FOUNDATIONONE®HEME

PATIENT
Sample, Jane

TUMOR TYPE
Acute myeloid leukemia (AML) (NOS)

REPORT DATE
01 Jan 2018

TRF#
XXXXXXXX

ABOUT THE TEST FoundationOne®Heme is a comprehensive genomic profiling test designed to identify genomic alterations within hundreds of cancer-related genes in hematologic malignancies, sarcomas, and pediatric cancers.

PATIENT

DISEASE Acute myeloid leukemia (AML) (NOS)
NAME Not Given
DATE OF BIRTH Not Given
SEX Female
MEDICAL RECORD # Not Given

PHYSICIAN

ORDERING PHYSICIAN Not Given
MEDICAL FACILITY Not Given
ADDITIONAL RECIPIENT Not Given
MEDICAL FACILITY ID Not Given
PATHOLOGIST Not Given

SPECIMEN

SPECIMEN SITE Not Given
SPECIMEN ID Not Given
SPECIMEN TYPE Not Given
DATE OF COLLECTION Not Given
SPECIMEN RECEIVED Not Given

Biomarker Findings

Microsatellite status - MS-Stable
Tumor Mutational Burden - TMB-Low (2 Muts/Mb)

Genomic Findings

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

KIT D816V
NRAS Q61H
MYH11 CBFB-MYH11 fusion

5 Therapies with Clinical Benefit
2 Therapies with Lack of Response
10 Clinical Trials

BIOMARKER FINDINGS	THERAPIES WITH CLINICAL BENEFIT (IN PATIENT'S TUMOR TYPE)	THERAPIES WITH CLINICAL BENEFIT (IN OTHER TUMOR TYPE)
Microsatellite status - MS-Stable	No therapies or clinical trials. see Biomarker Findings section	
Tumor Mutational Burden - TMB-Low (2 Muts/Mb)	No therapies or clinical trials. see Biomarker Findings section	

GENOMIC FINDINGS	THERAPIES WITH CLINICAL BENEFIT (IN PATIENT'S TUMOR TYPE)	THERAPIES WITH CLINICAL BENEFIT (IN OTHER TUMOR TYPE)
KIT **-** D816V **7 Trials** see p. 16	None	Dasatinib Nilotinib Ponatinib ⚠ Imatinib[1] ⚠ Sunitinib[1]
NRAS **-** Q61H **3 Trials** see p. 17	None	Cobimetinib Trametinib

⚠ *1. Patient may be resistant to indicated therapy*

GENOMIC FINDINGS WITH NO REPORTABLE THERAPEUTIC OR CLINICAL TRIALS OPTIONS

For more information regarding biological and clinical significance, including prognostic, diagnostic, germline, and potential chemosensitivity implications, see the Genomic Findings section.

MYH11 - CBFB-MYH11 fusion ..... p. 18

NOTE Genomic alterations detected may be associated with activity of certain FDA-approved drugs; however, the agents listed in this report may have varied clinical 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.

BIOMARKER

Tumor Mutational Burden

CATEGORY

TMB-Low (2 Muts/Mb)

POTENTIAL TREATMENT STRATEGIES

On the basis of emerging clinical evidence, increased TMB may be associated with greater sensitivity to immunotherapeutic agents, including anti-CTLA-4148, anti-PDL1149,151,152, and anti-PD-1 therapies122,142,153; FDA-approved agents include ipilimumab, atezolizumab, avelumab, durvalumab, pembrolizumab, and nivolumab. In multiple solid tumor types, higher mutational burden has corresponded with response and improved prognosis. Pembrolizumab improved progression-free survival (14.5 vs. 3.4-3.7 months) in patients with non-small cell lung cancer (NSCLC) and higher mutational load (greater than 200 nonsynonymous mutations; hazard ratio = 0.19)142. In studies of patients with either NSCLC or colorectal cancer (CRC), patients whose tumors harbor elevated mutational burden reported higher overall response rates to pembrolizumab122,142,153. Anti-PD-1 therapies have achieved clinical benefit for certain patients with high mutational burden, including 3 patients with endometrial adenocarcinoma who reported sustained partial responses following treatment with pembrolizumab154 or nivolumab155, a patient with hypermutant glioblastoma who obtained clinical benefit from pembrolizumab156, and two pediatric patients with biallelic mismatch repair deficiency (bMMRD)-associated ultrahypermutant glioblastoma who experienced clinically and radiologically significant responses to ivolumab157. In patients with melanoma, mutational load was associated with long-term clinical benefit from ipilimumab148,158 and anti-PD-1/anti-PD-L1 treatments151. For patients with metastatic urothelial carcinoma, those who responded to atezolizumab treatment had a significantly increased mutational load [12.4 mutations (mut) per megabase (Mb)] compared to nonresponders (6.4 mut/Mb)149, and mutational load of 16 mut/Mb or higher was associated with significantly longer overall survival152.

FREQUENCY & PROGNOSIS

Reports of high TMB are generally rare in leukemia150. In a study of 92 patients with various hematologic malignancies, elevated TMB levels [>10 mutations per megabase (mut/Mb)] were not detected in AML (0/5) or ALL (0/1) cases analyzed (Karim et al., 2017; AACR Abstract 3724).

FINDING SUMMARY

Tumor mutation burden (TMB, also known as mutation load) is a measure of the number of somatic protein-coding base substitution and insertion/deletion mutations occurring in a tumor specimen. TMB is affected by a variety of causes, including exposure to mutagens such as ultraviolet light in melanoma139,140 and cigarette smoke in lung cancer141,142, mutations in the proofreading domains of DNA polymerases encoded by the POLE and POLD1 genes143,144,145,146,147, and microsatellite instability (MSI)143,146,147. The tumor seen here harbors a low TMB. Compared to patients with tumors harboring higher TMB levels, patients with tumors harboring low TMB levels have experienced lower rates of clinical benefit from treatment with immune checkpoint inhibitors, including anti-CTLA-4 therapy in melanoma148, anti-PD-L1 therapy in urothelial carcinoma149, and anti-PD-1 therapy in non-small cell lung cancer and colorectal cancer122,142.

BIOMARKER

Microsatellite status

CATEGORY

MS-Stable

POTENTIAL TREATMENT STRATEGIES

On the basis of clinical evidence, microsatellite stable (MSS) tumors are significantly less likely than MSI-high (MSI-H) tumors to respond to anti-PD-1 immune checkpoint inhibitors119,120,121, including approved therapies nivolumab and pembrolizumab (Overman et al., 2016;ASCO Abstract 3501)122. In a retrospective analysis of 361 patients with solid tumors treated with pembrolizumab, 3% were MSI-H and experienced a significantly higher ORR compared with non-MSI-H cases (70% vs. 12%, p=0.001) (Ayers et al., ASCO-SITC 2016; Abstract P60). Pembrolizumab therapy resulted in a significantly lower objective response rate (ORR) in MSS colorectal cancer (CRC) compared with MSI-H CRC (0% vs. 40%)122. Similarly, a clinical study of nivolumab, alone or in combination with ipilimumab, in patients with CRC reported a significantly higher response rate in patients with MSI-H tumors than those without (Overman et al., 2016; ASCO Abstract 3501).

FREQUENCY & PROGNOSIS

High MSI (MSI-H) is generally rare in hematologic malignancies compared with solid tumors. Moreover, reports of MSI in hematologic malignancies in the literature are conflicting and varied due to substantial heterogeneity, lack of consensus on the markers and methods used for MSI assessment, small sample size in most studies, and possible elimination of MSI positive cells in the bloodstream by immunosurveillance99. In studies of acute myeloid leukemia (AML), MSI at any level has been reported at incidences from 6-56%100,101,102,103,104,105,106,107; however, contradicting studies reported an absence of MSI in AML (Bonneville et al., 2017; DOI: 10.1200/PO.17.00073)108. Similarly, MSI-H has been observed with incidences of 3–32%102,104,105,107 or reported as absent in AML (Bonneville et al., 2017; DOI: 10.1200/PO.17.00073)100. In a large study of 1,394 patients with de novo or therapy-related AML, MSI-H was not observed; however, 4.8% of cases demonstrated instability at one microsatellite locus109. In addition, a small number of studies have not found a significant correlation of MSI with relapsed AML104, nor with progression from MDS to AML110, and other publications have reported a high incidence (20–32%) of MSI in de novo AML/MDS105,106,107,111. In contrast, other studies have reported increased incidences of MSI in relapsed or therapy-related AML/MDS compared to de novo disease103,107,111,112,113,114,115,116, and a cell lineage analysis of AML/CML progression found increased MSI associated with relapsed disease after chemotherapy in 3/6 patients117. Therefore, the role of MSI in MDS/AML progression and resistance to chemotherapy is unclear. One study has suggested that organ transplant patients are at higher risk of developing AML/MDS as a result of prolonged immunosuppression, and reported all 7 such patients analyzed exhibited MSI, with 6/7 being MSI-H118.

FINDING SUMMARY

Microsatellite instability (MSI) is a condition of genetic hypermutability that generates excessive amounts of short insertion/deletion mutations in the genome; it generally occurs at microsatellite DNA sequences and is caused by a deficiency in DNA mismatch repair (MMR) in the tumor93. Defective MMR and consequent MSI occur as a result of genetic or epigenetic inactivation of one of the MMR pathway proteins, primarily MLH1, MSH2, MSH6, or PMS293,94,95. The tumor

seen here is microsatellite-stable (MSS), equivalent to the clinical definition of an MSS tumor: one with mutations in none of the tested microsatellite markers[96,97,98]. MSS status indicates MMR proficiency and typically correlates with intact expression of all MMR family proteins[93,95,97,98].

GENE
KIT

ALTERATION
D816V

POTENTIAL TREATMENT STRATEGIES

On the basis of clinical evidence, primarily in GIST, AML and systemic mastocytosis, KIT activating alterations are associated with sensitivity to KIT tyrosine kinase inhibitors including imatinib, sunitinib, sorafenib, dasatinib, nilotinib, pazopanib, regorafenib, ponatinib, midostaurin and avapritinib (Heinrich et al., 2014; ASCO Abstract 10506, Heinrich et al., 2017; ASCO Abstract 11011, DeAngelo et al., 2017; ASH Abstract 2)21,28,29,30,31,32,33,34. However, mutation of residues within the KIT activation-loop (A-loop) has been associated with preclinical and clinical resistance to imatinib (Heinrich et al., 2013; ASCO Abstract 10509)5,6,7,8,9,10,11,12,13,14,15,16,17,35,36 and sunitinib (Heinrich et al., 2013; ASCO Abstract 10509)5,10,14,17,37 in patients with GIST. KIT exon 17 mutations, including at D816 were reported to be sensitive to avapritinib in clinical (Heinrich et al., 2017; ASCO Abstract 11011, DeAngelo et al., 2017; ASH Abstract 2)21 and preclinical21 studies. Preclinical38,39,40 and limited clinical41,42,43 data indicate that KIT A-loop mutants are sensitive to sorafenib, although preclinical evidence of potential resistance has been reported specifically for the D816V mutation39,44,45. Several Aloop alterations at residues D816, D820, N822, and A829 have exhibited ponatinib sensitivity in preclinical evaluations (Schrock et al., 2013; ASCO Abstract B266)17,46,47,48,49. Limited clinical evidence in melanoma suggests that mTOR inhibitors as a monotherapy or in combination with first-line kinase inhibitors may be a useful strategy to target kinase inhibitor-resistant tumors (Si et al., 2012; ASCO Abstract 8562)50.

FREQUENCY & PROGNOSIS

KIT mutations have been reported in 4-17% of acute myeloid leukemia (AML) samples22,23, and in 30% of CBF-AML cases24. KIT expression has been reported 60-80% of patients with AML23. Reports on the prognostic value of KIT mutations in AML have been mixed24,25, but some studies have found that patients with KIT mutations have a poorer prognosis than those without24,26,27.

FINDING SUMMARY

KIT (also called c-KIT) encodes a cell surface tyrosine kinase receptor that, upon ligand binding and dimerization, activates the PI3K-AKT and RAS-MAPK signaling pathways1. KIT aberrations, including point mutations, translocations, amplification, and overexpression, have been associated with various malignancies, and KIT is considered an oncoprotein2. Activating alterations in KIT, or the related gene PDGFRA, are known to underlie inherited predisposition to gastrointestinal stromal tumor (GIST) development3,4. Mutations of KIT within the activation loop, including amino acids C809, D816, D820, N822, Y823, and A829, as also observed here, have been reported to confer preclinical and clinical resistance to imatinib (Heinrich et al., 2013; ASCO Abstract 10509)5,6,7,8,9,10,11,12,13,14,15,16,17 and sunitinib (Heinrich et al., 2013; ASCO Abstract 10509)5,10,17,18 in GIST. KIT D816G has also beenreported as an emergent mutation conferring resistance to crizotinib in a patient with ROS1-rearranged non-small cell lung carcinoma (NSCLC)19. In a rare case, a partial response to imatinib was observed in a patient with a KIT activation loop mutation20. KIT exon 17 mutations, including at D816 were reported to be sensitive to avapritinib in clinical (Heinrich et al., 2017; ASCO Abstract 11011, DeAngelo et al., 2017; ASH Abstract 2)21 and preclinical21 studies.

GENE
MYH11

ALTERATION
CBFB-MYH11 fusion

POTENTIAL TREATMENT STRATEGIES

There are no targeted therapies are available to address genomic alterations in CBFB.

FREQUENCY & PROGNOSIS

CBFB-MYH11 fusions are present in 5% of acute myeloid leukemia (AML) cases124. The presence of either the CBFB-MYH11 fusion or RUNX1-RUNX1T1 (AML-ETO) fusion defines a subtype of AML (CBF-AML) that is associated with favorableprognosis124,130,131,132,133. No difference in prognosis has been noted between the different variants of CBFB-MYH11 fusions observed thus far134,135. However, high copy numbers of the CBFB-MYH11 fusion after induction therapy has been associated with higher relapse rate in CBF-AML versus patients with low copy numbers136,137. CBFBMYH11 fusion is thought to be insufficient for leukemic transformation by itself, and is frequently accompanied by other rearrangements or alterations, including alterations to the RAS pathway in up to 90% of samples71,124,127,138.

FINDING SUMMARY

CBFB encodes the regulatory beta subunit of core binding factor, CBF-beta, that complexes with CBF-alpha (RUNX1, 2, or 3) to regulate transcription of genes required for normal hematopoiesis and osteogenesis123. CBFB-MYH11 fusions commonly result from inv(16)(p13.1q22) or t(16;16)(p13.1;q22)124 and share the N-terminus (most commonly exons 1-5, aa 1-165) of CBFB and Cterminal dimerization domain of smooth muscle myosin heavy chain125,126. CBFB-MYH11 fusion interferes with the transcriptional regulation mediated by RUNX1 (and other RUNX family members), leading to impaired hematopoietic cell differentiation and predisposition to leukemic transformation127,128; however, additional, RUNX1-independent mechanisms have also been proposed129.

GENE
NRAS

ALTERATION
Q61H

POTENTIAL TREATMENT STRATEGIES

Preclinical evidence suggests that NRAS activation may predict sensitivity to MEK inhibitors, such as cobimetinib and trametinib51,73,74,75,76. Clinical responses to MEK inhibitor-based therapy regimens have been observed in patients with NRAS mutation in certain tumor types (Heuck et al., 2014; ASH Abstract 4775)77,78,79. Preclinical data in cancer cell lines indicates that NRAS mutation predicts sensitivity to the PI3K-alpha-specific inhibitor BYL71980. The reovirus Reolysin targets cells with activated RAS signaling81,82,83 and is in clinical trials in patients with some tumor types. Reolysin has demonstrated mixed clinical efficacy, with the highest rate of response reported for patients with head and neck cancer84,85,86,87,88,89,90,91,92.

FREQUENCY & PROGNOSIS

NRAS mutation has been reported in 8-25%22,66 of acute myeloid leukemia (AML) cases. Studies have reported that RAS mutation (NRAS or KRAS) had no influence on clinical outcome in pediatric patients with AML, in patients under 60 years old with AML, in patients with secondary AML, or in patients with AML harboring CBFB-MYH11 fusions67,68,69,70,71. A preclinical study suggested that acquired resistance to FLT3 tyrosine kinase inhibitors (TKIs) in AML cell lines may be driven by activating mutations in NRAS, leading to upregulation of AKT and MAPK signaling; combination of FLT3 TKIs with inhibitors of PI3K or MEK may circumvent this resistance72.

FINDING SUMMARY

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 pathways51. NRAS alterations affecting amino acids G12, G13, G60, and Q61 as well as mutations I24N, T50I, and A146T have been characterized to be activating and oncogenic51,52,53,54,55,56,57,58,59,60,61,62,63,64,65.

Cobimetinib

Assay findings associations

NRAS
Q61H

AREAS OF THERAPEUTIC USE

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

On the basis of a case study in an NRAS-mutant chronic myelomonocytic leukemia (CMML)208, NRAS amplification or activating mutations may predict sensitivity to MEK inhibitors such as cobimetinib. Significant clinical responses to various other MEK inhibitors have been documented in NRAS-mutant melanoma77,209,210,211.

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)212. 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 inhibitor213. 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 CMML208. 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).

Dasatinib

Assay findings associations

KIT
D816V

AREAS OF THERAPEUTIC USE

Dasatinib is a kinase inhibitor that targets the BCR-ABL fusion protein, SRC family kinase receptors, KIT, EPHA2, and PDGFR-beta. It is FDA approved for the treatment of certain subtypes of Philadelphia chromosome-positive (Ph+) chronic myeloid leukemia (CML) and Ph+ acute lymphoblastic leukemia (ALL).

GENE ASSOCIATION

On the basis of clinical evidence in melanoma (Kalinsky et al., 2016; ASCO 2016 Abstract 9501)159 and preclinical evidence in other cancer types160,161, activating KIT alterations may confer sensitivity to dasatinib. Preclinical and limited clinical data indicate that secondary imatinibresistant mutations, including V654A and A-loop alterations at residues D816, D820, N822, and A829, remain sensitive to dasatinib38,161,162,163,164,165.

SUPPORTING DATA

A case study has reported long term remission in an acute myeloid leukemia (AML) patient with a KIT D816V mutation treated with a combination of chemotherapy and dasatinib165. Preclinical studies have reported mixed responses to dasatinib in small studies of unselected AML patients166. Inhibition of cell lines expressing KIT D816V following treatment with dasatinib has been achieved, but the levels of drug required exceed those that are likely to be achievable in patients167,168.

Nilotinib

Assay findings associations

KIT
D816V

AREAS OF THERAPEUTIC USE

Nilotinib targets tyrosine kinases such as ABL (including BCR-ABL), PDGFRs, KIT, CSF1R, DDR1, and DDR2. It is FDA approved to treat newly diagnosed pediatric or adult patients with Philadelphia chromosome-positive (Ph+) chronic myeloid leukemia (CML) in chronic phase, adults with Ph+ CML in chronic or accelerated phase with resistance or intolerance to prior therapy including imatinib, and pediatric patients with Ph+ CML in chronic phase with resistance or intolerance to prior tyrosine-kinase inhibitor therapy.

GENE ASSOCIATION

On the basis of strong clinical186,187,188,189 and preclinical190,191 data in multiple tumor types, KIT amplification or activation may confer sensitivity to nilotinib. Additionally, imatinib-resistant KIT mutations such as V654A and A-loop alterations at residues D816, D820, N822, and A829 maintain sensitivity to nilotinib as demonstrated in multiple clinical and preclinical studies38,162,163,188,192,193,194.

SUPPORTING DATA

A Phase 2 study of nilotinib in combination with daunorubicin and cytarabine in patients with newly diagnosed acute myeloid leukemia (AML) and KIT expression but no KIT mutations reported a complete response (CR) in 80% (12/15) of evaluable patients, and a low relapse rate of 17% (2/12) in patients who achieved CR (Al-Kali et al., 2015; ASH Annual Meeting Abstract 3808). A Phase 1 study of nilotinib in combination with re-induction chemotherapy in patients with relapsed and refractory KIT-positive AML reported a high CR rate (83%, 10/12 patients overall) but noted that the concentrations needed to inhibit the KIT pathway were generally higher than clinically achievable (Brandwein et al., 2013; ASH Annual Meeting Abstract 3961).

Ponatinib

Assay findings associations

KIT
D816V

AREAS OF THERAPEUTIC USE

Ponatinib is a multi-kinase inhibitor targeting BCR-ABL, RET, KIT, FLT-3, PDGFRs, VEGFRs, FGFRs, and other tyrosine kinases. Ponatinib was approved by the FDA for use in advanced, T315I-mutated chronic myeloid leukemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL), as well as for CML and Ph+ ALL patients for whom no other tyrosine kinase inhibitor is indicated.

GENE ASSOCIATION

KIT exon 9 and 11 activating mutations have demonstrated sensitivity to ponatinib in several preclinical cell models (Schrock et al., 2013; ASCO Abstract B266, Garner et al., 2013; AACR Abstract 3394)46,47,48,49, with clinical efficacy demonstrated in patients with GIST exhibiting exon 11 mutations (Heinrich et al., 2014; ASCO Abstract 1050)17 and imatinib resistance (Heinrich et al., 2014; ASCO Abstract 10506). Secondary imatinib-resistant mutations, including T670I (gatekeeper) and Aloop alterations at residues D816, D820, N822, and A829, have exhibited ponatinib sensitivity in preclinical evaluations (Schrock et al., 2013; ASCO Abstract B266)17,46,47,48,49.

SUPPORTING DATA

Clinically, ponatinib has been most extensively studied in patients with BCR-ABLpositive hematological malignancies. Ponatinib has shown efficacy in preclinical models of endometrial, bladder, gastric, breast, lung, colon, and medullary thyroid carcinomas (Gozgit et al., 2013; AACR Abstract 2084)195. A Phase 1 study of ponatinib in patients with acute myeloid leukemia (AML), all of whom had FLT3 alterations, reported 3/12 response rate, with two complete responses and one partial response196.

Trametinib

Assay findings associations

NRAS
Q61H

AREAS OF THERAPEUTIC USE

Trametinib is a MEK inhibitor that is FDA approved as a monotherapy and in combination with dabrafenib to treat patients with unresectable or metastatic melanoma with BRAF V600E or V600K mutations, as well as in combination with dabrafenib as adjuvant treatment for completely resected advanced BRAF V600E- or V600K-positive melanoma. It is also approved in combination with dabrafenib to treat patients with metastatic non-small cell lung cancer (NSCLC) with a BRAF V600E mutation and to treat patients with BRAF V600E-positive anaplastic thyroid cancer (ATC) who lack satisfactory locoregional treatment options.

GENE ASSOCIATION

Activating mutations in NRAS may result in activation of downstream pathways, including the MAPK pathway, and may therefore predict sensitivity to inhibitors of MAPK pathway components, including the MEK inhibitor trametinib. A patient with atypical chronic myeloid leukemia harboring an NRAS mutation exhibited a durable, near-complete response on trametinib treatment79. Significant clinical responses to various other MEK inhibitors have been documented in NRAS-mutant melanoma77,209,210,211.

SUPPORTING DATA

A Phase 1/2 study evaluated trametinib for the treatment of relapsed or refractory myeloid malignancies214. Patients with KRAS- or NRAS-mutated acute myeloid leukemia (AML) or myelodysplastic syndrome achieved an overall response rate (ORR) of 20% (10/50), including 6 complete remissions (CRs), and a median overall survival (OS) of 4.9 months. Patients with KRAS- or NRAS-mutated chronic myelomonocytic leukemia had an ORR of 27% (3/11), including 3 CRs, and a median OS of 14.5 months. In contrast, the study reported an ORR of 3% (1/30) and a median OS of 3.0 months for patients with wild-type or unknown RAS status214. Retrospective genomic analysis of RASmutated cases suggested that mutations in epigenetic regulators (e.g., MLL2, SETD2, TET2, IDH1/2) were more frequent among nonresponders than responders (70% vs. 33%) (Johnson et al., 2015; ASH Abstract 1386). Preclinical data support the sensitivity of RAS-mutated AML to MEK inhibitors, including trametinib73,215. A patient with NRAS-mutated atypical chronic myeloid leukemia (CML) experienced an exceptional hematologic response and disease control for at least 14 months on trametinib therapy79. Another patient with CML and concomitant melanoma reported a complete hematological response to treatment with dabrafenib and trametinib216.

Imatinib

Patient may be resistant to Imatinib

Assay findings associations

KIT
D816V

APPROVED INDICATIONS

Imatinib targets the BCR-ABL fusion protein, PDGFR, and KIT. It is FDA approved for the treatment of KIT-positive gastrointestinal stromal tumors (GIST), Ph+ chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL), myelodysplastic syndrome/myeloproliferative syndrome (MDS/MPS), aggressive systemic mastocytosis without a D816V KIT mutation, hypereosinophilic syndrome and/or chronic eosinophilic leukemia, and dermatofibrosarcoma protuberans.

GENE ASSOCIATION

KIT activating mutations or amplification may confer sensitivity to tyrosine kinase inhibitors such as imatinib20,169,170,171,172,173,174,175,176,177. However, mutation of residues within or adjacent to the KIT activation loop, as observed here, have demonstrated resistance to imatinib in patients with GIST9,11,13,35,36,178,179,180,181,182(Liegl B, et al. 2008; 18623623).

SUPPORTING DATA

A Phase 2 study of imatinib in patients with KIT-positive acute myeloid leukemia (AML) reported responses in 5 out of 21 cases, including 2 complete hematologic remissions, 1 patient with no evidence of leukemia, and 2 sustained minor responses175. However, other studies reported limited efficacy of imatinib in KIT-positive AML, with a Phase 2 study reporting clinical response consisting of hematologic improvement in 1 out of 36 patients183 and another study reporting no response in any of 10 included patients with AML or 8 with myelodysplastic syndrome (MDS)184. A Phase 1/2 study of imatinib with mitoxantrone, etoposide, and cytarabine in KIT-positive AML reported a complete response rate of 62% (13/21)169. A case study reported a complete molecular response to single-agent imatinib maintenance lasting more than 6 months in a patient with BCR-ABL-positive AML185.

Sunitinib

Patient may be resistant to Sunitinib

Assay findings associations

KIT
D816V

APPROVED INDICATIONS

Sunitinib is a small-molecule tyrosine kinase inhibitor that targets PDGFRs, VEGFRs, KIT, FLT3, CSF-1R, and RET. It is FDA approved for the treatment of advanced or metastatic pancreatic neuroendocrine tumors, gastrointestinal stromal tumors (GISTs) in patients who have progressed on or are intolerant to imatinib, and advanced renal cell carcinoma (RCC) as well as for the adjuvant treatment of patients at high risk of recurrent RCC after nephrectomy.

GENE ASSOCIATION

On the basis of extensive clinical and preclinical data in multiple tumor types10,197,198,199,200,201,202,203, KIT amplification or activating mutation may predict sensitivity to sunitinib. However, mutation of residues within the KIT activation loop (A-loop) is predicted to confer resistance to sunitinib based on extensive clinical data in GIST5,10,14,37.

SUPPORTING DATA

Sunitinib has been shown to inhibit activated FLT3 and the proliferation of cells with FLT3-activating mutations in preclinical studies of acute myeloid leukemia (AML)204,205. In one clinical study, 4/4 AML patients with activating FLT3 mutations exhibited morphologic or partial responses to sunitinib206. A case study reported complete though short-term hematologic responses for a patient with an eosinophilia-associated myeloid neoplasm and ETV6-FLT3 fusion, after sequential sunitinib and sorafenib therapeutic regimens207.

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.

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

GENE
KIT

ALTERATION
D816V

RATIONALE
KIT amplification or activating mutations may predict sensitivity to small molecule tyrosine kinase inhibitors. Also, because KIT activation leads to activation of the PI3K-AKT-mTOR pathway, PI3K and mTOR inhibitors may be relevant in a tumor with KIT activation. However, mutation of the KIT activation loop, as seen here, is associated with secondary resistance to imatinib and sunitinib; therefore, imatinib and sunitinib may not be effective in this case. 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 "KIT", "PI3K", "mTOR", "sorafenib", "dasatinib", "nilotinib", "PKC412", "ponatinib", "everolimus", "temsirolimus", "AML", and/or "leukemia".

NCT01643603

PHASE 1 / 2

Phase I/II Study of Dasatinib in Recipients of Allogeneic Stem Cell Transplantation for Hematologic Malignancies.

TARGETS
ABL, SRC, DDR2, KIT, PDGFRs

LOCATIONS: Michigan

NCT01806571

PHASE 2

A Phase II Study of Combination Daunorubicin and Cytarabine (Ara-c) and Nilotinib (Tasigna) (DATA) in Patients Newly Diagnosed With Acute Myeloid Leukemia and KIT Overexpression

TARGETS
ABL, KIT, PDGFRs

LOCATIONS: Arizona, Minnesota

NCT01552434

PHASE 1

A Phase I Trial of Bevacizumab, Temsirolimus Alone and in Combination With Valproic Acid or Cetuximab in Patients With Advanced Malignancy and Other Indications

TARGETS
HDAC, EGFR, VEGFA, mTOR

LOCATIONS: Texas

NCT01582191

PHASE 1

A Phase 1 Trial of Vandetanib (a Multi-kinase Inhibitor of EGFR, VEGFR and RET Inhibitor) in Combination With Everolimus (an mTOR Inhibitor) in Advanced Cancer

TARGETS
EGFR, RET, SRC, VEGFRs, mTOR

LOCATIONS: Texas

NCT01620216

PHASE 2

A Phase II Pilot Study of Kinase Inhibition in Relapsed/Refractory Acute Leukemias: Using a Comprehensive in Vitro Kinase Inhibitor Panel to Select Individualized, Targeted Therapies.

TARGETS
CSFR1, FLT3, KIT, PDGFRs, RET, VEGFRs, BCR-ABL, BTK, EPHA2, LYN, SRC, DDR1, BRAF, CRAF, EPH, TIE2, FGFRs

LOCATIONS: Texas

NCT02779283

PHASE 1

A Phase Ib Feasibility Study of Personalized Kinase Inhibitor Therapy Combined With Induction Chemotherapy in Acute Myeloid Leukemia in Patients Who Exhibit In Vitro Kinase Inhibitor Sensitivity

TARGETS
ABL, FGFRs, RAFs, RET, SRC, CSF1R, DDR2, FLT3, KIT, PDGFRs, VEGFRs

LOCATIONS: Oregon

NCT02013648

PHASE 3

Randomized Phase III Study of Intensive Chemotherapy With or Without Dasatinib (Sprycel™) in Adult Patients With Newly Diagnosed Core-Binding Factor Acute Myeloid Leukemia (CBF-AML)

TARGETS
ABL1, SRC, KIT, EPHA2, PDFGRs, LYN

LOCATIONS: Multiple ex-US locations

GENE
NRAS

ALTERATION
Q61H

RATIONALE
Activating mutations in NRAS leads to activation of the RAF-MEK-ERK, PI3K, and other pathways, and may predict sensitivity 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", "binimetinib", "refametinib", "E6201", "reolysin", "AML", "leukemia", and/or "advanced cancer".

NCT02670044

PHASE 1 / 2

A Phase IB/II Multi-Arm Study With Venetoclax in Combination With Cobimetinib and Venetoclax in Combination With Idasanutlin in Patients Aged >/= 60 Years With Relapsed or Refractory Acute Myeloid Leukemia Who Are Not Eligible for Cytotoxic Therapy

TARGETS
BCL2, MDM2, MEK

LOCATIONS: Colorado, Roma (Italy), Marseille (France), Toronto (Canada), Massachusetts, Bobigny (France), Bologna (Italy), North Carolina, Texas, Montreal (Canada), Pesaro (Italy), California, New York, Edmonton (Canada), Pessac (France)

NCT02089230

PHASE 1 / 2

Phase I/II Trial of MEK Inhibitor MEK162 in Patients With Relapsed and or Refractory Acute Myeloid Leukemia and Patients With Poor Prognosis Acute Myeloid Leukemia Not Suitable for or Unwilling to Receive Standard Therapy

TARGETS
MEK

LOCATIONS: Texas

NCT02551718

N/A

Individualized Treatment for Relapsed/Refractory Acute Leukemia Based on Chemosensitivity and Genomics/Gene Expression Data

TARGETS
Multiple

LOCATIONS: Washington

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.

CHEK2
N186S and R145W

CSF1R
E920D

GATA2
P161A

KDM2B
E928V

NTRK2
S167Y

PC
A615S

RAD50
D855Y

FoundationOne Heme is designed to include genes known to be somatically altered in human hematologic malignancies, sarcomas, and pediatric cancers 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 utilizes DNA sequencing to interrogate 406 genes as well as selected introns of 31 genes involved in rearrangements, in addition to RNA sequencing of 265 genes. The assay will be updated periodically to reflect new knowledge about cancer biology.

HEMATOLOGICAL MALIGNANCY DNA GENE LIST: ENTIRE CODING SEQUENCE FOR THE DETECTION OF BASE SUBSTITUTIONS, INSERTION/DELETIONS, AND COPY NUMBER ALTERATIONS

ABL1	*ACTB*	*AKT1*	*AKT2*	*AKT3*	*ALK*	*AMER1 (FAM123B or WTX)*		*APC*
APH1A	*AR*	*ARAF*	*ARFRP1*	*ARHGAP26 (GRAF)*	*ARID1A*	*ARID2*	*ASMTL*	*ASXL1*
ATM	*ATR*	*ATRX*	*AURKA*	*AURKB*	*AXIN1*	*AXL*	*B2M*	*BAP1*
BARD1	*BCL10*	*BCL11B*	*BCL2*	*BCL2L2*	*BCL6*	*BCL7A*	*BCOR*	*BCORL1*
BIRC3	*BLM*	*BRAF*	*BRCA1*	*BRCA2*	*BRD4*	*BRIP1*	*BRSK1*	*BTG2*
BTK	*BTLA*	*C11orf30 (EMSY)*	*CAD*	*CALR**	*CARD11*	*CBFB*	*CBL*	*CCND1*
CCND2	*CCND3*	*CCNE1*	*CCT6B*	*CD22*	*CD274 (PD-L1)*	*CD36*	*CD58*	*CD70*
CD79A	*CD79B*	*CDC73*	*CDH1*	*CDK12*	*CDK4*	*CDK6*	*CDK8*	*CDKN1B*
CDKN2A	*CDKN2B*	*CDKN2C*	*CEBPA*	*CHD2*	*CHEK1*	*CHEK2*	*CIC*	*CIITA*
CKS1B	*CPS1*	*CREBBP*	*CRKL*	*CRLF2*	*CSF1R*	*CSF3R*	*CTCF*	*CTNNA1*
CTNNB1	*CUX1*	*CXCR4*	*DAXX*	*DDR2*	*DDX3X*	*DNM2*	*DNMT3A*	*DOT1L*
DTX1	*DUSP2*	*DUSP9*	*EBF1*	*ECT2L*	*EED*	*EGFR*	*ELP2*	*EP300*
EPHA3	*EPHA5*	*EPHA7*	*EPHB1*	*ERBB2*	*ERBB3*	*ERBB4*	*ERG*	*ESR1*
ETS1	*ETV6*	*EXOSC6*	*EZH2*	*FAF1*	*FAM46C*	*FANCA*	*FANCC*	*FANCD2*
FANCE	*FANCF*	*FANCG*	*FANCL*	*FAS (TNFRSF6)*	*FBXO11*	*FBXO31*	*FBXW7*	*FGF10*
FGF14	*FGF19*	*FGF23*	*FGF3*	*FGF4*	*FGF6*	*FGFR1*	*FGFR2*	*FGFR3*
FGFR4	*FHIT*	*FLCN*	*FLT1*	*FLT3*	*FLT4*	*FLYWCH1*	*FOXL2*	*FOXO1*
FOXO3	*FOXP1*	*FRS2*	*GADD45B*	*GATA1*	*GATA2*	*GATA3*	*GID4 (C17orf39)*	*GNA11*
GNA12	*GNA13*	*GNAQ*	*GNAS*	*GPR124*	*GRIN2A*	*GSK3B*	*GTSE1*	*HDAC1*
HDAC4	*HDAC7*	*HGF*	*HIST1H1C*	*HIST1H1D*	*HIST1H1E*	*HIST1H2AC*	*HIST1H2AG*	*HIST1H2AL*
HIST1H2AM	*HIST1H2BC*	*HIST1H2BJ*	*HIST1H2BK*	*HIST1H2BO*	*HIST1H3B*	*HNF1A*	*HRAS*	*HSP90AA1*
ICK	*ID3*	*IDH1*	*IDH2*	*IGF1R*	*IKBKE*	*IKZF1*	*IKZF2*	*IKZF3*
IL7R	*INHBA*	*INPP4B*	*INPP5D (SHIP)*	*IRF1*	*IRF4*	*IRF8*	*IRS2*	*JAK1*
JAK2	*JAK3*	*JARID2*	*JUN*	*KAT6A (MYST3)*	*KDM2B*	*KDM4C*	*KDM5A*	*KDM5C*
KDM6A	*KDR*	*KEAP1*	*KIT*	*KLHL6*	*KMT2A (MLL)*	*KMT2C (MLL3)*	*KMT2D (MLL2)*	*KRAS*
LEF1	*LRP1B*	*LRRK2*	*MAF*	*MAFB*	*MAGED1*	*MALT1*	*MAP2K1*	*MAP2K2*
MAP2K4	*MAP3K1*	*MAP3K14*	*MAP3K6*	*MAP3K7*	*MAPK1*	*MCL1*	*MDM2*	*MDM4*
MED12	*MEF2B*	*MEF2C*	*MEN1*	*MET*	*MIB1*	*MITF*	*MKI67*	*MLH1*
MPL	*MRE11A*	*MSH2*	*MSH3*	*MSH6*	*MTOR*	*MUTYH*	*MYC*	*MYCL (MYCL1)*
MYCN	*MYD88*	*MYO18A*	*NCOR2*	*NCSTN*	*NF1*	*NF2*	*NFE2L2*	*NFKBIA*
NKX2-1	*NOD1*	*NOTCH1*	*NOTCH2*	*NPM1*	*NRAS*	*NT5C2*	*NTRK1*	*NTRK2*
NTRK3	*NUP93*	*NUP98*	*P2RY8*	*PAG1*	*PAK3*	*PALB2*	*PASK*	*PAX5*
PBRM1	*PC*	*PCBP1*	*PCLO*	*PDCD1*	*PDCD11*	*PDCD1LG2 (PD-L2)*	*PDGFRA*	*PDGFRB*
PDK1	*PHF6*	*PIK3CA*	*PIK3CG*	*PIK3R1*	*PIK3R2*	*PIM1*	*PLCG2*	*POT1*
PPP2R1A	*PRDM1*	*PRKAR1A*	*PRKDC*	*PRSS8*	*PTCH1*	*PTEN*	*PTPN11*	*PTPN2*
PTPN6 (SHP-1)	*PTPRO*	*RAD21*	*RAD50*	*RAD51*	*RAF1*	*RARA*	*RASGEF1A*	*RB1*
RELN	*RET*	*RHOA*	*RICTOR*	*RNF43*	*ROS1*	*RPTOR*	*RUNX1*	*S1PR2*
SDHA	*SDHB*	*SDHC*	*SDHD*	*SERP2*	*SETBP1*	*SETD2*	*SF3B1*	*SGK1*
SMAD2	*SMAD4*	*SMARCA1*	*SMARCA4*	*SMARCB1*	*SMC1A*	*SMC3*	*SMO*	*SOCS1*
SOCS2	*SOCS3*	*SOX10*	*SOX2*	*SPEN*	*SPOP*	*SRC*	*SRSF2*	*STAG2*
STAT3	*STAT4*	*STAT5A*	*STAT5B*	*STAT6*	*STK11*	*SUFU*	*SUZ12*	*TAF1*
TBL1XR1	*TCF3 (E2A)*	*TCL1A (TCL1)*	*TET2*	*TGFBR2*	*TLL2*	*TMEM30A*	*TMSB4XP8 (TMSL3)*	
TNFAIP3	*TNFRSF11A*	*TNFRSF14*	*TNFRSF17*	*TOP1*	*TP53*	*TP63*	*TRAF2*	*TRAF3*
TRAF5	*TSC1*	*TSC2*	*TSHR*	*TUSC3*	*TYK2*	*U2AF1*	*U2AF2*	*VHL*
WDR90	*WHSC1 (MMSET or NSD2)*		*WISP3*	*WT1*	*XBP1*	*XPO1*	*YY1AP1*	*ZMYM3*
ZNF217	*ZNF24 (ZSCAN3)*	*ZNF703*	*ZRSR2*					

*Note: the assay was updated on 11/8/2016 to include the detection of alterations in CALR

HEMATOLOGICAL MALIGNANCY DNA GENE LIST: FOR THE DETECTION OF SELECT REARRANGEMENTS

ALK	*BCL2*	*BCL6*	*BCR*	*BRAF*	*CCND1*	*IGH*	*IGK*	*CRLF2*
EGFR	*EPOR*	*ETV1*	*ETV4*	*ETV5*	*ETV6*	*EWSR1*	*FGFR2*	*IGL*
JAK1	*JAK2*	*KMT2A (MLL)*	*MYC*	*NTRK1*	*PDGFRA*	*PDGFRB*	*RAF1*	*RARA*
RET	*ROS1*	*TMPRSS2*	*TRG*					

HEMATOLOGICAL MALIGNANCY RNA GENE LIST: FOR THE DETECTION OF SELECT REARRANGEMENTS

ABI1	*ABL1*	*ABL2*	*ACSL6*	*AFF1*	*AFF4*	*ALK*	*ARHGAP26 (GRAF)*	
ARHGEF12	*ARID1A*	*ARNT*	*ASXL1*	*ATF1*	*ATG5*	*ATIC*	*BCL10*	*BCL11A*
BCL11B	*BCL2*	*BCL3*	*BCL6*	*BCL7A*	*BCL9*	*BCOR*	*BCR*	*BIRC3*
BRAF	*BTG1*	*CAMTA1*	*CARS*	*CBFA2T3*	*CBFB*	*CBL*	*CCND1*	*CCND2*
CCND3	*CD274 (PD-L1)*	*CDK6*	*CDX2*	*CHIC2*	*CHN1*	*CIC*	*CIITA*	*CLP1*
CLTC	*CLTCL1*	*CNTRL (CEP110)*	*COL1A1*	*CREB3L1*	*CREB3L2*	*CREBBP*	*CRLF2*	*CSF1*
CTNNB1	*DDIT3*	*DDX10*	*DDX6*	*DEK*	*DUSP22*	*EGFR*	*EIF4A2*	*ELF4*
ELL	*ELN*	*EML4*	*EP300*	*EPOR*	*EPS15*	*ERBB2*	*ERG*	*ETS1*
ETV1	*ETV4*	*ETV5*	*ETV6*	*EWSR1*	*FCGR2B*	*FCRL4*	*FEV*	*FGFR1*
FGFR1OP	*FGFR2*	*FGFR3*	*FLI1*	*FNBP1*	*FOXO1*	*FOXO3*	*FOXO4*	*FOXP1*
FSTL3	*FUS*	*GAS7*	*GLI1*	*GMPS*	*GPHN*	*HERPUD1*	*HEY1*	*HIP1*
HIST1H4I	*HLF*	*HMGA1*	*HMGA2*	*HOXA11*	*HOXA13*	*HOXA3*	*HOXA9*	*HOXC11*
HOXC13	*HOXD11*	*HOXD13*	*HSP90AA1*	*HSP90AB1*	*IGH*	*IGK*	*IGL*	*IKZF1*
IL21R	*IL3*	*IRF4*	*ITK*	*JAK1*	*JAK2*	*JAK3*	*JAZF1*	*KAT6A (MYST3)*
KDSR	*KIF5B*	*KMT2A (MLL)*	*LASP1*	*LCP1*	*LMO1*	*LMO2*	*LPP*	*LYL1*
MAF	*MAFB*	*MALT1*	*MDS2*	*MECOM*	*MKL1*	*MLF1*	*MLLT1 (ENL)*	*MLLT10 (AF10)*
MLLT3	*MLLT4 (AF6)*	*MLLT6*	*MN1*	*MNX1*	*MSI2*	*MSN*	*MUC1*	*MYB*
MYC	*MYH11*	*MYH9*	*NACA*	*NBEAP1 (BCL8)*	*NCOA2*	*NDRG1*	*NF1*	*NF2*
NFKB2	*NIN*	*NOTCH1*	*NPM1*	*NR4A3*	*NSD1*	*NTRK1*	*NTRK2*	*NTRK3*
NUMA1	*NUP214*	*NUP98*	*NUTM2A*	*OMD*	*P2RY8*	*PAFAH1B2*	*PAX3*	*PAX5*
PAX7	*PBX1*	*PCM1*	*PCSK7*	*PDCD1LG2 (PD-L2)*	*PDE4DIP*	*PDGFB*	*PDGFRA*	*PDGFRB*
PER1	*PHF1*	*PICALM*	*PIM1*	*PLAG1*	*PML*	*POU2AF1*	*PPP1CB*	*PRDM1*
PRDM16	*PRRX1*	*PSIP1*	*PTCH1*	*PTK7*	*RABEP1*	*RAF1*	*RALGDS*	*RAP1GDS1*
RARA	*RBM15*	*RET*	*RHOH*	*RNF213*	*ROS1*	*RPL22*	*RPN1*	*RUNX1*
RUNX1T1 (ETO)	*RUNX2*	*SEC31A*	*SEPT5*	*SEPT6*	*SEPT9*	*SET*	*SH3GL1*	*SLC1A2*
SNX29 (RUNDC2A)	*SRSF3*	*SS18*	*SSX1*	*SSX2*	*SSX4*	*STAT6*	*STL*	*SYK*
TAF15	*TAL1*	*TAL2*	*TBL1XR1*	*TCF3 (E2A)*	*TCL1A (TCL1)*	*TEC*	*TET1*	*TFE3*
TFG	*TFPT*	*TFRC*	*TLX1*	*TLX3*	*TMPRSS2*	*TNFRSF11A*	*TOP1*	*TP63*
TPM3	*TPM4*	*TRIM24*	*TRIP11*	*TTL*	*TYK2*	*USP6*	*WHSC1 (MMSET or NSD2)*	
WHSC1L1	*YPEL5*	*ZBTB16*	*ZMYM2*	*ZNF384*	*ZNF521*			

ADDITIONAL ASSAYS: FOR THE DETECTION OF SELECT CANCER BIOMARKERS

Microsatellite (MS) status
Tumor Mutational Burden (TMB)

The median exon coverage for this sample is 843X

ACCURACY		
Sensitivity: Base Substitutions	At ≥5% Minor Allele Frequency	>99.0%
Sensitivity: Insertions/Deletions (1-40bp)	At ≥10% Minor Allele Frequency	98.0%
Sensitivity: Focal Copy Number Alterations (Homozygous Deletions or Amplifications)	At ≥8% copies	>95.0%
Sensitivity: Microsatellite status	At ≥20% tumor nuclei	97.0%
Sensitivity: Known Gene Fusions	>95.0%	
Specificity: Base Substitutions, Insertions/Deletions, and Focal Copy Number Alterations	Positive Predictive Value (PPV)	>99.0%
Specificity: Known Gene Fusions	Positive Predictive Value (PPV)	>95.0%
Specificity: Microsatellite status	Positive Predictive Value (PPV)	>95.0%
Accuracy: Tumor Mutational Burden	At ≥20% tumor nuclei	>90.0%
Reproducibility (average concordance between replicates)	97.0% inter-batch precision 97.0% intra-batch precision 95.0% microsatellite status precision 96.0% tumor mutation burden precision	

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, which is a measure of microsatellite instability (MSI), is determined by assessing indel characteristics at 114 homopolymer repeat loci in or near the targeted gene regions of the FoundationOne Heme test. Microsatellite status is assayed for all FoundationOne Heme samples and may be reported as "MSI-High", "MSI-Intermediate", "MS-Stable", or "Cannot Be Determined". Microsatellite status is reported as "Cannot Be Determined" if the sample is not of sufficient quality to be confidently determined.

Tumor Mutational 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 and may be reported as “TMB-High”, “TMB-Intermediate”, “TMB-Low”, or “Cannot Be Determined”. TMB results, which are rounded to the nearest integer, 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 fewer than or equal to 5 Muts/Mb. Tumor Mutational Burden is reported as “Cannot Be Determined” if the sample is not of sufficient quality to confidently determine Tumor Mutational Burden.

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

ABOUT FOUNDATIONONE HEME

FoundationOne Heme is a comprehensive genomic profiling test for hematologic malignancies, sarcomas and pediatric cancers. The test is designed to provide physicians with clinically actionable information to help with diagnostic sub-classification, prognosis assessment, and targeted therapeutic selection. Test results provide information about clinically significant alterations, potential targeted therapies, available clinical trials, and quantitative markers that may support immunotherapy clinical trial enrollment. FoundationOne Heme is analytically validated to detect all classes of genomic alterations in more than 400 cancer-related genes. In addition to DNA sequencing, FoundationOne Heme employs RNA sequencing across more than 250 genes to capture a broad range of gene fusions, common drivers of hematologic malignancies and sarcomas, pediatric cancers.

FoundationOne Heme 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 Heme 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.

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.

Diagnostic Significance

FoundationOne Heme identifies alterations to select cancer-associated genes or portions of genes (biomarkers). In some cases, the Report also highlights selected negative test results regarding biomarkers of clinical significance.

Qualified Alteration Calls (Equivocal and Subclonal)

An alteration denoted as "amplification – equivocal" implies that FoundationOne Heme 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 Heme 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 FoundationOne Heme data provide some, but not unambiguous, evidence for homozygous deletion of the gene in question. An alteration denoted as "subclonal" is one that FoundationOne Heme analytical methodology has identified as being present in <10% of the assayed tumor DNA.

Ranking of Alterations and Therapies

Biomarker Findings
Appear at the top of the report, but are not ranked higher than Genomic Findings.

Genomic Findings
Therapies with Clinical Benefit In Patient's Tumor Type → Therapies with Clinical Benefit in Other Tumor Type → Clinical Trial Options → No Known Options (If multiple findings exist within any of these categories, the results are listed alphabetically by gene name.)

Therapies
Sensitizing therapies → Resistant therapies. (If multiple therapies exist within any of these categories, they are listed alphabetically by therapy name.)

Clinical Trials
Pediatric trial qualification → Geographical proximity → Later trial phase.

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 Heme.

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 Heme is performed using DNA and RNA 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 4, TNFRSF11A exon1, and TP53 exon 1.

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

CE

SELECT ABBREVIATIONS

ABBREVIATION	DEFINITION
CR	Complete response
DCR	Disease control rate
DNMT	DNA methyltransferase
HR	Hazard ratio
ITD	Internal tandem duplication
MMR	Mismatch repair
muts/Mb	Mutations per megabase
NOS	Not otherwise specified
ORR	Objective response rate
OS	Overall survival
PD	Progressive disease
PFS	Progression-free survival
PR	Partial response
SD	Stable disease
TKI	Tyrosine kinase inhibitor

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