FOUNDATIONONE®

Patient Name	Report Date	Tumor Type
Bansal, Sonia	**11 May 2016**	**Breast carcinoma (NOS)**

Date of Birth	03 May 1964	**Medical Facility**	Max Healthcare	**Specimen Received**	02 May 2016
Sex	Female	**Ordering Physician**	Verma, Amit	**Specimen Site**	Not Provided
FMI Case #	TRF151330	**Additional Recipient**	Not Given	**Date of Collection**	28 April 2016
Medical Record #	Not Given	**Medical Facility ID #**	201107	**Specimen Type**	Block
Specimen ID	M829/16A	**Pathologist**	Not Provided		

ABOUT THE TEST:

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

PATIENT RESULTS

4 genomic alterations

2 therapies associated with potential clinical benefit

0 therapies associated with lack of response

6 clinical trials

TUMOR TYPE: BREAST CARCINOMA (NOS)

Genomic Alterations Identified†

AKT2 amplification
CCNE1 amplification
MCL1 amplification
TP53 R248Q

Additional Disease-relevant Genes with No Reportable Alterations Identified†

ERBB2

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

THERAPEUTIC IMPLICATIONS

Genomic Alterations Detected	FDA-Approved Therapies (in patient's tumor type)	FDA-Approved Therapies (in another tumor type)	Potential Clinical Trials
AKT2 amplification	Everolimus	Temsirolimus	Yes, see clinical trials section
CCNE1 amplification	None	None	None
MCL1 amplification	None	None	None
TP53 R248Q	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 Breast Invasive Carcinoma TCGA dataset, putative high-level amplification of AKT2 has been reported in 2.2% of cases [9]; a similar incidence of 2.8% has been reported in the scientific literature[10]. Although AKT2 amplification has been reported to be rare in breast cancer, preclinical studies suggest that it may be associated with increased tumor invasion and metastasis[3,10]. However, AKT2 expression has been found to be associated with reduced risk of distant recurrence in estrogen receptor positive (ER+) breast cancer patients[11]. **Potential Treatment Strategies:** Amplification of AKT2 may promote AKT-mTOR pathway activation and may predict sensitivity to inhibitors of this pathway. AKT inhibitors are in clinical trials in various tumor types and mTOR inhibitors have been FDA approved in breast cancer and other tumor types [12]. In preclinical studies, the AKT inhibitor MK-2206 showed evidence of enhancing anti-tumor activity of other chemotherapeutic agents in lung and ovarian tumor cells[13]. In addition, a preclinical study in breast and ovarian cancer cells correlated AKT2 activation with resistance to docetaxel[14].
● ***CCNE1*** amplification	**Gene and Alteration:** CCNE1 encodes the protein cyclin E1, 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 [15]. Amplification of chromosomal region 19q12-q13, where CCNE1 resides, has been demonstrated in multiple tumor types[16,17,18]. Increased copy number of CCNE1 is highly linked with overexpression of the cyclin E1 protein[17,19,20]. Cyclin E1 overexpression can lead to cell transformation as a result of increased cyclin E1 activity[15,21]. **Frequency and Prognosis:** In the Breast Invasive Carcinoma TCGA dataset, putative high-level amplification of CCNE1 has been reported in 2.8% of cases [9]. An analysis of HER2-positive breast cancer samples found CCNE1 amplification in 18-35% of patients[22]. However, a separate study reported that CCNE1 gene overexpression occurred mainly in basal-like breast cancer, whereas overexpression of CCNE2 was associated with HER2-positive and luminal B breast cancer subtypes[23]. CCNE1 amplification and cyclin E1 overexpression have been correlated with poor prognosis in patients with breast cancer[24,25,26,27].

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GENE ALTERATION	INTERPRETATION
	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 [15], 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[28]. 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[22,29,30,31]. 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[32]. 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[22]. CCNE1 amplification has also been implicated in resistance to platinum-based therapies in patients with ovarian carcinoma[20,33,34,35], correlating with inferior survival in this population[20,33].
● ***MCL1*** amplification	**Gene and Alteration:** MCL1 (myeloid cell leukemia 1) encodes a member of the BCL2 family that regulates programmed cell death or apoptosis [36]. MCL1 has been reported to be amplified in cancer[37] and may be biologically relevant in this context[38,39]. **Frequency and Prognosis:** In the TCGA datasets, MCL1 amplification was observed in 9-14% of invasive breast carcinoma cases [9,40]. Additionally, increased copy number of the MCL1 region has been reported in 36% of breast tumor samples in other studies[39]. Elevated MCL1 protein expression has been associated with high tumor grade and poor patient prognosis in breast cancer[41]. **Potential Treatment Strategies:** There are no FDA-approved therapies to address MCL1 amplification, but investigations focused on inhibitors of MCL1 are under way [42]. In addition, clinical trials of some agents that target BCL2 may be relevant for tumors with MCL1 amplification, although MCL1 expression has been associated with resistance to other BCL2-targeted agents (including ABT-263 and ABT-737)[43,44,45,46,47,48]. Indirect approaches using therapeutic agents that reduce MCL1 expression are also being investigated[49]. Preclinical studies have shown that the multikinase inhibitor sorafenib indirectly downregulates MCL1[50,51,52,53,54] and synergizes with other agents, such as TRAIL[50,53,55,56], a BCL-XL inhibitor[51], or an mTOR inhibitor[52], to induce cell death. Other preclinical studies suggest that another avenue to address MCL1 amplification may be the use of CDK2/7/9 inhibitors in combination with other agents[57,58]. Clinical trials are investigating the use of CDK2/7/9 inhibitors, alone or in combination with other therapies, in solid tumors. In addition, preclinical studies of patient-derived tumor cells suggest that increased MCL1 levels may confer resistance to anti-tubulin therapies such as paclitaxel[59].
● ***TP53*** R248Q	**Gene and Alteration:** Functional loss of the tumor suppressor p53, which is encoded by the TP53 gene, is common in aggressive advanced cancers [60]. 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[61,62,63,64]. Germline mutations in TP53 are associated with the very rare disorder Li-Fraumeni syndrome and the early onset of many cancers[65,66,67,68,69,70]. Estimates for the prevalence of germline TP53 mutations in the general population range from 1:5,000[71] to 1:20,000[70], and in the appropriate clinical context, germline testing of TP53 is recommended.

GENE ALTERATION	INTERPRETATION

Frequency and Prognosis: TP53 is one of the most commonly mutated genes in breast cancer, and mutations in this gene have been identified in 27-37% of breast carcinoma samples [9,72,73,74,75,76]. TP53 mutations within the region encoding the DNA binding domain have been reported to be associated with poor prognosis in patients with breast cancer[76,77,78]. In addition, TP53 mutation carriers have an 18-60 fold increased risk for early onset breast cancer[79,80,81].

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[82,83,84,85], therapies that reactivate mutant p53 such as APR-246[86], or p53 gene therapy and immunotherapeutics such as SGT-53[87,88,89,90] 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[91]. 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[92]. 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.

THERAPIES

FDA-APPROVED THERAPIES IN PATIENT TUMOR TYPE

THERAPY	SUMMARY OF DATA IN PATIENT TUMOR TYPE
Everolimus	**Approved Indications:** Everolimus is an orally available mTOR inhibitor that is FDA approved to treat renal cell carcinoma 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. **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:** Addition of everolimus to exemestane as second-line therapy for hormone receptor-positive (HR+), HER2-negative breast cancer improved median progression-free survival (PFS) compared to exemestane alone (11.5 vs. 4.1 months) and showed a trend to longer overall survival (31.0 vs. 26.6 months) [12,93,94]. Clinical studies for patients with HR+ breast cancer indicate that everolimus may potentiate letrozole or tamoxifen efficacy and can be safely combined with anastrozole[95,96,97]. Two Phase 3 trials have evaluated whether addition of everolimus would circumvent or overcome resistance of HER2-positive (HER2+) breast cancer to trastuzumab-based therapy: As first-line treatment for patients with HER2+ breast cancer, everolimus combined with trastuzumab plus paclitaxel did not significantly improve median PFS in the full study population (15.0 months with everolimus vs. 14.5 months with placebo) but increased PFS in the HR-negative subpopulation (20.3 vs. 13.1 months)[98]. For patients with trastuzumab-resistant HER2+ breast cancer, the addition of everolimus to trastuzumab plus vinorelbine prolonged median PFS (7.0 vs. 5.8 months)[99]. Follow-up exploratory analysis showed that patients with PIK3CA alterations achieved longer median PFS with everolimus vs. placebo (hazard ratio [HR] = 0.69), when combined with trastuzumab plus paclitaxel (12.0 vs. 7.6 months) or vinorelbine (6.9 vs. 5.7 months)(Slamon et al., 2015; ASCO Abstract 512). Low PTEN expression or PTEN loss also was significantly associated with benefit from added everolimus in the combined analysis of both studies (HR = 0.50) (Slamon et al., 2015; ASCO Abstract 512)[99]. For patients with metastatic triple-negative breast cancer, everolimus plus carboplatin achieved a clinical benefit rate of 36% (9/25)[100]. A Phase 1b trial of a combination of everolimus and the MEK inhibitor trametinib in patients with solid tumors reported frequent adverse events, and the study was unable to identify a recommended Phase 2 dose and schedule for the combination[101].

ADDITIONAL THERAPIES – FDA-APPROVED IN OTHER TUMOR TYPES

THERAPY	SUMMARY OF DATA IN OTHER TUMOR TYPE
Temsirolimus	**Approved Indications:** Temsirolimus is an intravenous mTOR inhibitor that is FDA approved for the treatment of advanced renal cell carcinoma. **Gene Association:** Amplification of AKT2 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 1 trial examining the combination of temsirolimus, liposomal doxorubicin, and bevacizumab in 74 patients with breast and gynecological malignancies reported that 37.9% of patients experienced either a complete response (1.4%), partial response (18.9%), or stable disease (17.6%); among 25 patients with PIK3CA mutation or PTEN loss, 52% experienced a complete or partial response (36%) or stable disease (16%) [102]. Another Phase 1 trial including patients with several types of cancer reported a 42% incidence of complete or partial responses in patients with metastatic breast cancer[103]. However, a Phase 2 study of temsirolimus in pretreated patients with metastatic breast cancer reported minimal clinical activity and no association with PTEN protein or PIK3CA mutation status[104]. A Phase 3 placebo-controlled trial of letrozole plus oral temsirolimus as first-line endocrine therapy in postmenopausal women with locally advanced or metastatic breast cancer was terminated at the second interim since the addition of temsirolimus to letrozole did not improve progression-free survival as a first-line therapy[105]. A study examining the efficacy of temsirolimus-involving regimens in 24 patients with mesenchymal/metaplastic breast cancer (MpBCs) reported 2 complete responses, 4 partial responses, 2 instances of stable disease >6 months, and 4 instances of stable disease <6 months[106].

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.

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", "breast carcinoma", "solid tumor", and/or "advanced cancer".

TITLE	PHASE	TARGETS	LOCATIONS	NCT ID
A Phase Ib Study of the Oral PARP Inhibitor Olaparib With the Oral mTORC1/2 Inhibitor AZD2014 or the Oral AKT Inhibitor AZD5363 for Recurrent Endometrial, Triple Negative Breast, and Ovarian, Primary Peritoneal, or Fallopian Tube Cancer	Phase 1/Phase 2	mTORC1, mTORC2, AKT	Texas	NCT02208375
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 I Multi-centre Trial of the Combination of Olaparib (PARP Inhibitor) and AZD5363 (AKT Inhibitor) in Patients With Advanced Solid Tumours	Phase 1	PARP, AKT	Newcastle upon Tyne (United Kingdom), Surrey (United Kingdom)	NCT02338622
A Phase Ib Trial of LEE011 in Combination With Everolimus (RAD001) and Exemestane in the Treatment of Postmenopausal Women With Hormone Receptor Positive, HER2 Negative Locally Advanced or Metastatic Breast Cancer	Phase 1/Phase 2	mTOR, Aromatase, CDK4, CDK6	Massachusetts, Michigan, New York, Texas, Catalunya (Spain), Hong Kong (Hong Kong), Saint Herblain cedex (France), Wilrijk (Belgium)	NCT01857193
A Multicenter, Open-label, Phase 1b Study of MLN0128 (an Oral mTORC1/2 Inhibitor) in Combination With MLN1117 (an Oral PI3Kα Inhibitor) in Adult Patients With Advanced Nonhematologic Malignancies	Phase 1	mTORC1, mTORC2, PI3K-alpha	Massachusetts, Tennessee, Texas, Barcelona (Spain), Sutton (United Kingdom)	NCT01899053
A RANDOMIZED, PHASE II, MULTI-CENTER, PLACEBO-CONTROLLED STUDY OF IPATASERTIB (GDC-0068), AN INHIBITOR OF AKT, IN COMBINATION WITH PACLITAXEL AS FRONT-LINE TREATMENT FOR PATIENTS WITH METASTATIC TRIPLE-NEGATIVE BREAST CANCER	Phase 2	AKT	Campania (Italy), Lombardia (Italy), Singapore (Singapore), Taichung (Taiwan), Taipei (Taiwan), Taoyuan (Taiwan), Veneto (Italy), Wilrijk (Belgium)	NCT02162719

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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 make their significance unclear. We choose to include them here in the event that they become clinically meaningful in the future.

ATM T1156A	***BRCA1*** G1801D	***CD274*** amplification	***CDKN2A*** amplification	***CDKN2B*** amplification	***CEBPA*** amplification
CYLD G431E	***ERRFI1*** A435S	***GATA3*** amplification	***GATA4*** P394T	***IKBKE*** S31C	***JAK2*** amplification
LZTR1 A662V,E522K	***MLL3*** L2420V,V125I	***PARK2*** P180L	***PBRM1*** rearrangement	***PDCD1LG2*** amplification	***POLE*** V240L
ROS1 T2052N	***RPTOR*** K948R	***ZNF703*** H402_D403>PTHL GGSSCSTCSAHD			

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 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*		

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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%)
Specificity of all variant types	Positive Predictive Value (PPV)	>99.0%
REPRODUCIBILITY (average concordance between replicates)		96.4% inter-batch precision 98.9% intra-batch 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 re-arrangement 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 re-arrangement 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).

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

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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.

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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.

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