FOUNDATIONONE®

Patient Name	Report Date	Tumor Type
Singh, Digamber	**17 August 2016**	**Pancreas ductal adenocarcinoma**

Date of Birth	10 January 1951	**Medical Facility**	Max Healthcare		
Sex	Male	**Ordering Physician**	Verma, Amit	**Specimen Received**	20 July 2016
FMI Case #	TRF167394	**Additional Recipient**	Anker Behl	**Specimen Site**	Pancreas
Medical Record #	Not Given	**Medical Facility ID #**	201107	**Date of Collection**	18 July 2016
Specimen ID	8943 VIV A1	**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[II]

4 genomic findings

4 therapies associated with potential clinical benefit

0 therapies associated with lack of response

9 clinical trials

[II] Reduced sensitivity due to sample quality – See Appendix: Performance Specifications for details.

TUMOR TYPE: PANCREAS DUCTAL ADENOCARCINOMA

Genomic Alterations Identified[†]

AKT2 amplification
KRAS G12D
TP53 I255F
CCNE1 amplification

[†] 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
AKT2 amplification	None	Everolimus Temsirolimus	Yes, see clinical trials section
KRAS G12D	None	Cobimetinib Trametinib	Yes, see clinical trials section
TP53 I255F	None	None	Yes, see clinical trials section
CCNE1 amplification	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
● ***AKT2*** amplification	**Gene and Alteration:** AKT2 encodes an intracellular serine/threonine kinase that is also known as PKB-beta. AKT2 is one of three members of the AKT gene family, and activation of AKT2 has been implicated in multiple malignancies[1,2]. AKT isoforms appear to have different roles in tumorigenesis; AKT1 appears to contribute to tumor initiation, whereas AKT2 promotes invasion and metastasis in breast tumors[3]. Although AKT2 amplification has been reported to associate with AKT2 overexpression[4,5,6], studies in various cancers suggest that AKT2 phosphorylation may have greater clinical relevance than AKT2 amplification or mRNA overexpression[7,8]. **Frequency and Prognosis:** In the Pancreatic Adenocarcinoma TCGA dataset, putative high-level amplification of AKT2 has been reported in 8% of cases (cBioPortal, Apr 2016). AKT2 amplification has been identified in one of 10 primary pancreatic tumors, and in two of 18 pancreatic cancer cell lines in one study[9]. AKT2 activation, through various mechanisms, has been reported to occur frequently in pancreatic cancer and may contribute to pathogenesis[10]. **Potential Treatment Strategies:** Amplification of AKT2 may promote AKT-mTOR pathway activation and may predict sensitivity to inhibitors of this pathway. However, studies in various cancers suggest that AKT2 phosphorylation may have greater clinical relevance than AKT2 amplification or mRNA overexpression[7,8]. AKT inhibitors are in clinical trials in patients with various tumor types. The mTOR inhibitors everolimus and temsirolimus have received FDA approval in other tumor types, and these agents as well as other mTOR inhibitors are in clinical trials in multiple solid tumor types, alone or in combination with other therapies. The AKT inhibitor MK-2206 has shown preclinical and preliminary clinical evidence of enhancing the antitumor activity of chemotherapeutic agents[11,12].
● ***KRAS*** G12D	**Gene and Alteration:** KRAS encodes a member of the RAS family of small GTPases. Activating mutations in RAS genes can cause uncontrolled cell proliferation and tumor formation[13,14]. The KRAS gene is one of the most commonly mutated genes in human malignancies[15,16,17]. KRAS alterations affecting amino acids G12, G13, Q22, P34, A59, Q61, and A146, as well as mutations G10_A11insG, A18D, L19F, and K117N have been characterized to be activating and oncogenic[13,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35]. **Frequency and Prognosis:** KRAS mutation has been observed in 91-95% of pancreatic adenocarcinoma cases[36,37], with the majority of mutations found at codon 12[16,38,39,40]; KRAS amplification was observed in 6.4% of pancreatic ductal adenocarcinoma cases[37], but has been observed more frequently in undifferentiated carcinomas of the pancreas (42%, 10/24)[41]. Activating KRAS mutations were shown to promote transdifferentiation of pancreas acinar cells to ductal cells in mouse models of pancreatic ductal adenocarcinoma[42,43].

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GENE ALTERATION	INTERPRETATION
	Potential Treatment Strategies: Preclinical evidence suggests that KRAS activation may predict sensitivity to MEK inhibitors, including trametinib and cobimetinib, alone or in combination with other targeted therapies[13,44,45,46,47,48]. Phase 1 monotherapy trials of MEK inhibitors in patients with pancreatic cancer reported 0-25% partial response (PR) rates and disease control [PR and/or stable disease (SD) rates ranging from 0/2 (0%) to 5/5 (100%)](Rosen et al., 2008; ASCO Abstract 14585)[49,50,51,52,53]. The largest trial tested selumetinib in 38 patients with pancreatic cancer and reported 37% disease control, including 2 PRs[51]. Prolonged PRs were seen in patients treated with CI-1040[49] and trametinib[52,54]. However, trials testing combination treatment with a MEK inhibitor (trametinib, refametinib, or pimasertib) and gemcitabine reported no additional benefit compared to gemcitabine alone and no significant association of KRAS mutation status with response rate or survival (Riess et al., 2014; ASCO Abstract 4129, Van Laethem et al., 2014; ASCO Abstract 4025, Van Cutsem et al., 2015; ASCO GI Abstract 344)[55,56], with refametinib and gemcitabine even showing a trend towards worse response and survival in patients with KRAS-mutant pancreatic tumors than in those with KRAS-wild-type tumors (Riess et al., 2014; ASCO Abstract 4129, Van Laethem et al., 2014; ASCO Abstract 4025). Furthermore, multiple trials that combined MEK inhibitors with other targeted therapies, such as the EGFR inhibitor erlotinib (Ko et al., 2013; ASCO Abstract 4014) or various inhibitors of the PI3K-AKT pathway (LoRusso et al., 2012; ASCO Abstract 2566, Juric et al., 2014; ASCO Abstract 9051, Chung et al., 2015; ASCO Abstract 4119)[57], reported no PRs and frequent adverse events in patients with KRAS-mutant pancreatic cancer. But other approaches based on promising preclinical data, such as combinations of MEK inhibitors with BCL-XL inhibitors[58] or CDK4/6 inhibitors[59,60], are in clinical trials for KRAS-mutant pancreatic cancer. The reovirus Reolysin targets cells with activated RAS signaling[61,62,63] and is in clinical trials in some tumor types. Two case studies have reported clinical benefit of combination therapy including Reolysin for patients with pancreatic cancer[64,65]. Although KRAS mutation status may predict lack of response to EGFR-targeted therapies in some tumor types[66,67,68,69,70], KRAS mutation was not associated with objective response in pancreatic patients treated with erlotinib and chemotherapy[71].
● ***TP53*** I255F	**Gene and Alteration:** Functional loss of the tumor suppressor p53, which is encoded by the TP53 gene, is common in aggressive advanced cancers[72]. Mutations affecting the DNA binding domain (aa 100-292), the tetramerization domain (aa 325-356), or the C-terminal regulatory domain (aa 356-393), such as observed here, are thought to disrupt the transactivation of p53-dependent genes and are predicted to promote tumorigenesis[73,74,75,76]. Germline mutations in TP53 are associated with the very rare disorder Li-Fraumeni syndrome and the early onset of many cancers[77,78,79,80,81,82]. Estimates for the prevalence of germline TP53 mutations in the general population range from 1:5,000[83] to 1:20,000[82], and in the appropriate clinical context, germline testing of TP53 is recommended. **Frequency and Prognosis:** TP53 mutations are common in pancreatic ductal adenocarcinomas and are known to occur in the process of pancreatic carcinogenesis[84,85]. TP53 mutations have been reported in 33-75% of pancreatic carcinomas, with the majority occurring as missense mutations, while deletion of TP53 has been found in 65.7% of pancreatic ductal adenocarcinoma cases[36,86,87,88]. Additionally, aberrant expression of p53 has been found in 54-81% of pancreatic ductal adenocarcinoma cases[87,89,90,91]. Studies have found inconsistent results regarding the prognostic significance of p53 expression in pancreatic ductal adenocarcinoma, although one study correlated low levels of TP53 mRNA with poor patient prognosis[89,92,93].

GENE ALTERATION	INTERPRETATION
	Potential Treatment Strategies: There are no approved therapies to address TP53 mutation or loss. However, tumors with TP53 loss of function alterations may be sensitive to the WEE1 inhibitor AZD1775[94,95,96,97], therapies that reactivate mutant p53 such as APR-246[98], or p53 gene therapy and immunotherapeutics such as SGT-53[99,100,101,102] and ALT-801 (Hajdenberg et al., 2012; ASCO Abstract e15010). Combination of AZD1775 with paclitaxel and carboplatin achieved significantly longer progression-free survival than paclitaxel and carboplatin alone in patients with TP53-mutant ovarian cancer (Oza et al., 2015; ASCO Abstract 5506). Furthermore, AZD1775 in combination with carboplatin achieved a 27% (6/22) response rate and 41% (9/22) stable disease rate in patients with TP53-mutant ovarian cancer refractory or resistant to carboplatin plus paclitaxel (Leijen et al., 2015; ASCO Abstract 2507). In a Phase 1 clinical trial, 8 of 11 evaluable patients receiving SGT-53 as a single agent exhibited stable disease[103]. Clinical trials of SGT-53 in combination with chemotherapy are underway. Additionally, the combination of a CHK1 inhibitor and irinotecan reportedly reduced tumor growth and prolonged survival in a TP53 mutant, but not TP53 wild-type, breast cancer xenotransplant mouse model[104]. Kevetrin has also been reported to activate p53 in preclinical studies and might be relevant in the context of mutant p53 (Kumar et al., 2012; AACR Abstract 2874). Clinical trials of these agents are under way for some tumor types for patients with a TP53 mutation.
● ***CCNE1*** amplification	**Gene and Alteration:** CCNE1 encodes the cyclin E1 protein, which plays a role in the regulated transition from the G1 to S phase by binding to and activating cyclin-dependent protein kinase 2 (CDK2). It also has a direct role in initiation of replication and maintenance of genomic stability[105]. Amplification of chromosomal region 19q12-q13 has been demonstrated in many types of cancer, and CCNE1 is a well-studied gene within this amplicon[106]. Increased copy number of CCNE1 is highly associated with overexpression of the cyclin E1 protein[107,108]. **Frequency and Prognosis:** Putative high-level amplification of CCNE1 has been found in 6.2% of cases in the Pancreatic Adenocarcinoma TCGA dataset (cBioPortal, May 2016). Multiple studies have reported increased cyclin E1 expression in pancreatic cancer[109,110,111]. Cyclin E overexpression has been correlated with increased metastasis and poor outcome in patients with pancreatic ductal adenocarcinoma[111]. **Potential Treatment Strategies:** There are no approved therapies that directly target CCNE1 alterations. Because cyclin E1 promotes cell cycle progression in a complex with CDK2[105], preclinical studies have investigated CDK2 inhibitors as a potential therapeutic approach for tumors with CCNE1 activation. One preclinical study reported that CCNE1 amplification and/or overexpression largely correlated with sensitivity of cultured and xenografted ovarian carcinoma cell lines to a CDK2 inhibitor SNS-032[112]. However, other studies showed that sensitivity of various cell lines to CDK2 inhibitors, including SNS-032, dinaciclib, and seliciclib, at clinically achievable doses, is largely independent of CCNE1 copy number or expression[113,114,115,116]. One study reported a reduction in tumor CCNE1 levels in 4/6 lung and esophageal cancer cases following treatment with the HDAC inhibitor vorinostat, paralleling findings from a CCNE1-driven mouse model of lung cancer, where vorinostat treatment led to tumor reduction and a decrease in CCNE1 levels[117]. Amplification of CCNE1 has been linked to inferior clinical benefit rate and progression-free survival in patients with HER2-positive breast cancer treated with trastuzumab[115]. CCNE1 amplification has also been implicated in resistance to platinum-based therapies in patients with ovarian carcinoma[108,118,119,120], correlating with inferior survival in this population[108,118].

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
Everolimus	**Approved Indications:** 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; and, in association with tuberous sclerosis complex (TSC), renal angiomyolipoma and subependymal giant cell astrocytoma. Everolimus is also approved to treat hormone receptor-positive, HER2-negative advanced breast cancer in combination with exemestane following prior therapy with letrozole or anastrozole, as well as in combination with the multikinase inhibitor lenvatinib to treat advanced RCC following prior antiangiogenic therapy. **Gene Association:** Amplification of AKT2 may promote AKT-mTOR pathway activation and may predict sensitivity to inhibitors of this pathway such as everolimus. However, studies in various cancers suggest that AKT2 phosphorylation may have greater clinical relevance than AKT2 amplification or mRNA overexpression[7,8]. **Supporting Data:** In a Phase 1/2 study of patients with advanced pancreatic adenocarcinoma, the combination of everolimus, cetuximab, and capecitabine was found to be excessively toxic with minimal efficacy[121]. Early studies with single agent everolimus in pancreatic cancer also did not show efficacy[122,123]; however, clinical trials examining mTOR inhibitors in combination with other chemotherapeutics are underway in pancreatic cancer. In some tumor types, including pancreatic cancer, it has been observed that monotherapy with mTOR inhibitors can activate a feedback loop involving the PI3K-AKT pathway, sometimes causing rapid progression of the tumor[123]. Treatment with a dual mTOR/PI3K inhibitor, or with a combination of these inhibitors, may circumvent this phenomenon. 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[124].
Temsirolimus	**Approved Indications:** Temsirolimus is an intravenous mTOR inhibitor that is FDA approved for the treatment of advanced renal cell carcinoma. **Gene Association:** AKT2 amplification may promote AKT-mTOR pathway activation and may predict sensitivity to inhibitors of this pathway such as temsirolimus. However, studies in various cancers suggest that AKT2 phosphorylation may have greater clinical relevance than AKT2 amplification or mRNA overexpression[7,8]. **Supporting Data:** A Phase 2 clinical trial in patients with pancreatic cancer reported that temsirolimus monotherapy was ineffective and may have contributed to disease progression[123]. A Phase 1 trial of bevacizumab and temsirolimus plus liposomal doxorubicin in patients with advanced solid tumors showed that the combination was well tolerated and resulted in 21% of patients having stable disease for over 6 months, with a 21% rate of partial or complete remission[125]. In some tumor types, including pancreatic cancer, monotherapy with mTOR inhibitors has been observed to activate a feedback loop involving the PI3K-AKT pathway, sometimes causing rapid tumor progression[123]. Treatment with a dual mTOR/PI3K inhibitor, or with a combination of these inhibitors, may circumvent this phenomenon.

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 clinical studies (Bendell et al., 2014; AACR Abstract CT328) and preclinical studies[126], KRAS amplification or activating mutations may predict sensitivity to MEK inhibitors such as cobimetinib. **Supporting Data:** A clinical trial of cobimetinib in combination with the pan-PI3K inhibitor GDC-0941 in patients with solid tumors reported a partial response in a patient with BRAF-mutant pancreatic cancer but no responses in patients with KRAS-mutant pancreatic cancers (LoRusso et al., 2012; ASCO Abstract 2566). Other trials of MEK inhibitors in combination with PI3K pathway inhibitors (Juric et al., 2014; ASCO Abstract 9051, Chung et al., 2015; ASCO Abstract 4119)[57] or the EGFR inhibitor erlotinib (Ko et al., 2013; ASCO Abstract 4014) also showed no responses in patients with KRAS-mutant pancreatic cancer. Although no published data are available on the use of cobimetinib in combination with chemotherapy to treat pancreatic cancers, clinical trials combining other MEK inhibitors with gemcitabine reported no additional benefit compared to gemcitabine alone and no significant association of KRAS mutation status with response rate or survival (Riess et al., 2014; ASCO Abstract 4129, Van Laethem et al., 2014; ASCO Abstract 4025, Van Cutsem et al., 2015; ASCO GI Abstract 344)[55,56].
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:** KRAS amplification or activating mutations may activate the downstream MAPK pathway and may indicate sensitivity to MEK inhibitors such as trametinib. **Supporting Data:** A Phase 1 trial of trametinib monotherapy reported disease control [partial response (PR) and/or stable disease (SD)] in 15/22 (60%) patients with pancreatic cancer, including 2 long-term PRs; 100% disease control (1 PR and 4 SD among 5 patients) was seen in patients with KRAS-mutant tumors[52,54]. However, clinical trials of combined treatment with trametinib and gemcitabine reported no additional benefit compared to gemcitabine alone and no significant association of KRAS mutation status with response rate or survival[55,56]. A Phase 1b combination trial of trametinib and the pan-PI3K inhibitor BKM120 reported no responses as well as prevalent and often severe adverse effects in patients with pancreatic cancer[57], similar to findings in other combination trials of MEK and PI3K pathway inhibitors (LoRusso et al., 2012; ASCO Abstract 2566, Juric et al., 2014; ASCO Abstract 9051, Chung et al., 2015; ASCO Abstract 4119). 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[124].

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
● ***AKT2*** amplification	AKT2 amplification may lead to AKT-mTOR pathway activation and may predict sensitivity to inhibitors of this pathway. 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 "AKT", "mTOR", "everolimus", "temsirolimus", "API-1", "MK-2206", "perifosine", "pancreatic carcinoma", "solid tumor", and/or "advanced cancer".

TITLE	PHASE	TARGETS	LOCATIONS	NCT ID
A Phase I Trial of the IGF-1R Antibody AMG 479 in Combination With Everolimus (RAD001) and Panitumumab in Patients With Advanced Cancer (The RAP Trial)	Phase 1	EGFR, IGF1R, mTOR	North Carolina	NCT01061788
An Exploratory Study of Metformin With or Without Rapamycin as Maintenance Therapy After Induction Chemotherapy in Subjects With Metastatic Pancreatic Adenocarcinoma	Phase 1/Phase 2	mTOR	Arizona, Maryland	NCT02048384
A Phase I, First-in-Human, Dose Escalation Trial of MSC2363318A, a Dual p70S6K/Akt Inhibitor, in Subjects With Advanced Malignancies	Phase 1	AKT, p70S6K	California, Michigan, Texas, Vermont	NCT01971515
A Phase 1, Open-label Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of MLN0128 (an Oral mTORC 1/2 Inhibitor) as a Single Agent and in Combination With Paclitaxel in Adult Patients With Advanced Nonhematologic Malignancies	Phase 1	mTORC1, mTORC2	Florida, Oklahoma, Tennessee	NCT02412722

CLINICAL TRIALS TO CONSIDER

GENE	RATIONALE FOR POTENTIAL CLINICAL TRIALS
● ***KRAS*** G12D	KRAS amplification or activating mutations may activate downstream pathways, including the MAPK pathway, and indicate sensitivity to MEK inhibitors. 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 "KRAS", "MEK", "reolysin", "trametinib", "cobimetinib", "MEK162", "PD-0325901", "pancreatic carcinoma", "solid tumor", and/or "advanced cancer".

TITLE	PHASE	TARGETS	LOCATIONS	NCT ID
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, Texas, Washington, Dresden (Germany), Freiburg (Germany), Ontario (Canada), Quebec (Canada), Seoul (Korea, Republic of), Singapore (Singapore), Victoria (Australia)	NCT01988896
Phase I/II Study With Lapatinib Plus Trametinib in Patients With Metastatic KRAS Mutant Colorectal, Non-small Cell Lung and Pancreatic Cancer	Phase 1/Phase 2	MEK, EGFR, ERBB2, ERBB4	Amsterdam (Netherlands)	NCT02230553
Molecular Basket Trial In Multiple Malignancies With Common Target Pathway Aberrancies: A Phase II Trial of GSK2256098 and Trametinib in Patients With Advanced Pancreatic Cancer	Phase 2	MEK, FAK	Ontario (Canada)	NCT02428270

CLINICAL TRIALS TO CONSIDER

GENE	RATIONALE FOR POTENTIAL CLINICAL TRIALS
● ***TP53*** I255F	TP53 loss of function alterations may predict sensitivity to WEE1 inhibitors, therapies that reactivate mutant p53, or p53 gene therapy and immunotherapeutics. 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 "p53", "SGT-53", "pancreatic carcinoma", "solid tumor", and/or "advanced cancer".

TITLE	PHASE	TARGETS	LOCATIONS	NCT ID
Phase II Study of Combined Targeted p53 Gene Therapy (SGT-53) Plus Gemcitabine/Nab-Paclitaxel for Treatment of Metastatic Pancreatic Cancer	Phase 2	p53	Texas	NCT02340117

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.

ALK E405D	***BRCA2*** P389Q	***ERBB4*** A158E	***FANCA*** V985L	***FANCL*** F36L	***IRS2*** G879S,G882A
Microsatellite status MS-Stable	***NOTCH3*** H170R	***SOX9*** P353Q	***TET2*** R581C	***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

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.

[II] Reduced Sensitivity: Although we can definitively confirm the presence of the genomic alterations detailed in this report, the data obtained may have been insufficient for comprehensive detection of genomic alterations. Reduced sensitivity may be due to poor sample quality or, in rare cases, to patient transplant history. Any Tumor Mutation Burden (TMB) value (Muts/Mb) shown on a report with reduced sensitivity reflects an estimate of the lowest possible TMB.

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APPENDIX

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.