity to PD-1- or PD-L1-targeting immune checkpoint inhibitors, alone or in combination with other agents[19,29-33].

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GENE
NF1

ALTERATION
R192*, G2397R, Y2331fs*4, rearrangement intron 25

TRANSCRIPT ID
NM_001042492, NM_001042492, NM_001042492

CODING SEQUENCE EFFECT
574C>T, 7189G>A, 6992_7005delATTCAGCAGGTACC

VARIANT ALLELE FREQUENCY (% VAF)
20.9%, 9.5%, 30.4%

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

On the basis of clinical evidence in neurofibromatosis Type 1-associated neurofibroma[50-53], glioma or glioblastoma[53-57], and non-small cell lung cancer[58], NF1 inactivation may predict sensitivity to MEK inhibitors such as cobimetinib, trametinib, binimetinib, and selumetinib. Loss or inactivation of NF1 may also predict sensitivity to mTOR inhibitors, including everolimus and temsirolimus, based on limited clinical data[59-61] and strong preclinical data in models of malignant peripheral nerve sheath tumor (MPNST)[62-63]. A preclinical study suggests that combined mTOR and MEK inhibition is effective in a model of NF1-deficient MPNST[64]. Whereas frequent adverse events precluded a recommended Phase 2 dose and schedule for the combination of trametinib and everolimus in a Phase 1b trial for solid tumors[65], a retrospective study for heavily pretreated patients with solid tumors reported tolerable regimens of the combination for 23/31 patients, with 16 patients treated >3 months and evaluable patients achieving a median PFS of 6.5 months[66].

FREQUENCY & PROGNOSIS

NF1 mutation has been observed in 5-6% of lower grade gliomas and 9-14% of glioblastoma multiforme (GBM) cases; homozygous deletion of NF1 was observed in 1% of lower grade gliomas and 2-3% of GBMs[43,67-69]. NF1 loss was significantly associated with decreased overall and disease-specific survival in patients with lower grade gliomas (II-III), but not in those with GBM[70].

FINDING SUMMARY

NF1 encodes neurofibromin, a GTPase-activating protein (GAP) that is a key negative regulator of the RAS signaling pathway[71]. Neurofibromin acts as a tumor suppressor by repressing RAS signaling[72]. Alterations such as seen here may disrupt NF1 function or expression[72-81]. The consequences of alterations that may leave the GAP-related domain intact, such as seen here, are unclear; however, similar alterations have been previously reported in the context of cancer, which may indicate biological relevance.

POTENTIAL GERMLINE IMPLICATIONS

One or more of the NF1 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 neurofibromatosis type 1 (ClinVar, Mar 2022)[82]. Follow-up germline testing would be needed to distinguish whether the finding in this patient is somatic or germline. Germline mutations in NF1 cause the autosomal dominant disorder neurofibromatosis type 1, which is characterized in part by increased risk of developing various tumors, including sarcoma, glioma, breast carcinoma, and neuroendocrine and hematological neoplasms[83-85]. Estimates for the prevalence of the disorder in the general population range from 1:2,500 to 1:3,000[86-87], and in the appropriate clinical context, germline testing of NF1 is recommended.

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GENE
PIK3CA

ALTERATION
H1047L - subclonal

TRANSCRIPT ID
NM_006218

CODING SEQUENCE EFFECT
3140A>T

VARIANT ALLELE FREQUENCY (% VAF)
0.58%

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[88-95], AKT[96-97], or mTOR[59,98-104]. 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[95]. However, the Phase 2 study of copanlisib for patients with endometrial carcinoma harboring PIK3CA hotspot mutations failed to report any objective responses (n=11)[94].

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[92-93]. In the Phase 2 MATCH trial for patients with PIK3CA-mutated solid tumors, 28% (18/65) of patients experienced PFS lasting at least 6 months after treatment with taselisib; however, no ORs were observed in this study[105]. A separate Phase 1b study of taselisib in combination with the CDK4/6 inhibitor palbociclib for patients with PIK3CA-mutated solid tumors reported an ORR of 0% (n=12) and a DCR of 17% (2/12)[106]. In a Phase 1 trial of the dual PI3K/mTOR kinase inhibitor apitolisib, 79% (11/14) of patients with PIK3CA-mutated advanced solid tumors experienced disease control (3 PRs, 8 SDs)[107]. The PI3K inhibitor alpelisib is approved as a single agent for the treatment of patients with PIK3CA-related overgrowth spectrum (PROS)[108], but has shown limited activity as monotherapy for PIK3CA-mutated solid tumors with a Phase 1a study reporting an ORR of 6.0% (8/134) and a DCR of 58% (78/134)[109].

FREQUENCY & PROGNOSIS

PIK3CA mutations have been reported in 5-23% of high-grade gliomas (including glioblastomas, anaplastic astrocytomas, and anaplastic oligodendrogliomas)[69,110-113]. While another study did not observe PIK3CA mutations in low-grade astrocytomas or in anaplastic astrocytomas, it did report high ERK and AKT activity[110]. One study found that PIK3CA mutation in glioblastoma (GBM) was associated with shorter median PFS in both a discovery cohort (6.9 vs. 12.4 months, HR=2.89, p=0.01) and in the TCGA cohort (6.1 vs. 9 months, p=0.008), but was not consistently associated with median OS[114]. In a study of IDH-wildtype GBM, patients with alterations in PI3K class I genes (PIK3CA, PIK3R1, PIK3CG, and PIK3R2) had significantly longer OS (20.0 months altered vs. 16.9 months wildtype, HR=0.62, p=0.002) and PFS (11.0 months altered vs. 7.4 months wildtype, p=0.0043); patients with PIK3CA alterations experienced an improved OS but this association was not highly significant (20.0 months altered vs. 18.1 months wildtype, p=0.0407)[115].

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[116-117]. PIK3CA alterations that have been characterized as activating, such as observed here, are predicted to be oncogenic[118-139].

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GENE
H3F3A

ALTERATION
K28M

TRANSCRIPT ID
NM_002107

CODING SEQUENCE EFFECT
83A>T

VARIANT ALLELE FREQUENCY (% VAF)
43.3%

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

Prospective data from pooled clinical studies and preclinical evidence suggest that H3F3A K28M mutation predicts benefit from the investigational selective dopamine receptor D2 (DRD2) antagonist ONC201[140-141], which is supported by increased expression of the ONC201 target DRD2 in H3F3A K28M-mutant versus wild-type gliomas[141]. Among adult patients with recurrent H3F3A K28M-mutant gliomas, ONC201 achieved a DCR of 64% (7/11) and a 6-month PFS rate of 36% (5/14), with 3 patients experiencing complete and durable regressions of thalamic lesions[142]. Data from pooled ONC201 monotherapy trials showed that 31% (9/29) of patients with recurrent H3F3A K28M-mutated glioma remain progression free at 6.5 months median follow-up[143]. Although other H3F3A mutations have been reported[36], it is unclear whether these therapeutic strategies would be relevant in gliomas with H3F3A mutations other than K28M.

— Nontargeted Approaches —

Patients with pediatric H3 K27-altered diffuse midline glioma may benefit from radiotherapy, either as a single modality or combined with alkylating agents (NCCN Pediatric Central Nervous Systems Cancers, v1.2023).

FREQUENCY & PROGNOSIS

Recurrent mutations in the histone tail of H3F3A, at sites involved in critical post-translational modifications, have been reported at high frequency in pediatric and young adult brain tumors, including diffuse midline gliomas[144], diffuse hemispheric glioma[145], glioblastomas[146-148], aggressive pediatric gliomas[149], pilocytic astrocytomas[150], gangliogliomas[151], glial and glioneuronal tumors[152], as well as in low-grade gliomas undergoing transformation and secondary high-grade gliomas[153]. These mutations were commonly found concurrently with mutations in TP53 or in ATRX and DAXX, which form a complex required for H3.3 recruitment to DNA, and were mutually exclusive with IDH1 mutations, which indirectly affect methylation of critical H3.3 residues[147]. H3F3A K28M (also known as K27M) is a poor prognostic marker in glioma (NCCN CNS Cancers Guidelines, v1.2022). H3F3A G35 mutations are associated with disease onset during adolescence, whereas K28 mutations affect younger children and predict poorer IOS[148,154]. H3F3A K28M mutation has also been identified in 58% of adult midline gliomas, and is associated with shorter OS for patients with brainstem gliomas but not for patients with thalamic gliomas[155]. Mutations of H3F3A or H3F3B, the other gene encoding histone H3.3, have also been detected in giant cell tumor of bone and chondroblastoma, with low mutation frequencies in other tumors of cartilage and bone[156-158]. H3F3B K37M (commonly known as K36M) has been identified in head and neck squamous cell carcinoma, specifically in tumors of the oral cavity[159]. Overexpression of H3F3A is associated with poor survival in lung adenocarcinomas, and is thought to promote cancer cell invasion[160].

FINDING SUMMARY

H3F3A encodes the histone 3 variant H3.3. Histones form part of the nucleosome complex around which DNA is coiled in the cell. H3F3A mutations affecting different hotspot residues, such as G35 (commonly referred to as G34 in the literature) and K28 (commonly known as K27), form different subgroups based on methylation and gene expression differences, the region of the brain affected, and clinical parameters[154].

POTENTIAL DIAGNOSTIC IMPLICATIONS

H3F3A K27M mutation is characteristic of diffuse midline glioma, H3 K27M-altered (NCCN CNS Cancers Guidelines, v1.2022)(NCCN Pediatric CNS Cancers Guidelines, v1.2023)[161-162].

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GENE
TP53

ALTERATION
P278A - subclonal, R337C, R267W, splice site 993+2T>C - subclonal, S94* - subclonal

TRANSCRIPT ID
NM_000546, NM_000546, NM_000546, NM_000546, NM_000546

CODING SEQUENCE EFFECT
832C>G, 1009C>T, 799C>T, 993+2T>C, 281C>A

VARIANT ALLELE FREQUENCY (% VAF)
4.4%, 41.1%, 6.3%, 1.5%, 1.3%

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[163-166], or p53 gene therapy and immunotherapeutics such as SGT-53[167-171] and ALT-801[172]. 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[173]. 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[174]. 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[175]. 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[176]. 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[177]. 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[178]. 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[179]. 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[171]. Missense mutations leading to TP53 inactivation may also be sensitive to therapies that reactivate mutated p53 such as APR-246[180-182]. 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[183]. 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[184-185]; however, ATR inhibitors as monotherapy had little effect on these parameters in solid tumor models in other preclinical studies[186-187]. Therefore, it is unclear whether TP53 inactivation predicts sensitivity to ATR inhibition.

FREQUENCY & PROGNOSIS

TP53 mutations have been reported in 18-40% of astrocytoma samples, and preferentially in anaplastic astrocytoma; one study reported TP53 loss of function and partially/fully functional mutations in 15% and 25% of anaplastic astrocytomas, respectively[188-193]. Some studies suggest that the presence of a TP53 mutation is correlated with a favorable prognosis in patients with glioblastoma (GBM)[194]. One study reported that TP53 alterations were associated with poorer OS (12.9 months altered vs. 19.7 months wildtype, HR=1.58, p=0.0054) in IDH-wildtype GBM[115]. Mutation of TP53 is thought to be an early step in the tumorigenesis of astrocytomas, which can progress into anaplastic astrocytoma and then glioblastoma through gain of other genetic abnormalities such as loss of CDKN2A or RB1, followed by loss of PTEN[195].

FINDING SUMMARY

Functional loss of the tumor suppressor p53, which is encoded by the TP53 gene, is common in aggressive advanced cancers[196]. Alterations such as seen here may disrupt TP53 function or expression[197-201].

POTENTIAL GERMLINE IMPLICATIONS

One or more of the TP53 variants observed here has been described in the ClinVar database as a likely pathogenic or pathogenic germline mutation (by an expert panel or multiple submitters) associated with Li-Fraumeni syndrome (ClinVar, Mar 2022)[82]. Follow-up germline testing would be needed to distinguish whether the finding in this patient is somatic or germline. Germline mutations in TP53 are associated with the very rare autosomal dominant disorder Li-Fraumeni syndrome and the early onset of many cancers[202-204], including sarcomas[205-206]. Estimates for the prevalence of germline TP53 mutations in the general population range from 1:5,000[207] to 1:20,000[206]. 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[208]. 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[209-214]. 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[209-210]. Clinical management of patients with CH in this gene may include monitoring for hematologic changes and reduction of controllable risk factors for cardiovascular disease[215]. Comprehensive genomic profiling of solid tumors detects nontumor alterations that are due to CH[213,216-217]. Patient-matched peripheral blood mononuclear cell sequencing is required to conclusively determine if this alteration is present in tumor or is secondary to CH.

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Everolimus

Assay findings association

PIK3CA
H1047L - subclonal

AREAS OF THERAPEUTIC USE

Everolimus is an orally available mTOR inhibitor that is FDA approved to treat renal cell carcinoma (RCC) following antiangiogenic therapy; pancreatic neuroendocrine tumors; and well-differentiated non-functional neuroendocrine tumors of the lung or gastrointestinal tract. Everolimus is also approved to treat either renal angiomyolipoma or subependymal giant cell astrocytoma in association with tuberous sclerosis complex (TSC). Please see the drug label for full prescribing information.

GENE ASSOCIATION

On the basis of clinical evidence[59,98-104] , PIK3CA activating mutations may predict sensitivity to mTOR inhibitors such as everolimus and temsirolimus. Studies have reported modest activity of these therapies as single agents (ORRs of 0-4%), but improved activity has been observed when they are combined with other agents such as bevacizumab and doxorubicin (ORRs of 25-44%), for patients with PIK3CA-mutated solid tumors[101-104,218-222] .

SUPPORTING DATA

Case reports have described 2 children with PIK3CA-mutated diffuse glioma or glioneuronal tumor who benefited from treatment with everolimus alone or in combination with temozolomide[223-224] , and 1 adult with glioblastoma (GBM) harboring PIK3CA mutation and KRAS amplification who experienced disease progression with single-agent everolimus[225]. A Phase 2 trial of radiotherapy (RT), temozolomide (TMZ), and bevacizumab followed by everolimus and bevacizumab reported that 61% (31/51) of patients with newly diagnosed glioblastoma had objective responses with a median progression-free survival (PFS) of 11.3 months and median overall survival (OS) of 13.9 months[226]. A Phase 2 study of everolimus combined with TMZ and RT for the treatment of newly diagnosed glioblastoma reported a median PFS of 6.4 months and median OS of 15.8 months[227]. A Phase 1 trial of everolimus plus TMZ for patients with newly diagnosed or progressive glioblastoma reported partial responses (PR) in 11% (3/28) and stable disease (SD) in 57% (16/28) of cases[228]. A pilot study of everolimus with gefitinib in patients with recurrent glioblastoma reported 14% (3/22) PRs, 36% (8/22) SDs, and median PFS and OS of 2.6 months and 5.8 months, respectively[229]. Everolimus treatment achieved SD in 45% (5/11) of pediatric patients with heavily pretreated low-grade CNS tumors; median PFS of these responses was 14 months[230]. Whereas frequent adverse events precluded a recommended Phase 2 dose and schedule for the combination of trametinib and everolimus in a Phase 1b trial for solid tumors[65], a retrospective study for heavily pretreated patients with solid tumors reported tolerable regimens of the combination for 23/31 patients, with 16 patients treated >3 months and evaluable patients achieving a median PFS of 6.5 months[66].

Selumetinib

Assay findings association

NF1
R192*, G2397R, Y2331fs*4, rearrangement intron 25

AREAS OF THERAPEUTIC USE

Selumetinib is a MEK inhibitor that is FDA approved to treat pediatric patients 2 years of age and older with neurofibromatosis type 1 (NF1) who have symptomatic, inoperable plexiform neurofibromas (PNs). Please see the drug label for full prescribing information.

GENE ASSOCIATION

On the basis of clinical evidence in neurofibromatosis type 1 (NF1)-associated neurofibroma[50-53,231-235] , glioma[53-57,236] , and non-small cell lung cancer[58], NF1 inactivation may predict sensitivity to MEK inhibitors.

SUPPORTING DATA

Selumetinib has demonstrated clinical activity in low-grade glioma. A Phase 2 study of selumetinib for patients with low-grade glioma (LGG) reported 8/25 PRs for patients with BRAF alterations and 10/25 PRs for those with NF1-associated LGG[54]; a Phase 1 study of selumetinib reported 5/25 PRs for patients with LGG[237]. A Phase 2 study of selumetinib for patients with tumors with activating alterations in the MAPK pathway evaluated 8 patients with high-grade glioma (HGG); 2 SDs and no objective responses were observed in this subset[238].

Temsirolimus

Assay findings association

PIK3CA
H1047L - subclonal

AREAS OF THERAPEUTIC USE

Temsirolimus is an intravenous mTOR inhibitor that is FDA approved for the treatment of advanced renal cell carcinoma. Please see the drug label for full prescribing information.

GENE ASSOCIATION

On the basis of clinical evidence[59,98-104] , PIK3CA activating mutations may predict sensitivity to mTOR inhibitors such as everolimus and temsirolimus. Studies have reported modest activity of these therapies as single agents (ORRs of 0-4%), but improved activity has been observed when they are combined with other agents such as bevacizumab and doxorubicin (ORRs of 25-44%), for patients with PIK3CA-mutated solid tumors[101-104,218-222] .

SUPPORTING DATA

A Phase 1, dose-escalation trial combining temsirolimus and radiation/temozolomide therapy, with or without adjuvant temozolomide monotherapy, in patients with newly diagnosed glioblastoma reported no clinical responses but 24/25 patients experienced a period of stable disease; increased infection rates were noted with this regimen[239]. A Phase 1/2 trial of temsirolimus in combination with sorafenib in glioblastoma was terminated at the Phase 2 interim analysis after patients failed to meet the primary endpoint of 6 month progression-free survival; significant toxicity was also observed in the combination therapy, even at low doses of temsirolimus[240]. A Phase 2 study showed that addition of temsirolimus to bevacizumab therapy in patients with recurrent glioblastoma did not add clinical benefit[241]. A Phase 2 clinical trial of temsirolimus in pediatric glioma reported disease stabilization in 7/17 patients including one patient with anaplastic astrocytoma[242]. A Phase 1/2 study of temsirolimus in combination with erlotinib reported 6% (1/16) complete responses, 6% (1/16) partial responses, and 12.5% (2/16) instances of stable disease in patients with anaplastic glioma[243].

Trametinib

Assay findings association

NF1
R192*, G2397R, Y2331fs*4, rearrangement intron 25

AREAS OF THERAPEUTIC USE

Trametinib is a MEK inhibitor that is FDA approved as a monotherapy 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 neurofibromatosis type 1 (NF1)-associated neurofibroma[50-53,231-235] , glioma[53-57,236] , and non-small cell lung cancer[58], NF1 inactivation may predict sensitivity to MEK inhibitors.

SUPPORTING DATA

Case studies of trametinib in NF1-associated low-grade glioma have reported 7 PRs, including 2 patients with pilocytic astrocytoma, 2 patients with diffuse astrocytoma, 3 patients with low-grade glioma experiencing PRs of over 6 months[53,55-56,236] . A study of 2 pediatric patients with optic astrocytomas harboring BRAF duplications reported clinical benefit in response to trametinib with reductions in tumor volume (56-66%) and treatment ongoing at 484 and 468 days[244]. A study of 5 patients with KIAA1549-BRAF-fusion-positive pilocytic astrocytoma reported 1 PR and 3 minor responses[56] and, similarly, a patient with low-grade glioma harboring this fusion benefited from trametinib[245]. A patient with pilocytic astrocytoma harboring an NFIA-RAF1 fusion who had progressed on multiple lines of prior treatment exhibited ongoing SD following treatment with trametinib[246]. Whereas frequent adverse events precluded a recommended Phase 2 dose and schedule for the combination of trametinib and everolimus in a Phase 1b trial for solid tumors[65], a retrospective study for heavily pretreated patients with solid tumors reported tolerable regimens of the combination for 23/31 patients, with 16 patients treated >3 months and evaluable patients achieving a median PFS of 6.5 months[66].

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.

Electronically signed by Erik Williams, M.D. | 28 July 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
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NOTE Clinical trials are ordered by gene and prioritized by: age range inclusion criteria for pediatric patients, proximity to ordering medical facility, later trial phase, and verification of trial information within the last two months. While every effort is made to ensure the accuracy of the information contained below, the information available in the public domain is continually updated and should be investigated by the physician or research staff. This is not a comprehensive list of all available clinical trials. Foundation Medicine displays a subset of trial options and ranks them in this order of descending priority: Qualification for pediatric trial → Geographical proximity → Later trial phase. Clinical trials listed here may have additional enrollment criteria that may require medical screening to determine final eligibility. For additional information about listed clinical trials or to conduct a search for additional trials, please see clinicaltrials.gov. Or visit https://www.foundationmedicine.com/genomic-testing#support-services.

GENE

NF1

ALTERATION

R192*, G2397R, Y2331fs*4, rearrangement intron 25

RATIONALE

On the basis of clinical evidence and strong preclinical evidence, NF1 inactivation may predict sensitivity to MEK inhibitors. Limited clinical data and strong preclinical data indicate that loss or inactivation of NF1 may also predict sensitivity to mTOR inhibitors.

NCT03239015

PHASE 2

Efficacy and Safety of Targeted Precision Therapy in Refractory Tumor With Druggable Molecular Event

TARGETS
EGFR, ERBB2, ERBB4, PARP, mTOR, MET, RET, ROS1, VEGFRs, BRAF, CDK4, CDK6

LOCATIONS: Shanghai (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, KIT, PDGFRA, RET, VEGFRs, MEK

LOCATIONS: Guangzhou (China)

NCT05125523

PHASE 1

A Study of Sirolimus for Injection (Albumin Bound) in Patients With Advanced Solid Tumors

TARGETS
mTOR

LOCATIONS: Tianjin (China)

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)

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NCT05159245

PHASE 2

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

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

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

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

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
EGFR, RAFs, MEK

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

NCT04185831

PHASE 2

A MolEcularly Guided Anti-Cancer Drug Off-Label Trial

TARGETS
PD-L1, MEK, mTOR

LOCATIONS: Uppsala (Sweden), Gothenburg (Sweden)

NCT04720976

PHASE 1/2

JAB-3312 Activity in Adult Patients With Advanced Solid Tumors

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

LOCATIONS: Utah

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GENE

PIK3CA

ALTERATION

H1047L - subclonal

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.

NCT04589845

PHASE 2

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

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

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

NCT04341259

PHASE 1

A Study Of The Pharmacokinetics And Safety Of Ipatasertib In Chinese Participants With Locally Advanced Or Metastatic Solid Tumors.

TARGETS
AKTs

LOCATIONS: Shanghai City (China)

NCT03239015

PHASE 2

Efficacy and Safety of Targeted Precision Therapy in Refractory Tumor With Druggable Molecular Event

TARGETS
EGFR, ERBB2, ERBB4, PARP, mTOR, MET, RET, ROS1, VEGFRs, BRAF, CDK4, CDK6

LOCATIONS: Shanghai (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, KIT, PDGFRA, RET, VEGFRs, 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)

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NCT05125523

PHASE 1

A Study of Sirolimus for Injection (Albumin Bound) in Patients With Advanced Solid Tumors

TARGETS
mTOR

LOCATIONS: Tianjin (China)

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)

NCT03893487

PHASE NULL

Fimepinostat in Treating Brain Tumors in Children and Young Adults

TARGETS
HDAC, PI3K

LOCATIONS: Zürich (Switzerland), Washington, Oregon, California, Utah, Minnesota, Illinois, Michigan

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)

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

AR
S176R

DDR1
R825W

HNF1A
T285M

MAP2K2 (MEK2)
P298L

RICTOR
R873H

SOX9
P359S

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APPENDIX Genes Assayed in FoundationOne®CDx

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*	*ETV1*
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)

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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 PRINCIPLES

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 7.0.0

The median exon coverage for this sample is 886x

Electronically signed by Erik Williams, M.D. | 28 July 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

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1. Gatalica Z, et al. Cancer Epidemiol. Biomarkers Prev. (2014) pmid: 25392179
2. Kroemer G, et al. Oncoimmunology (2015) pmid: 26140250
3. Lal N, et al. Oncoimmunology (2015) pmid: 25949894
4. Le DT, et al. N. Engl. J. Med. (2015) pmid: 26028255
5. Ayers et al., 2016; ASCO-SITC Abstract P60
6. Alonso M, et al. Cancer Res. (2001) pmid: 11280776
7. Rodríguez-Hernández I, et al. PLoS ONE (2013) pmid: 24073290
8. Vladimirova V, et al. Neuropathol. Appl. Neurobiol. (2008) pmid: 18053027
9. Martinez R, et al. Oncology (2004) pmid: 15331927
10. Martinez R, et al. J. Cancer Res. Clin. Oncol. (2005) pmid: 15672285
11. Martinez R, et al. Cancer Genet. Cytogenet. (2007) pmid: 17498554
12. Szybka M, et al. Clin. Neuropathol. () pmid: 12908754
13. Kocarnik JM, et al. Gastroenterol Rep (Oxf) (2015) pmid: 26337942
14. You JF, et al. Br. J. Cancer (2010) pmid: 21081928
15. Bairwa NK, et al. Methods Mol. Biol. (2014) pmid: 24623249
16. Boland CR, et al. Cancer Res. (1998) pmid: 9823339
17. Pawlik TM, et al. Dis. Markers (2004) pmid: 15528785
18. Boland CR, et al. Gastroenterology (2010) pmid: 20420947
19. Samstein RM, et al. Nat. Genet. (2019) pmid: 30643254
20. Goodman AM, et al. Mol. Cancer Ther. (2017) pmid: 28835386
21. Goodman AM, et al. Cancer Immunol Res (2019) pmid: 31405947
22. Cristescu R, et al. Science (2018) pmid: 30309915
23. Ready N, et al. J. Clin. Oncol. (2019) pmid: 30785829
24. Hellmann MD, et al. N. Engl. J. Med. (2018) pmid: 29658845
25. Hellmann MD, et al. Cancer Cell (2018) pmid: 29657128
26. Hellmann MD, et al. Cancer Cell (2018) pmid: 29731394
27. Rozeman EA, et al. Nat Med (2021) pmid: 33558721
28. Sharma P, et al. Cancer Cell (2020) pmid: 32916128
29. Zhao J, et al. Nat. Med. (2019) pmid: 30742119
30. Touat M, et al. Nature (2020) pmid: 32322066
31. Bouffet E, et al. J. Clin. Oncol. (2016) pmid: 27001570
32. Johanns TM, et al. Cancer Discov (2016) pmid: 27683556
33. Lukas RV, et al. J. Neurooncol. (2018) pmid: 30073642
34. Chalmers ZR, et al. Genome Med (2017) pmid: 28420421
35. Patel RR, et al. Pediatr Blood Cancer (2020) pmid: 32386112
36. Johnson A, et al. Oncologist (2017) pmid: 28912153
37. Draaisma K, et al. Acta Neuropathol Commun (2015) pmid: 26699864
38. Wang L, et al. BMC Cancer (2020) pmid: 32164609
39. Pfeifer GP, et al. Mutat. Res. (2005) pmid: 15748635
40. Hill VK, et al. Annu Rev Genomics Hum Genet (2013) pmid: 23875803
41. Pfeifer GP, et al. Oncogene (2002) pmid: 12379884
42. Rizvi NA, et al. Science (2015) pmid: 25765070
43. Johnson BE, et al. Science (2014) pmid: 24336570
44. Choi S, et al. Neuro-oncology (2018) pmid: 29452419
45. Cancer Genome Atlas Research Network, et al. Nature (2013) pmid: 23636398
46. Briggs S, et al. J. Pathol. (2013) pmid: 23447401
47. Heitzer E, et al. Curr. Opin. Genet. Dev. (2014) pmid: 24583393
48. Nature (2012) pmid: 22810696
49. Roberts SA, et al. Nat. Rev. Cancer (2014) pmid: 25568919
50. Dombi E, et al. N. Engl. J. Med. (2016) pmid: 28029918
51. Schalkwijk S, et al. Cancer Chemother Pharmacol (2021) pmid: 33903938
52. Toledano H, et al. Childs Nerv Syst (2021) pmid: 33751171
53. Ronsley R, et al. Cancer Med (2021) pmid: 33939292
54. Fangusaro J, et al. Lancet Oncol. (2019) pmid: 31151904
55. Manoharan N, et al. J Neurooncol (2020) pmid: 32780261
56. Kondyli M, et al. J Neurooncol (2018) pmid: 30097824
57. Awada G, et al. Case Rep Oncol () pmid: 33082744
58. Middleton G, et al. Nature (2020) pmid: 32669708
59. Lim SM, et al. Oncotarget (2016) pmid: 26859683
60. Weiss B, et al. Neuro-oncology (2015) pmid: 25314964
61. Janku F, et al. Oncotarget (2014) pmid: 24931142
62. Johannessen CM, et al. Curr. Biol. (2008) pmid: 18164202
63. Johannessen CM, et al. Proc. Natl. Acad. Sci. U.S.A. (2005) pmid: 15937108
64. Malone CF, et al. Cancer Discov (2014) pmid: 24913553
65. Tolcher AW, et al. Ann. Oncol. (2015) pmid: 25344362
66. Patterson et al., 2018; AACR Abstract 3891
67. Cancer Genome Atlas Research Network, et al. N. Engl. J. Med. (2015) pmid: 26061751
68. Brennan CW, et al. Cell (2013) pmid: 24120142
69. Nature (2008) pmid: 18772890
70. Vizcaíno MA, et al. Hum. Pathol. (2015) pmid: 26190195
71. Hattori S, et al. Biochem. Biophys. Res. Commun. (1991) pmid: 1904223
72. Morcos P, et al. Mol. Cell. Biol. (1996) pmid: 8628317
73. Ballester R, et al. Cell (1990) pmid: 2121371
74. Xu GF, et al. Cell (1990) pmid: 2116237
75. Martin GA, et al. Cell (1990) pmid: 2121370
76. Thomas L, et al. Hum. Mutat. (2012) pmid: 22807134
77. Skuse GR, et al. Hum. Mol. Genet. (1997) pmid: 9300663
78. Messiaen LM, et al. Genet. Med. () pmid: 11258625
79. Ars E, et al. Hum. Mol. Genet. (2000) pmid: 10607834
80. Messiaen LM, et al. J. Med. Genet. (2005) pmid: 15863657
81. Poullet P, et al. Mol. Cell. Biol. (1994) pmid: 8264648
82. Landrum MJ, et al. Nucleic Acids Res. (2018) pmid: 29165669
83. Jett K, et al. Genet. Med. (2010) pmid: 20027112
84. Patil S, et al. Oncologist (2012) pmid: 22240541
85. Evans DG, et al. Clin Sarcoma Res (2012) pmid: 23036231
86. Upadhyaya M, et al. J. Med. Genet. (1995) pmid: 8544190
87. Williams VC, et al. Pediatrics (2009) pmid: 19117870
88. Fritsch C, et al. Mol. Cancer Ther. (2014) pmid: 24608574
89. Juric D, et al. J. Clin. Oncol. (2018) pmid: 29401002
90. Gallant JN, et al. NPJ Precis Oncol (2019) pmid: 30793038
91. Delestre F, et al. Sci Transl Med (2021) pmid: 34613809
92. Morschhauser F, et al. Mol Cancer Ther (2020) pmid: 31619463
93. Patnaik A, et al. Ann. Oncol. (2016) pmid: 27672108
94. Santin AD, et al. Gynecol Oncol Rep (2020) pmid: 31934607
95. Damodaran S, et al. J Clin Oncol (2022) pmid: 35133871
96. André F, et al. N. Engl. J. Med. (2019) pmid: 31091374
97. Smyth LM, et al. NPJ Breast Cancer (2021) pmid: 33863913
98. Park HS, et al. PLoS ONE (2016) pmid: 27105424
99. Hou MM, et al. Oncotarget (2014) pmid: 25426553
100. Varnier R, et al. Eur J Cancer (2019) pmid: 31351267
101. Janku F, et al. Cell Rep (2014) pmid: 24440717
102. Moroney J, et al. Clin. Cancer Res. (2012) pmid: 22927482
103. Basho RK, et al. JAMA Oncol (2017) pmid: 27893038
104. Moroney JW, et al. Clin. Cancer Res. (2011) pmid: 21890452
105. Krop et al., 2018; ASCO Abstract 101
106. Pascual J, et al. Cancer Discov (2021) pmid: 32958578
107. Dolly SO, et al. Clin. Cancer Res. (2016) pmid: 26787751
108. Canaud et al., 2021; ESMO Abstract LBA23
109. Aust Fam Physician (1986) pmid: 2941002
110. El-Habr EA, et al. Clin. Neuropathol. () pmid: 20569675
111. Gallia GL, et al. Mol. Cancer Res. (2006) pmid: 17050665
112. Broderick DK, et al. Cancer Res. (2004) pmid: 15289301
113. Derakhshandeh-Peykar P, et al. J. Neurogenet. (2011) pmid: 22026810
114. Tanaka S, et al. Acta Neuropathol Commun (2019) pmid: 31036078
115. Yan et al. 2020; DOI:10.1200/PO.19.00385
116. Samuels Y, et al. Cancer Cell (2005) pmid: 15950905
117. Nat. Rev. Cancer (2009) pmid: 19629070
118. Kang S, et al. Proc. Natl. Acad. Sci. U.S.A. (2005) pmid: 15647370
119. Ikenoue T, et al. Cancer Res. (2005) pmid: 15930273
120. Gymnopoulos M, et al. Proc. Natl. Acad. Sci. U.S.A. (2007) pmid: 17376864
121. Horn S, et al. Oncogene (2008) pmid: 18317450
122. Rudd ML, et al. Clin. Cancer Res. (2011) pmid: 21266528
123. Hon WC, et al. Oncogene (2012) pmid: 22120714
124. Burke JE, et al. Proc. Natl. Acad. Sci. U.S.A. (2012) pmid: 22949682
125. Wu H, et al. Proc. Natl. Acad. Sci. U.S.A. (2009) pmid: 19915146
126. Laurenti R, et al. Rev Saude Publica (1990) pmid: 2103068
127. Dan S, et al. Cancer Res. (2010) pmid: 20530683
128. Oda K, et al. Cancer Res. (2008) pmid: 18829572
129. Zhao L, et al. Oncogene (2008) pmid: 18794883
130. Lui VW, et al. Cancer Discov (2013) pmid: 23619167
131. Ross RL, et al. Oncogene (2013) pmid: 22430209
132. Rivière JB, et al. Nat. Genet. (2012) pmid: 22729224
133. Shibata T, et al. Cancer Lett. (2009) pmid: 19394761
134. Dogruluk T, et al. Cancer Res. (2015) pmid: 26627007
135. Croessmann S, et al. Clin. Cancer Res. (2018) pmid: 29284706
136. Ng PK, et al. Cancer Cell (2018) pmid: 29533785
137. Spangle JM, et al. (2020) pmid: 32929011
138. Chen L, et al. Nat Commun (2018) pmid: 29636477
139. Jin N, et al. J Clin Invest (2021) pmid: 34779417
140. Arrillaga-Romany I, et al. Oncotarget (2017) pmid: 29108308
141. Prabhu VV, et al. Clin. Cancer Res. (2018) pmid: 30559168
142. Chi et al., 2018; SNO abstract ACTR-34
143. Arrillaga et al., 2019; ASCO abstract 3005
144. Solomon DA, et al. Brain Pathol. (2016) pmid: 26517431
145. Fontebasso AM, et al. Acta Neuropathol (2013) pmid: 23417712
146. Pandit et al., 2016; ISPNO Abstract HG-58
147. Schwartzentruber J, et al. Nature (2012) pmid: 22286061
148. Venneti S, et al. Acta Neuropathol. (2014) pmid:

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25200322
149. Wu G, et al. Nat. Genet. (2012) pmid: 22286216
150. Hochart A, et al. Ann Clin Transl Neurol (2015) pmid: 25909089
151. Joyon N, et al. Neuropathol. Appl. Neurobiol. (2017) pmid: 27219822
152. Nguyen AT, et al. Neuropathol. Appl. Neurobiol. (2015) pmid: 25389051
153. Mistry M, et al. J. Clin. Oncol. (2015) pmid: 25667294
154. Sturm D, et al. Cancer Cell (2012) pmid: 23079654
155. Feng J, et al. Hum. Pathol. (2015) pmid: 26297251
156. Behjati S, et al. Nat. Genet. (2013) pmid: 24162739
157. Kervarrec T, et al. Mod Pathol (2017) pmid: 28059095
158. Righi A, et al. Hum Pathol (2017) pmid: 28899740
159. Papillon-Cavanagh S, et al. Nat. Genet. (2017) pmid: 28067913
160. Park SM, et al. Nat Commun (2016) pmid: 27694942
161. Louis DN, et al. Neuro Oncol (2021) pmid: 34185076
162. Pfister SM, et al. Cancer Discov (2022) pmid: 34921008
163. Hirai H, et al. Cancer Biol. Ther. (2010) pmid: 20107315
164. Bridges KA, et al. Clin. Cancer Res. (2011) pmid: 21799033
165. Rajeshkumar NV, et al. Clin. Cancer Res. (2011) pmid: 21389100
166. Osman AA, et al. Mol. Cancer Ther. (2015) pmid: 25504633
167. Xu L, et al. Mol. Cancer Ther. (2002) pmid: 12489850
168. Xu L, et al. Mol. Med. (2001) pmid: 11713371
169. Camp ER, et al. Cancer Gene Ther. (2013) pmid: 23470564
170. Kim SS, et al. Nanomedicine (2015) pmid: 25240597
171. Pirollo KF, et al. Mol. Ther. (2016) pmid: 27357628
172. Hajdenberg et al., 2012; ASCO Abstract e15010
173. Leijen S, et al. J. Clin. Oncol. (2016) pmid: 27601554
174. Moore et al., 2019; ASCO Abstract 5513
175. Leijen S, et al. J. Clin. Oncol. (2016) pmid: 27998224
176. Oza et al., 2015; ASCO Abstract 5506
177. Lee J, et al. Cancer Discov (2019) pmid: 31315834
178. Méndez E, et al. Clin. Cancer Res. (2018) pmid: 29535125
179. Seligmann JF, et al. J Clin Oncol (2021) pmid: 34538072
180. Lehmann S, et al. J. Clin. Oncol. (2012) pmid: 22965953
181. Mohell N, et al. Cell Death Dis (2015) pmid: 26086967
182. Fransson Å, et al. J Ovarian Res (2016) pmid: 27179933
183. Gourley et al., 2016; ASCO Abstract 5571
184. Kwok M, et al. Blood (2016) pmid: 26563132
185. Boudny M, et al. Haematologica (2019) pmid: 30975914
186. Dillon MT, et al. Mol. Cancer Ther. (2017) pmid: 28062704
187. Middleton FK, et al. Cancers (Basel) (2018) pmid: 30127241
188. Uno M, et al. Cancer Lett. (2005) pmid: 15914282
189. Uno M, et al. Int. J. Biol. Markers () pmid: 16711514
190. Lass U, et al. PLoS ONE (2012) pmid: 22844452
191. Faria MH, et al. APMIS (2012) pmid: 23009112
192. Milinkovic V, et al. PLoS ONE (2013) pmid: 24358143
193. Galatro TF, et al. PLoS ONE (2013) pmid: 23613880
194. Schmidt MC, et al. J. Neuropathol. Exp. Neurol. (2002) pmid: 11939587
195. Nozaki M, et al. Neuro-oncology (1999) pmid: 11550308
196. Brown CJ, et al. Nat. Rev. Cancer (2009) pmid: 19935675
197. Joerger AC, et al. Annu. Rev. Biochem. (2008) pmid: 18410249
198. Kato S, et al. Proc. Natl. Acad. Sci. U.S.A. (2003) pmid: 12826609
199. Kamada R, et al. J. Biol. Chem. (2011) pmid: 20978130
200. Zerdoumi Y, et al. Hum. Mol. Genet. (2017) pmid: 28472496
201. Yamada H, et al. Carcinogenesis (2007) pmid: 17690113
202. Bougeard G, et al. J. Clin. Oncol. (2015) pmid: 26014290
203. Sorrell AD, et al. Mol Diagn Ther (2013) pmid: 23355100
204. Nichols KE, et al. Cancer Epidemiol. Biomarkers Prev. (2001) pmid: 11219776
205. Kleihues P, et al. Am. J. Pathol. (1997) pmid: 9006316
206. Gonzalez KD, et al. J. Clin. Oncol. (2009) pmid: 19204208
207. Lalloo F, et al. Lancet (2003) pmid: 12672316
208. Mandelker D, et al. Ann. Oncol. (2019) pmid: 31050713
209. Jaiswal S, et al. N. Engl. J. Med. (2014) pmid: 25426837
210. Genovese G, et al. N. Engl. J. Med. (2014) pmid: 25426838
211. Xie M, et al. Nat. Med. (2014) pmid: 25326804
212. Acuna-Hidalgo R, et al. Am. J. Hum. Genet. (2017) pmid: 28669404
213. Severson EA, et al. Blood (2018) pmid: 29678827
214. Fuster JJ, et al. Circ. Res. (2018) pmid: 29420212
215. Hematology Am Soc Hematol Educ Program (2018) pmid: 30504320
216. Chabon JJ, et al. Nature (2020) pmid: 32269342
217. Razavi P, et al. Nat. Med. (2019) pmid: 31768066
218. Janku F, et al. Cancer Res. (2013) pmid: 23066039
219. Janku F, et al. J. Clin. Oncol. (2012) pmid: 22271473
220. Janku F, et al. Mol. Cancer Ther. (2011) pmid: 21216929
221. Moulder S, et al. Ann. Oncol. (2015) pmid: 25878190
222. Byeon et al., 2020; doi: 10.21037/tcr.2020.04.07
223. Gojo J, et al. Front Oncol (2019) pmid: 31998633
224. McNall-Knapp et al., 2020; SNO Abstract LGG-47
225. Blumenthal DT, et al. J. Neurooncol. (2016) pmid: 27531351
226. Hainsworth JD, et al. Clin Adv Hematol Oncol (2012) pmid: 22706484
227. Ma DJ, et al. Neuro-oncology (2015) pmid: 25526733
228. Mason WP, et al. Invest New Drugs (2012) pmid: 22160854
229. Kreisl TN, et al. J. Neurooncol. (2009) pmid: 19018475
230. Segal et al., 2016; ISPNO Abstract EPT-21
231. Glassberg et al., 2020; ASPHO Abstract 2015
232. Coyne et al., 2020; ASCO Abstract 3612
233. McCowage et al., 2018; ASCO Abstract 10504
234. Mueller et al., 2020; SNO Abstract NFB-17
235. Waldner et al., 2020; DOI: 10.1055/s-0040-1715638
236. Romo et al., 2019; SNO Abstract RARE-54
237. Banerjee A, et al. Neuro-oncology (2017) pmid: 28339824
238. Eckstein OS, et al. J Clin Oncol (2022) pmid: 35363510
239. Sarkaria JN, et al. Clin. Cancer Res. (2010) pmid: 20921209
240. Lee EQ, et al. Neuro-oncology (2012) pmid: 23099651
241. Lassen U, et al. Anticancer Res. (2013) pmid: 23564811
242. Geoerger B, et al. Eur. J. Cancer (2012) pmid: 22033322
243. Wen PY, et al. Neuro-oncology (2014) pmid: 24470557
244. Miller C, et al. J Neurosurg Pediatr (2017) pmid: 28009226
245. Wagner LM, et al. Pediatr Blood Cancer (2018) pmid: 29369501
246. Yde CW, et al. Cancer Genet (2016) pmid: 27810072

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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