FOUNDATIONONE®

Patient Name	Report Date	Tumor Type
Arya, Krishna	**17 September 2016**	**Thyroid anaplastic carcinoma**

Date of Birth	04 March 1949	**Medical Facility**	Max Healthcare		
Sex	Female	**Ordering Physician**	Verma, Amit	**Specimen Received**	08 September 2016
FMI Case #	TRF172343	**Additional Recipient**	Not Given	**Specimen Site**	Thyroid
Medical Record #	Not Given	**Medical Facility ID #**	201107	**Date of Collection**	06 September 2016
Specimen ID	S-10740/16 B	**Pathologist**	Not Provided	**Specimen Type**	Block

ABOUT THE TEST:

FoundationOne™ is a next-generation sequencing (NGS) based assay that identifies genomic alterations within hundreds of cancer-related genes.

PATIENT RESULTS

5 genomic findings

2 therapies associated with potential clinical benefit

0 therapies associated with lack of response

8 clinical trials

TUMOR TYPE: THYROID ANAPLASTIC CARCINOMA

Genomic Alterations Identified†

NRAS Q61R
NTRK3 L209I
CDKN2A/B loss
PARK2 loss exons 2-4
TERT promoter -124C>T

† For a complete list of the genes assayed and performance specifications, please refer to the Appendix

THERAPEUTIC IMPLICATIONS

Genomic Findings Detected	FDA-Approved Therapies (in patient's tumor type)	FDA-Approved Therapies (in another tumor type)	Potential Clinical Trials
NRAS Q61R	None	Cobimetinib Trametinib	Yes, see clinical trials section
NTRK3 L209I	None	None	Yes, see clinical trials section
CDKN2A/B loss	None	None	None
PARK2 loss exons 2-4	None	None	None
TERT promoter -124C>T	None	None	None

Note: Genomic alterations detected may be associated with activity of certain FDA-approved drugs; however, the agents listed in this report may have little or no evidence in the patient's tumor type. Neither the therapeutic agents nor the trials identified are ranked in order of potential or predicted efficacy for this patient, nor are they ranked in order of level of evidence for this patient's tumor type.

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GENOMIC ALTERATIONS

GENE ALTERATION	INTERPRETATION
● ***NRAS*** Q61R	**Gene and Alteration:** NRAS encodes a member of the RAS family of small GTPases that mediate transduction of growth signals. Activation of RAS signaling causes cell growth, differentiation, and survival by activating the RAF/MAPK/ERK, PI3K, and other pathways[1]. NRAS alterations affecting amino acids G12, G13, and Q61 as well as mutations I24N, T50I, G60E, and A146T have been characterized to be activating and oncogenic[1,2,3,4,5,6,7,8,9,10,11,12,13,14]. **Frequency and Prognosis:** NRAS mutations have been reported in 6-8% of thyroid carcinomas, including 15.7% of anaplastic thyroid carcinomas (ATC) (COSMIC, cBioPortal, Apr 2016). In the literature, NRAS mutations have been found in 17-28% of ATCs[15,16]. Other studies have reported NRAS mutations in 8.4% (9/107) of thyroid carcinomas, with an incidence of 2% (1/49) in well-differentiated carcinomas (WDCs), 13.8% (4/29) in poorly differentiated carcinomas (PDCs), and 13.8% (4/29) in undifferentiated tumors (UDCs). In this study, activating mutations in RAS genes (NRAS, KRAS, or HRAS) were associated with poorly or undifferentiated tumor phenotypes, and activating RAS mutations, as well as NRAS mutations specifically, were associated with poor prognosis. In addition, in the subset of WDC and PDCs, activating RAS mutation was associated with poor prognosis[17]. **Potential Treatment Strategies:** Constitutive activation of NRAS leads to activation of the RAF-MEK-ERK pathway, leading to tumorigenesis, and may predict sensitivity to inhibitors of this pathway[1]. In a nonrandomized Phase 2 study of the MEK inhibitor MEK162 in 30 NRAS mutant melanoma patients, 20% (6/30) had a partial response, 63% (19/30) had stable disease, and the size of brain metastases was reduced in 2 patients treated with MEK162[18]. In a preclinical study, 5 out of 6 NRAS mutant non-small cell lung cancer cell lines were sensitive to the MEK inhibitors trametinib and selumetinib in vitro[19]. The MEK inhibitors cobimetinib and trametinib are FDA approved in melanoma and is in clinical trials for patients with solid tumors[20,21]. A clinical trial of the MEK inhibitor selumetinib in patients with thyroid cancer showed that the therapy increased radioiodine uptake in 12 of 20 cases, leading to clinical responses in all eight patients that reached the dosimetry threshold, including all five patients with NRAS mutations[22]. Clinical trials of these and other MEK inhibitors are under way. The reovirus Reolysin targets cells with activated RAS signaling[23,24,25] and is in clinical trials in some tumor types. Reolysin has demonstrated mixed clinical efficacy, with the highest rate of response reported for patients with head and neck cancer[26,27,28,29,30,31,32,33,34].
● ***NTRK3*** L209I	**Gene and Alteration:** NTRK3, also known as TRKC, encodes the NT-3 growth factor receptor, a member of the neurotrophic tyrosine receptor kinase family, including TRKA, TRKB, and TRKC; TRKs, which are activated by neurotrophins, play an important role in neuronal survival and differentiation and have also been shown to be involved in oncogenesis in both neurogenic and non-neurogenic cancers[35,36]. TRK signaling leads to activation of the RAS-MAPK and PI3K-AKT pathways, and TRKC, as well as the other TRKs, has generally been considered to be an oncogenic receptor; however, studies have found that in certain contexts, TRKC may function as a tumor suppressor[35,36,37,38,39,40]. Although this alteration has not been characterized and its functional effect is unknown, it has been reported in the context of cancer, which may indicate biological relevance. **Frequency and Prognosis:** No mutations of NTRK3 were observed in the 6 thyroid anaplastic carcinoma samples evaluated in COSMIC (Sep 2016), nor has NTRK3 been extensively studied in this context (PubMed, Sep 2016).

GENE ALTERATION	INTERPRETATION
	Potential Treatment Strategies: Clinical and preclinical data suggest that activating NTRK3 alterations may predict sensitivity to TRK inhibitors such as LOXO-101 and entrectinib, which are under clinical investigation, and to crizotinib, which is FDA approved for the treatment of patients with ALK-positive non-small cell lung cancer (NSCLC) (Hong et al., 2015; AACR-NCI-EORTC Abstract PR13)[41,42]. Preliminary results from a Phase 1 study evaluating LOXO-101 showed that the drug was well tolerated and treatment led to clinical benefit in all 6 response-evaluable patients with NTRK fusions including 5 confirmed partial responses (PRs); these responders included two patients with gastrointestinal stromal tumor or mammary analogue secretory carcinoma of the salivary gland harboring ETV6-NTRK3 fusions and a patient with an undifferentiated sarcoma harboring an LMNA-NTRK1 fusion (Hong et al., 2016; AACR Abstract CT008, Hong et al., 2015; AACR-NCI-EORTC Abstract PR13)[43]. In a Phase 1 study evaluating entrectinib, objective responses were observed in 3 of 4 TKI-naive patients harboring NTRK fusions (Drilon et al., 2016; AACR Abstract CT007). A partial response to entrectinib was observed in a patient with a mammary analogue secretory carcinoma (MASC) harboring ETV6-NTRK3 prior to progression with the acquisition of NTRK3 G623R, a mutation that was shown to reduce sensitivity to NTRK inhibitors in vitro[41]. Prior to treatment with entrectinib, the patient with MASC received crizotinib and exhibited stable disease[41]. Preclinical data suggest that ETV6-NTRK3 may be sensitive to crizotinib[42] and inhibitors of SRC or IGF1R[44,45,46]; however, these approaches are not expected to be relevant for the NTRK3 alteration seen here.
● ***CDKN2A/B*** loss	**Gene and Alteration:** CDKN2A encodes two different, unrelated tumor suppressor proteins, p16INK4a and p14ARF, whereas CDKN2B encodes the tumor suppressor p15INK4b[47,48]. 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[49,50]. The tumor suppressive functions of p14ARF involve stabilization and activation of p53, via a mechanism of MDM2 inhibition[51,52]. This alteration is predicted to inactivate p16INK4a[53,54,55,56], p15INK4b[57], and p14ARF[58,59]. **Frequency and Prognosis:** Homozygous deletion of CDKN2A and/or CDKN2B has been observed in 2.6% of anaplastic thyroid cancer cases[60]. Homozygous deletion of the region encoding p16INK4a and p14ARF has been found in 2.6% (1/39) and 7.7% (3/39) of papillary thyroid carcinomas (PTCs), respectively[61]. CDKN2A p16INK4a promoter methylation has been reported in 27-41% of PTCs and has been found to be more common in patients with high risk or advanced stage disease[62,63]. Loss of heterozygosity of the CDKN2A p16INK4a-encoding locus has also been reported in 6% and 44.4% of follicular thyroid adenomas and carcinomas, respectively[64]. Studies have found CDKN2A p16INK4a promoter methylation in 56%-86% of undifferentiated thyroid carcinomas, with methylation status correlating with more aggressive tumors[65,66,67]. In one study, p16INK4a expression was reported in 31.6% (6/19) of anaplastic thyroid carcinomas and in 66.7% (14/21) of PTCs, while another study has reported p16INK4a expression in 89% (39/44) of PTCs, with lack of p16INK4a expression frequently found in the follicular variant of PTC[62,68].

GENE ALTERATION	INTERPRETATION
	Potential Treatment Strategies: Preclinical data suggest that tumors with loss of p16INK4a function may be sensitive to CDK4/6 inhibitors, such as LEE011, LY2835219, and FDA-approved palbociclib[69,70,71,72]. However, multiple clinical studies have shown no significant correlation between p16INK4a loss or inactivation and therapeutic benefit of these agents (Gopalan et al., 2014; ASCO Abstract 8077)[73,74,75], and 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[76,77], the clinical relevance of p14ARF as a predictive biomarker is not clear.
● ***PARK2*** loss exons 2-4	**Gene and Alteration:** PARK2 (Parkin) is an E3 ubiquitin ligase involved in multiple cellular functions including protein turnover, stress response, metabolism, and cell growth and survival. Dysfunction of PARK2 is associated with the development and progression of Parkinson Disease[78]. While the role of PARK2 in neurodegenerative disease has been a primary focus of investigation, there is a growing body of evidence demonstrating PARK2 inactivation is involved in human cancer. In preclinical studies, PARK2 has been shown to inhibit cell proliferation and tumorigenesis in cancer cell line models[79] and Parkin-deficient mice show increased susceptibility to tumorigenesis, suggesting a role as a tumor suppressor[80,81,82]. In vitro functional studies have characterized PARK2 R42C, N254S, R275Q, and E344G as loss of function mutations[79], and truncation mutations that disrupt the RING finger domains (amino acids 238-293 and 418-449) are also predicted to result in loss of function[79]. **Frequency and Prognosis:** PARK2 is located on chromosome 6q25.2–27, a region that is frequently deleted in human cancer[79]. In a study across 11 cancer types, deletions of PARK2 were reported in 30% of tumors, with 11% harboring focal deletions in PARK2[83]. Deletions of PARK2 were most frequent in ovarian (62%), bladder (38%), and breast (32%) carcinomas[83]. Somatic mutations in PARK2 have also been reported in multiple cancer types[79], including in cervical cancer (5.6%), lung squamous cell cancer (5.6%), colorectal cancer (2.4-5.6%), gastric cancer (4.6%), skin cutaneous melanoma (3.5%), lung adenocarcinoma (2.7-3.1%), and endometrioid cancer (2.1%)[78]. **Potential Treatment Strategies:** PARK2 was reported to promote ubiquitination and turnover of cyclin D1 and cyclin E1, and depletion of PARK2 resulted in accumulation of cyclin D1 and cyclin E1 and enhanced cell proliferation[79,83]. Therefore, inhibitors of CDK4/CDK6 (the binding partners for cyclin D1) or CDK2 (the binding partner for cyclin E1) may be relevant in the case of PARK2 deletion or loss of function mutation[84].
● ***TERT*** promoter -124C>T	**Gene and Alteration:** Telomerase reverse transcriptase (TERT, or hTERT) is a catalytic subunit of the telomerase complex, which is required to maintain appropriate chromosomal length[85]. Activation of TERT is a hallmark of cancer, being detected in up to 80-90% of malignancies and absent in quiescent cells[86,87,88]. Mutations within the promoter region of TERT have been observed in melanoma, glioma, thyroid, and bladder cancers[89]. Mutations within the promoter region of TERT that confer enhanced TERT promoter activity have been reported in two hot spots, located at –124 bp and –146 bp upstream of the transcriptional start site (also termed C228T and C250T, respectively)[89,90,91], as well as tandem mutations at positions –124/–125 bp and –138/–139 bp[90].

GENE ALTERATION	INTERPRETATION

Frequency and Prognosis: In thyroid tumors specifically, hTERT activity or expression is observed in 71% of follicular thyroid carcinomas (FTC), 48% of papillary thyroid carcinomas (PTC), 18% of follicular thyroid adenomas, and 78% of anaplastic thyroid carcinomas (ATC), but only in 6% of benign thyroid tissue[92]. In thyroid cancers, TERT promoter mutations have been reported in 8-23% of PTCs, 11-17% of FTCs, 29-43% of poorly/undifferentiated thyroid carcinomas, and 33-73% of ATCs[60,93,94,95,96,97]. Promoter mutations have not been reported in either medullary thyroid carcinoma or normal/benign thyroid tissue. In thyroid tumors, these promoter mutations were shown to be associated with tumor aggressiveness and increased patient mortality, and often coincided with BRAF or RAS alterations[89,93,95,96,98].

Potential Treatment Strategies: Therapeutic options for targeting tumors with TERT mutations are limited, although a variety of approaches are under development, including immunotherapies utilizing TERT as a tumor-associated antigen, antisense oligonucleotide- or peptide-based therapies, and TERT promoter-directed cytotoxic molecules.

THERAPIES

There are no approved therapies in this patient's tumor type that are specific to the reported genomic alterations.

ADDITIONAL THERAPIES – FDA-APPROVED IN OTHER TUMOR TYPES

THERAPY	SUMMARY OF DATA IN OTHER TUMOR TYPE
Cobimetinib	**Approved Indications:** Cobimetinib is a MEK inhibitor that is FDA approved in combination with vemurafenib for the treatment of unresectable or metastatic melanoma with BRAF V600E or V600K mutations. **Gene Association:** Based on a case study in an NRAS-mutant chronic myelomonocytic leukemia (CMML)[99], NRAS amplification or activating mutations may predict sensitivity to MEK inhibitors such as cobimetinib. **Supporting Data:** Cobimetinib has been investigated primarily in the context of BRAF V600-mutant melanoma. A Phase 3 study with 495 patients treated either with the BRAF inhibitor vemurafenib plus cobimetinib or vemurafenib alone reported a 68-70% overall response rate, 9.9-12.3 months progression-free survival, and a lower rate of cutaneous squamous cell carcinoma in the combination group; disease progression did not correlate with concurrent alterations in the RAS pathway (Larkin et al., 2015; ASCO Abstract 9006)[100]. In a Phase 1b study, vemurafenib combined with cobimetinib achieved an objective response rate of 87% for patients with BRAF V600-mutant melanoma who had not previously received a BRAF inhibitor[101]. One study reported near-complete response to vemurafenib in a patient with BRAF V600K-mutant melanoma who subsequently developed chronic myelomonocytic leukemia (CMML) with NRAS G12R mutation, and concurrent cobimetinib treatment led to suppression of CMML[99]. In a Phase 1b study, out of 47 patients treated with cobimetinib and the AKT inhibitor ipatasertib, 3 patients with KRAS-mutant ovarian, mesonephric cervical, or endometrial carcinoma had a partial response, with prolonged stable disease lasting for >6 months (Bendell et al., 2014; AACR Abstract CT328).
Trametinib	**Approved Indications:** Trametinib is a MEK inhibitor that is FDA approved as both a single agent and in combination with dabrafenib for the treatment of unresectable or metastatic melanoma with BRAF V600E or V600K mutations. **Gene Association:** NRAS activation may lead to hyperactivation of the downstream RAF-MEK-ERK pathway, suggesting sensitivity to MEK inhibitors such as trametinib.

Supporting Data: A clinical trial of the MEK inhibitor selumetinib in patients with thyroid cancer showed that the therapy increased radioiodine uptake in 12 of 20 cases, leading to clinical responses in all eight patients that reached the dosimetry threshold, including all five patients with NRAS mutations[22]. In a preclinical study, trametinib potently inhibited growth of thyroid cancer cells in vitro; combination of trametinib and pazopanib led to sustained shrinkage in tumor volume by 50% in xenograft models[102]. A Phase 1 trial of trametinib in 206 patients with solid tumors reported 21 (10%) objective responses[103]. Phase 1 monotherapy trials of RO4987655, another MEK inhibitor, have shown significant response rates in patients with melanoma, including those with BRAF and NRAS mutations, but very low response rates in patients with other solid tumors, including those with KRAS mutations[104,105]. A Phase 1b trial of trametinib in combination with gemcitabine in patients with solid tumors showed a complete response in a breast cancer patient, as well as partial responses in pancreatic and salivary gland cancer[106]. A Phase 1b trial of combination treatment with the MEK inhibitor MEK162 and the PI3K-alpha inhibitor BYL719 reported disease control (partial responses or stable disease) in 47% (21/45) of patients, including partial responses in 2 of 3 patients with KRAS-mutant ovarian cancer and 1 of 3 patients with NRAS-mutant melanoma; a 43% rate of stable disease was observed in patients with KRAS-mutant colorectal cancer, with responses independent of PIK3CA mutation status (Juric et al., 2014; ASCO Abstract 9051). However, a Phase 1b trial of a combination of trametinib and the mTOR inhibitor everolimus in patients with solid tumors reported frequent adverse events and was unable to identify a recommended Phase 2 dose and schedule for the combination[107].

Genomic alterations detected may be associated with activity of certain approved drugs; however, the agents listed in this report may have little or no evidence in the patient's tumor type.

CLINICAL TRIALS TO CONSIDER

IMPORTANT: 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 meant to be a complete list of available trials. In order to conduct a more thorough search, please go to www.clinicaltrials.gov and use the search terms provided below. For more information about a specific clinical trial, type the NCT ID of the trial indicated below into the search bar.

GENE	RATIONALE FOR POTENTIAL CLINICAL TRIALS
● ***NRAS*** Q61R	Activation of RAS signaling causes cell growth, differentiation, and survival by activating the RAF-MEK-ERK, PI3K, and other pathways. NRAS activating mutations or amplification may therefore sensitize tumors to inhibitors of these downstream pathways. Examples of clinical trials that may be appropriate for this patient are listed below. These trials were identified through a search of the trial website clinicaltrials.gov using keyword terms such as "NRAS", "MEK", "PI3K", "MEK162", "BKM120", "selumetinib", "trametinib", "cobimetinib", "reolysin", "anaplastic thyroid carcinoma", "solid tumor", and/or "advanced cancer".

TITLE	PHASE	TARGETS	LOCATIONS	NCT ID
A Phase I Study of BKM120 and Everolimus in Advanced Solid Malignancies	Phase 1	PI3K, mTOR	Georgia	NCT01470209
A Cancer Research UK Phase I Dose Escalation Trial of Oral VEGFR and EGFR Inhibitor, Vandetanib in Combination With the Oral MEK Inhibitor, Selumetinib (VanSel-1) in Solid Tumours (Dose Escalation) and NSCLC (Expansion Cohort).	Phase 1	MEK, RET, VEGFRs, EGFR	Cambridge (United Kingdom), Manchester (United Kingdom), Newcastle (United Kingdom), Oxford (United Kingdom)	NCT01586624
Phase I/II Study of the CDK4/6 Inhibitor Palbociclib (PD-0332991) in Combination With the MEK Inhibitor PD-0325901 for Patients With KRAS Mutant Non-Small Cell Lung Cancer and Other Solid Tumors	Phase 1/Phase 2	MEK, CDK4, CDK6	Massachusetts	NCT02022982
A Phase 1b Study of the Safety and Pharmacology of Atezolizumab Administered With Cobimetinib in Patients With Locally Advanced or Metastatic Solid Tumors	Phase 1	MEK, PD-L1	California, Colorado, Connecticut, Massachusetts, New York, North Carolina, Tennessee, Texas, Washington, (Australia), (Canada), (Germany), (Korea, Republic of), (Singapore)	NCT01988896

CLINICAL TRIALS TO CONSIDER

GENE	RATIONALE FOR POTENTIAL CLINICAL TRIALS
● ***NTRK3*** L209I	Tumors with NTRK3 activating mutations may be sensitive to pan-TRK inhibitors. However, the functional effect of the mutation reported here is unknown; therefore, it is unclear if this therapeutic approach would be relevant. Examples of clinical trials that may be appropriate for this patient are listed below. These trials were identified through a search of the trial website clinicaltrials.gov using keyword terms such as "TRK", "AZD7451", "DS-6051B", "entrectinib", "lestaurtinib", "LOXO-101", "PLX7486", "TSR-011", "thyroid carcinoma", and/or "solid tumor".

TITLE	PHASE	TARGETS	LOCATIONS	NCT ID
A Phase 1 Study to Assess Safety, Pharmacokinetics, and Pharmacodynamics of PLX7486 as a Single Agent and in Combination With Gemcitabine and Nab-Paclitaxel in Patients With Advanced Solid Tumors	Phase 1	CSF1R, TRKA, TRKB, TRKC	Arizona, California, Maryland, Massachusetts, South Carolina	NCT01804530
A Phase 1 Study of the Oral TRK Inhibitor LOXO-101 in Adult Patients With Solid Tumors	Phase 1	TRKA, TRKB, TRKC	Colorado, Massachusetts, Ohio, Oregon, Pennsylvania, Tennessee, Texas	NCT02122913
A Phase 1/2a, Multicenter, Open-Label Study of Oral Entrectinib (RXDX-101) in Adult Patients With Locally Advanced or Metastatic Cancer Confirmed to be Positive for NTRK1, NTRK2, NTRK3, ROS1, or ALK Molecular Alterations	Phase 1	TRKs, ROS1, ALK	California, Colorado, District of Columbia, Florida, Massachusetts, New York, Tennessee, Texas, Barcelona (Spain), Seoul (Korea, Republic of)	NCT02097810
A Phase 1, Two-Part, Multi-Center, Non Randomized, Open-Label, Multiple Dose First-In-Human Study Of DS-6051b, An Oral ROS1 And NTRK Inhibitor, In Subjects With Advanced Solid Tumors	Phase 1	TRKA, TRKB, TRKC, ROS1	Arizona, California, Massachusetts, Texas	NCT02279433

APPENDIX

VARIANTS OF UNKNOWN SIGNIFICANCE

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
L54_L55>L

ARID1B
D1728_E1729>E

BRIP1
H478R

CBFB
G180S

CD79A
A10T

CHEK2
E351D

FGF3
R200_P201>T

FLT4
D581N

KEL
R428H

Microsatellite status
MS-Stable

MSH6
V1051I

NOTCH1
A1804S

POLE
T594I

PTCH1
R34H

ROS1
L567V

SPTA1
R661H

TOP2A
T1324A

TSC2
R1122C

Tumor Mutation Burden
TMB-Unknown

APPENDIX

GENES ASSAYED IN FOUNDATIONONE

FoundationOne is designed to include all 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 315 genes as well as introns of 28 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	*ABL2*	*ACVR1B*	*AKT1*	*AKT2*	*AKT3*	*ALK*	*AMER1 (FAM123B)*	*APC*	*AR*
ARAF	*ARFRP1*	*ARID1A*	*ARID1B*	*ARID2*	*ASXL1*	*ATM*	*ATR*	*ATRX*	*AURKA*
AURKB	*AXIN1*	*AXL*	*BAP1*	*BARD1*	*BCL2*	*BCL2L1*	*BCL2L2*	*BCL6*	*BCOR*
BCORL1	*BLM*	*BRAF*	*BRCA1*	*BRCA2*	*BRD4*	*BRIP1*	*BTG1*	*BTK*	*C11orf30 (EMSY)*
CARD11	*CBFB*	*CBL*	*CCND1*	*CCND2*	*CCND3*	*CCNE1*	*CD274*	*CD79A*	*CD79B*
CDC73	*CDH1*	*CDK12*	*CDK4*	*CDK6*	*CDK8*	*CDKN1A*	*CDKN1B*	*CDKN2A*	*CDKN2B*
CDKN2C	*CEBPA*	*CHD2*	*CHD4*	*CHEK1*	*CHEK2*	*CIC*	*CREBBP*	*CRKL*	*CRLF2*
CSF1R	*CTCF*	*CTNNA1*	*CTNNB1*	*CUL3*	*CYLD*	*DAXX*	*DDR2*	*DICER1*	*DNMT3A*
DOT1L	*EGFR*	*EP300*	*EPHA3*	*EPHA5*	*EPHA7*	*EPHB1*	*ERBB2*	*ERBB3*	*ERBB4*
ERG	*ERRFI1*	*ESR1*	*EZH2*	*FAM46C*	*FANCA*	*FANCC*	*FANCD2*	*FANCE*	*FANCF*
FANCG	*FANCL*	*FAS*	*FAT1*	*FBXW7*	*FGF10*	*FGF14*	*FGF19*	*FGF23*	*FGF3*
FGF4	*FGF6*	*FGFR1*	*FGFR2*	*FGFR3*	*FGFR4*	*FH*	*FLCN*	*FLT1*	*FLT3*
FLT4	*FOXL2*	*FOXP1*	*FRS2*	*FUBP1*	*GABRA6*	*GATA1*	*GATA2*	*GATA3*	*GATA4*
GATA6	*GID4 (C17orf39)*	*GLI1*	*GNA11*	*GNA13*	*GNAQ*	*GNAS*	*GPR124*	*GRIN2A*	*GRM3*
GSK3B	*H3F3A*	*HGF*	*HNF1A*	*HRAS*	*HSD3B1*	*HSP90AA1*	*IDH1*	*IDH2*	*IGF1R*
IGF2	*IKBKE*	*IKZF1*	*IL7R*	*INHBA*	*INPP4B*	*IRF2*	*IRF4*	*IRS2*	*JAK1*
JAK2	*JAK3*	*JUN*	*KAT6A (MYST3)*	*KDM5A*	*KDM5C*	*KDM6A*	*KDR*	*KEAP1*	*KEL*
KIT	*KLHL6*	*KMT2A (MLL)*	*KMT2C (MLL3)*	*KMT2D (MLL2)*	*KRAS*	*LMO1*	*LRP1B*	*LYN*	*LZTR1*
MAGI2	*MAP2K1*	*MAP2K2*	*MAP2K4*	*MAP3K1*	*MCL1*	*MDM2*	*MDM4*	*MED12*	*MEF2B*
MEN1	*MET*	*MITF*	*MLH1*	*MPL*	*MRE11A*	*MSH2*	*MSH6*	*MTOR*	*MUTYH*
MYC	*MYCL (MYCL1)*	*MYCN*	*MYD88*	*NF1*	*NF2*	*NFE2L2*	*NFKBIA*	*NKX2-1*	*NOTCH1*
NOTCH2	*NOTCH3*	*NPM1*	*NRAS*	*NSD1*	*NTRK1*	*NTRK2*	*NTRK3*	*NUP93*	*PAK3*
PALB2	*PARK2*	*PAX5*	*PBRM1*	*PDCD1LG2*	*PDGFRA*	*PDGFRB*	*PDK1*	*PIK3C2B*	*PIK3CA*
PIK3CB	*PIK3CG*	*PIK3R1*	*PIK3R2*	*PLCG2*	*PMS2*	*POLD1*	*POLE*	*PPP2R1A*	*PRDM1*
PREX2	*PRKAR1A*	*PRKCI*	*PRKDC*	*PRSS8*	*PTCH1*	*PTEN*	*PTPN11*	*QKI*	*RAC1*
RAD50	*RAD51*	*RAF1*	*RANBP2*	*RARA*	*RB1*	*RBM10*	*RET*	*RICTOR*	*RNF43*
ROS1	*RPTOR*	*RUNX1*	*RUNX1T1*	*SDHA*	*SDHB*	*SDHC*	*SDHD*	*SETD2*	*SF3B1*
SLIT2	*SMAD2*	*SMAD3*	*SMAD4*	*SMARCA4*	*SMARCB1*	*SMO*	*SNCAIP*	*SOCS1*	*SOX10*
SOX2	*SOX9*	*SPEN*	*SPOP*	*SPTA1*	*SRC*	*STAG2*	*STAT3*	*STAT4*	*STK11*
SUFU	*SYK*	*TAF1*	*TBX3*	*TERC*	*TERT (promoter only)*	*TET2*	*TGFBR2*	*TNFAIP3*	*TNFRSF14*
TOP1	*TOP2A*	*TP53*	*TSC1*	*TSC2*	*TSHR*	*U2AF1*	*VEGFA*	*VHL*	*WISP3*
WT1	*XPO1*	*ZBTB2*	*ZNF217*	*ZNF703*					

DNA Gene List: For the Detection of Select Rearrangements

ALK	*BCL2*	*BCR*	*BRAF*	*BRCA1*	*BRCA2*	*BRD4*	*EGFR*	*ETV1*	*ETV4*
ETV5	*ETV6*	*FGFR1*	*FGFR2*	*FGFR3*	*KIT*	*MSH2*	*MYB*	*MYC*	*NOTCH2*
NTRK1	*NTRK2*	*PDGFRA*	*RAF1*	*RARA*	*RET*	*ROS1*	*TMPRSS2*		

Additional Assays: For the Detection of Select Cancer Biomarkers

Microsatellite status

Tumor Mutation Burden

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APPENDIX

GENES ASSAYED IN FOUNDATIONONE

APPENDIX

FOUNDATIONONE PERFORMANCE SPECIFICATIONS

ACCURACY		
Sensitivity: Base Substitutions	At Mutant Allele Frequency ≥10%	>99.9% (CI* 99.6%-100%)
	At Mutant Allele Frequency 5-10%	99.3% (CI* 98.3%-99.8%)
Sensitivity: Insertions/Deletions (1-40 bp)	At Mutant Allele Frequency ≥20%	97.9% (CI* 92.5%-99.7%)
	At Mutant Allele Frequency 10-20%	97.3% (CI* 90.5%-99.7%)
Sensitivity: Copy Number Alterations—Amplifications (ploidy <4, Amplification with Copy Number ≥8)	At ≥30% tumor nuclei	>99.0% (CI* 93.6%-100%)
	At 20% tumor nuclei	92.6% (CI* 66.1%-99.8%)
Sensitivity: Copy Number Alterations—Deletions (ploidy <4, Homozygous Deletions)	At ≥30% tumor nuclei	97.2% (CI* 85.5%-99.9%)
	At 20% tumor nuclei	88.9% (CI* 51.8%-99.7%)
Sensitivity: Rearrangements (selected rearrangements in specimens with ≥20% tumor nuclei)**		>90.0% [1] >99.0% for ALK fusion[2] (CI* 89.1%-100%)
Sensitivity: Microsatellite status	At ≥20% tumor nuclei	97.0% (CI* 89.6%-99.6%)
Specificity: all variant types	Positive Predictive Value (PPV)	>99.0%
Specificity: Microsatellite status	Positive Predictive Value (PPV)	>95.0%
Accuracy: Tumor Mutation Burden	At ≥20% tumor nuclei	>90.0%
REPRODUCIBILITY (average concordance between replicates)		96.4% inter-batch precision 98.9% intra-batch precision 95.8% microsatellite status precision 96.4% tumor mutation burden precision

*95% Confidence Interval

** Performance for gene fusions within targeted introns only. Sensitivity for gene fusions occurring outside targeted introns or in highly repetitive intronic sequence contexts is reduced.

[1] Based on analysis of coverage and rearrangement structure in the COSMIC database for the solid tumor fusion genes where alteration prevalence could be established, complemented by detection of exemplar rearrangements in cell line titration experiments.

[2] Based on ALK rearrangement concordance analysis vs. a standard clinical FISH assay described in: Yelensky, R. et al. Analytical validation of solid tumor fusion gene detection in a comprehensive NGS-based clinical cancer genomic test, In: Proceedings of the 105th Annual Meeting of the American Association for Cancer Research; 2014 Apr 5-9; San Diego, CA. Philadelphia (PA): AACR; 2014. Abstract nr 4699

Assay specifications were determined for typical median exon coverage of approximately 500X. For additional information regarding the validation of FoundationOne, please refer to the article, Frampton, GM. et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing, Nat Biotechnol (2013 Oct. 20).

Microsatellite status (a measure of microsatellite instability, or "MSI") is determined by assessing indel characteristics at 114 homopolymer repeat loci in or near the targeted gene regions of the FoundationOne test. Microsatellite status is assayed for all FoundationOne samples. MSI-High results are reported in all tumor types. In select tumor types, other Microsatellite status results may be reported (MS-Stable, MSI-Ambiguous, MSI-Unknown) when relevant. Microsatellite status result may be reported as "Unknown" if the sample is not of sufficient quality to confidently determine Microsatellite status.

Tumor Mutation Burden (TMB) is determined by measuring the number of somatic mutations occurring in sequenced genes on the FoundationOne and FoundationOne Heme tests and extrapolating to the genome as a whole. TMB is assayed for all FoundationOne and FoundationOne Heme samples. TMB-High results are reported in all tumor types. In select tumor types, other TMB results may be reported (TMB-Intermediate, TMB-Low, TMB-Unknown) when relevant. TMB results are determined as follows: TMB-High corresponds to greater than or equal to 20 mutations per megabase (Muts/Mb); TMB-Intermediate corresponds to 6-19 Muts/Mb; TMB-Low corresponds to less than or equal to 5 Muts/Mb. Tumor Mutation Burden may be reported as "Unknown" if the sample is not of sufficient quality to confidently determine Tumor Mutation Burden.

For additional information specific to the performance of this specimen, please contact Foundation Medicine, Inc. at 1-888-988-3639.

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ABOUT FOUNDATIONONE™

FoundationOne™: FoundationOne was developed and its performance characteristics determined by Foundation Medicine, Inc. (Foundation Medicine). FoundationOne has not been cleared or approved by the United States Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. FoundationOne may be used for clinical purposes and should not be regarded as purely investigational or for research only. Foundation Medicine's clinical reference laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical testing.

Diagnostic Significance: FoundationOne identifies alterations to select cancer-associated genes or portions of genes (biomarkers). In some cases, the Test 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 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 for identifying a copy number amplification is five (5) for ERBB2 and six (6) for all other genes. Conversely, an alteration denoted as "loss – equivocal" implies that the FoundationOne 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 analytical methodology has identified as being present in <10% of the assayed tumor DNA.

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.

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.

Alterations and Drugs Not Presented in Ranked Order: In this Report, neither any biomarker alteration, nor any drug associated with potential clinical benefit (or potential lack of clinical benefit), are ranked in order of potential or predicted efficacy.

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.

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. These include: subclonal alterations in heterogeneous samples, low sample quality or with homozygous losses of <3 exons; and deletions and insertions >40bp, or in repetitive/high homology sequences. FoundationOne is performed using DNA derived from tumor, and as such germline events may not be reported. The following targets typically have low coverage resulting in a reduction in sensitivity: *SDHD* exon 6 and *TP53* exon 1.

FoundationOne complies with all European Union (EU) requirements of the IVD Directive 98/79EC. As such, the FoundationOne Assay has been registered for CE mark by our EU Authorized Representative, Qarad b.v.b.a, Cipalstraat 3, 2440 Geel, Belgium. CE

For more comprehensive information please log on to the Interactive Cancer Explorer™
To set up your Interactive Cancer Explorer account, contact your sales representative or call 1-888-988-3639.