FOUNDATIONONE Heme

Patient Name

Report Date

Tumor Type
Bone marrow leukemia non-lymphocytic acute myelocytic (AML)

Date of Birth		**Medical Facility**		**Specimen Received**	
Sex		**Ordering Physician**		**Specimen Site**	
FMI Case #	SRF201611	**Additional Recipient**		**Date of Collection**	
Medical Record #		**Medical Facility ID #**		**Specimen Type**	
Specimen ID		**Pathologist**			

ABOUT THE TEST:

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

PATIENT RESULTS[II]

4 genomic alterations

5 therapies associated with potential clinical benefit

0 therapies associated with lack of response

8 clinical trials

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

TUMOR TYPE: BONE MARROW LEUKEMIA NON-LYMPHOCYTIC ACUTE MYELOCYTIC (AML)

Genomic Alterations Identified[†]

FLT3 FLT3-ITD (R595_E596ins16)
NF1 L2149fs*20
NPM1 W288fs*10+
PTPN11 N308D – subclonal[#]

[†] For a complete list of the genes assayed and performance specifications, please refer to the Appendix
[#] See Appendix for details

THERAPEUTIC IMPLICATIONS

Genomic Alterations Detected	FDA-Approved Therapies (in patient's tumor type)	FDA-Approved Therapies (in another tumor type)	Potential Clinical Trials
FLT3 FLT3-ITD (R595_E596ins16)	None	Ponatinib Sorafenib Sunitinib	Yes, see clinical trials section
NF1 L2149fs*20	None	Cobimetinib Trametinib	Yes, see clinical trials section
NPM1 W288fs*10+	None	None	None
PTPN11 N308D - subclonal	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
● ***FLT3*** FLT3-ITD (R595_E596ins16)	**Gene and Alteration:** FLT3 encodes a receptor tyrosine kinase. Signaling through the FLT3 pathway leads to phosphorylation of Shc1 and AKT1 and activation of mTOR, as well as RAS activation and phosphorylation of ERK1/2[1,2,3]. The FLT3 internal tandem duplication (FLT3-ITD) observed here is predicted to be activating[4,5,6]. FLT3-ITD are frequent in acute myeloid leukemia (AML) and are associated with poor prognosis, as well as having implications for risk stratification in patients with normal karyotype[7,8,9,10]. **Frequency and Prognosis:** In the TCGA dataset, FLT3 mutation was observed in 27% of acute myeloid leukemia (AML) cases[11]. FLT3-ITDs have been reported in 31% of cases and mutations of the tyrosine kinase domain specifically in 11% of cases[9]. FLT3-ITDs in patients with AML are associated with poor prognosis as well as having implications for risk stratification in patients with normal karyotype[7,8,9,10]. **Potential Treatment Strategies:** Therapies targeting FLT3 are under clinical investigation, including sorafenib, ponatinib, sunitinib, crenolanib, midostaurin, quizartinib, lestaurtinib, gilteritinib, and pexidartinib[12,13,14]. The addition of sorafenib to standard chemotherapy regimens (Uy et al., 2015; ASH Abstract 319)[15], hematopoietic cell transplant regimens (Brunner et al., 2015; ASH Abstract 864)[16,17,18] or hypomethylating agents[19,20] has resulted in clinical efficacy for patients with AML and FLT3-ITD. In a Phase 1 trial of 12 patients with AML, ponatinib elicited an overall response rate (RR) of 30% (3/10) in patients with FLT3-ITD, with a higher RR (43%, 3/7) observed in patients who were FLT3 inhibitor-naive[21]. In another Phase 1 trial, 4/4 patients with AML harboring activating FLT3 mutations exhibited morphologic or partial responses to sunitinib[22]. Crenolanib has shown preclinical efficacy against tumors harboring FLT3 alterations, including mutations at D835 (FLT3-TKD) that confer resistance to sorafenib and sunitinib[23,23,24,25,26,27,28]. The Phase 3 RATIFY trial reported that addition of midostaurin improved overall survival (OS) for patients with AML and FLT3-ITDs or FLT3-TKDs relative to treatment with standard chemotherapy alone (Stone et al., 2015; ASH Abstract 6). Quizartinib is also being investigated for patients with AML and FLT3-ITDs and resulted in composite complete remission (CRc) in 43% of cases versus 11% in historical controls in a Phase 2 trial (Hills et al., 2015; ASH Abstract 2557). In Phase 1/2 trials of gilteritinib for AML, overall responses (ORs) were observed in 57-63% of patients with unspecified FLT3 mutations (Levis et al., 2015; ASCO Abstract 7003) and in 60% (68/114) of those with FLT3-ITDs only (Altman et al., 2015; ASH Abstract 321).
● ***NF1*** L2149fs*20	**Gene and Alteration:** NF1 encodes neurofibromin, a GTPase-activating protein (GAP) that is a key negative regulator of the RAS signaling pathway[29]. Neurofibromin acts as a tumor suppressor by repressing RAS signaling[30]. Although this alteration has not been fully characterized and its effect on NF1 function is unclear, it has been previously reported in the context of cancer, which may indicate biological relevance. Germline mutations in NF1 cause the autosomal dominant disorder neurofibromatosis type 1, which is characterized in part by increased risk of developing various tumors, including sarcoma, glioma, breast carcinoma, and neuroendocrine and hematological neoplasms[31,32,33]. Estimates for the prevalence of the disorder in the general population range from 1:2,500 to 1:3,000[34,35], and in the appropriate clinical context, germline testing of NF1 is recommended. **Frequency and Prognosis:** NF1 mutation has been reported in 1-7% of AML samples analyzed, which has been suggested to represent a subset of AML cases that may benefit from therapies with mTOR inhibitors (COSMIC, cBioPortal, Jan 2016)[36,37,38].

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GENE ALTERATION	INTERPRETATION
	Potential Treatment Strategies: On the basis of clinical evidence from a Phase 1 study in neurofibromatosis type 1 (Widemann et al., 2014; ASCO Abstract 10018) and a case report in neurofibromatosis-associated glioblastoma[39] as well as extensive preclinical evidence in several tumor types[40,41,42,43,44], NF1 inactivation may predict sensitivity to MEK inhibitors, including the approved therapies cobimetinib and trametinib. Loss or inactivation of NF1 may also predict sensitivity to mTOR inhibitors, including the approved agents everolimus and temsirolimus, based on limited clinical data[45,46,47] and strong preclinical data in models of malignant peripheral nerve sheath tumor (MPNST)[48,49]. These and other relevant compounds are being investigated in clinical trials. Limited clinical and preclinical evidence in melanoma suggest that in the context of BRAF mutation, NF1 loss or inactivation may reduce sensitivity to BRAF inhibitors[41,50,51]; data are conflicting on whether NF1 deficiency reduces sensitivity to MEK inhibition in BRAF-mutant melanoma[41,50,52,53]. Although a preclinical study suggests that combined mTOR and MEK inhibition is effective in a model of NF1-deficient MPNST[54], a Phase 1b trial of a combination of the mTOR inhibitor everolimus and the MEK inhibitor trametinib in patients with solid tumors reported frequent adverse events and was unable to identify a recommended Phase 2 dose and schedule for the combination[55]. However, it is not known whether these therapeutic approaches would be relevant in the context of alterations that have not been fully characterized, as seen here.
● ***NPM1*** W288fs*10+	**Gene and Alteration:** NPM1 encodes nucleophosmin, a protein involved in numerous critical cellular processes including the regulation of tumor suppressors such as p53 and ARF. NPM1 expression is elevated in many tumors, leading to enhanced c-MYC transformation; low NPM1 expression is associated with suppressed tumor growth. Thus, depending on the cellular level of NPM1, it may act as a proto-oncogene or a tumor suppressor[56,57]. The NPM1 truncating alteration observed here is similar to mutations that have been repeatedly observed in the context of acute myeloid leukemia (AML) and are known to be deleterious to NPM1 function[58,59,60,61]. **Frequency and Prognosis:** In the TCGA dataset, NPM1 mutation was observed in 27% of AML samples analyzed[11]. Abnormal cytoplasmic localization of nucleophosmin has been observed in 35% of AML cases, and analysis of 52 NPM1 mutations found all but one to be located in the last coding exon, exon 12[60]. Exon 12 mutations interrupting the nuclear localization signal of nucleophosmin were reported in 27% of patients with AML[58]. NPM1 mutations are generally associated with a better response to chemotherapy. NPM1 mutations in the absence of FLT3-ITD are associated with better recurrence-free survival and better overall survival, but may predict a lack of benefit from matched related donor transplantation in the first complete remission[58,62]. **Potential Treatment Strategies:** There are no approved therapies to address NPM1 alterations. However, several approaches to target cancers with mutant NPM1 are under preclinical and clinical development, including small molecule inhibitors that redirect mutant NPM1 to the nucleolus or interfere with the function of wild-type NPM1[63]. Additionally, NPM1 alterations in AML associate with a distinct DNA methylation profile[64] and frequently co-occur with alterations in epigenetic regulators (IDH1, IDH2, DNMT3A, or TET2), which may predict sensitivity to DNA methyltransferase (DNMT) inhibitors such as azacitidine and decitabine. Several studies have described complete responses (CR) to azacitidine or decitabine for patients with AML and NPM1 mutations; however, higher response rates were observed for patients with concurrent mutations in FLT3-ITD or DNMT3A[65,66,67,68,69].

GENE ALTERATION	INTERPRETATION
PTPN11 N308D - subclonal	**Gene and Alteration:** PTPN11 encodes for the protein tyrosine-protein phosphatase non-receptor type 11, also known as SHP2. PTPN11 plays a critical role in both embryonic development and cancer[70]. PTPN11 is also known to be somatically mutated in a variety of cancers, where both oncogenic and tumor suppressor roles for PTPN11 have been described[71,72,73]. The N-terminal SRC homology 2 (SH2) domain (aa 6-102) negatively regulates SHP2 activity by binding to the active site of the SHP-2 protein tyrosine phosphatase (PTP) domain (aa 247-521)[74]. Alterations that disrupt this interaction (such as mutation of residues D61, E69, A72, E76, and G503, as well as G60A, Y63C, F71I, T73I, T507K, and Q510E) or affect the specificity and structure of the SH2 and PTP domains (including T42A, E139D, N308S, N308D, and P491S) have been characterized as activating[71,75,76,77,78,79,80,81,81,82,83,84,85] and predicted to be oncogenic[71,76,77,78,79,86,87,88,89,90]. Germline mutations in PTPN11 have been found in the developmental disorder Noonan syndrome, which predisposes individuals to various cancers, including embryonal rhabdomyosarcoma, neuroblastoma, and juvenile myelomonocytic leukemia[87,91,92,93,94,95]. **Frequency and Prognosis:** PTPN11 mutations have been found in 4-5% of AML samples, including 9% (4/47) of AML associated with myelodysplastic syndrome samples and 18% of pediatric cases of acute monocytic leukemia French-American-British (FAB)-M5 subtype[71,96,97]. Mutations in PTPN11 are associated with poor prognosis in juvenile myelomonocytic leukemia[98]. A preclinical study has found that a small molecule inhibitor of SHP2 can reduce the proliferation of primary AML samples, suggesting inhibition of SHP2 as a potential therapeutic approach in AML[99]. In addition, preclinical studies have demonstrated activation of the AKT and ERK pathway in hematopoietic cells expressing a PTPN11 activating mutation[71,76,77,100]. **Potential Treatment Strategies:** SHP-2 has been reported to activate the RAS-MEK-ERK, PI3K, and SRC kinase pathways[77,101,102,103]. Preclinical studies in hematologic and solid cancer cell lines (Elamin et al., 2015; ASCO Abstract 11077)[77,100] and in animal models of developmental abnormalities associated with Noonan syndrome and LEOPARD syndrome[104,105,106] have suggested that PTPN11 mutations may predict sensitivity to MEK or PI3K inhibitors. The MEK inhibitors trametinib and cobimetinib are approved to treat unresectable or metastatic BRAF V600E or V600K mutant melanoma[107,108]. Various MEK and PI3K inhibitors are under investigation in clinical trials.

THERAPIES

There are no therapies FDA-approved 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
Ponatinib	**Approved Indications:** Ponatinib is a multikinase inhibitor targeting BCR-ABL, RET, KIT, FLT-3, PDGFRs, VEGFRs, FGFRs, and other tyrosine kinases. It is FDA approved to treat advanced, T315I-mutated chronic myeloid leukemia (CML) and Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL), as well as for CML and Ph+ ALL patients for whom no other tyrosine kinase inhibitor is indicated. **Gene Association:** Activating mutations in FLT3, including FLT3-ITD, may predict sensitivity to ponatinib[109]. Ponatinib has shown efficacy against FLT3-driven leukemic cells[110], and a Phase 1 study of ponatinib in patients with acute myeloid leukemia (AML), all of whom had FLT3 alterations, reported a 3/12 response rate, with 2 complete responses and one partial response[21]. **Supporting Data:** Clinically, ponatinib has been most extensively studied in patients with BCR-ABL-positive hematological malignancies. Ponatinib has shown efficacy in preclinical models of endometrial, bladder, gastric, breast, lung, colon, and medullary thyroid carcinomas, and is being clinically tested in some solid tumor types (Gozgit et al., 2013; AACR Abstract 2084)[111]. 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 response[21].
Sorafenib	**Approved Indications:** Sorafenib is a kinase inhibitor that targets the RAF kinases, KIT, FLT3, RET, VEGFRs, and PDGFRs. It is FDA approved for the treatment of unresectable hepatocellular carcinoma, advanced renal cell carcinoma, and recurrent or metastatic differentiated thyroid carcinoma. **Gene Association:** FLT3 activating mutations or amplification may predict sensitivity to sorafenib. Sorafenib has been shown to inhibit activated FLT3 in preclinical studies of acute myelogenous leukemia (AML)[13,112] and to provide clinical benefit to adult[18,19,113,114] and pediatric[115] patients with FLT3-ITD-mutated or other FLT3-mutated AML or CMML[116]. A patient with FLT3-amplified and KRAS-mutant colorectal cancer has been reported to achieve significant benefit from sorafenib treatment[117]. In patients with FLT3-mutated AML, addition of sorafenib to chemotherapy associated with a trend to longer overall survival[15].

	Supporting Data: For younger adults (18-60 years) with newly diagnosed AML, sorafenib added to standard therapy increased 3-year event-free survival (EFS) (40% vs. 22%) and relapse-free survival (RFS) (56% vs. 38%) compared with placebo; although the 3-year overall survival (OS) rates were similar (63% vs. 56%) in both treatment arms, sorafenib was associated with a trend for prolonged OS in patients with FLT3-ITD[15]. For patients aged 60 years or older with FLT3-mutated AML, addition of sorafenib to induction and post-remission chemotherapy prolonged the 1-year OS for patients with FLT3-ITD compared to historical controls (62% vs. 30%); patients with FLT3-ITD achieved a median disease-free survival (DFS) of 12.5 months and OS of 15.0 months, whereas DFS and OS for those with FLT3 kinase domain mutations (FLT3-TKD) were 9.0 months and 16.2 months, respectively (Uy et al., 2015; ASH Abstract 319). On the other hand, when sorafenib consolidation was used after induction chemotherapy, neither EFS nor OS for elderly patients with AML was significantly improved, irrespective of FLT-ITD status, and the regimen was associated with higher induction toxicity[118]. Use of sorafenib as maintenance therapy after hematopoietic cell transplantation (HCT) was reported in a retrospective study to significantly improve OS (hazard ratio for death, 0.146) for patients with FLT3-ITD-positive AML in first remission (Brunner et al., 2015; ASH Abstract 864), which is supported by the outcomes in a case series[18] and two additional retrospective analyses[16,17]. Addition of sorafenib to hypomethylating agents has also yielded clinical responses in AML. In a Phase 2 study for patients with refractory or relapsed AML and FLT3-ITD mutations, sorafenib plus azacitidine achieved a response rate of 46% (17/37)[19], and 5/6 patients with FLT3-ITD-positive AML had overall responses to sorafenib plus decitabine in another study[20].
Sunitinib	**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 renal cell carcinoma, advanced or metastatic pancreatic neuroendocrine tumors, and gastrointestinal stromal tumors (GIST) after progression on imatinib. **Gene Association:** Amplification or activating mutations in FLT3 may predict sensitivity to sunitinib based on clinical[22,115,119,120] and preclinical[12,112,121,122] evidence. Clinical evidence includes cases of AML with FLT3-ITD[22,115,119], FLT3 kinase domain mutations[119] and FLT3-ITD-mutated pediatric cases[115]. 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 regimens[120]. **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)[12,121]. In a clinical study, 4/4 patients with AML harboring activating FLT3 mutations exhibited morphologic or partial responses to sunitinib[22].
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:** On the basis of clinical evidence (Widemann et al., 2014; ASCO Abstract 10018)[39] and strong preclinical evidence[40,41,42,43,44], NF1 inactivation may predict sensitivity to MEK inhibitors such as cobimetinib. However, it is not known whether this therapeutic approach would be relevant in the context of alterations that have not been fully characterized, as seen here.

	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)[108]. In a Phase 1b study, vemurafenib combined with cobimetinib achieved an objective response rate of 87% for patients with BRAF V600-mutant melanoma who had not previously received a BRAF inhibitor[123]. One study reported near-complete response to vemurafenib in a patient with BRAF V600K-mutant melanoma who subsequently developed chronic myelomonocytic leukemia (CMML) with NRAS G12R mutation, and concurrent cobimetinib treatment led to suppression of CMML[124]. In a Phase 1b study, out of 47 patients treated with cobimetinib and the AKT inhibitor ipatasertib, 3 patients with KRAS-mutant ovarian, mesonephric cervical, or endometrial carcinoma had a partial response, with prolonged stable disease lasting for >6 months (Bendell et al., 2014; AACR Abstract CT328).
Trametinib	**Approved Indications:** Trametinib is a MEK inhibitor that is FDA approved as both a single agent and in combination with dabrafenib for the treatment of unresectable or metastatic melanoma with BRAF V600E or V600K mutations. **Gene Association:** On the basis of clinical evidence (Widemann et al., 2014; ASCO Abstract 10018)[39] and strong preclinical evidence[40,41,42,43,44], NF1 inactivation may predict sensitivity to MEK inhibitors such as trametinib. However, it is not known whether this therapeutic approach would be relevant in the context of alterations that have not been fully characterized, as seen here. **Supporting Data:** A Phase 1/2 study evaluated trametinib for the treatment of relapsed or refractory myeloid malignancies[125]. 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 status[125]. Retrospective genomic analysis of RAS-mutated 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 trametinib[126,127]. A patient with NRAS-mutated atypical chronic myeloid leukemia experienced an exceptional hematologic response and disease control for at least 14 months on trametinib therapy[128].

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
● ***FLT3*** FLT3-ITD (R595_E596ins16)	FLT3 activating alterations may predict sensitivity to FLT3 inhibitors and tyrosine kinase 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 "FLT3", "sorafenib", "sunitinib", "ponatinib", "crenolanib", "midostaurin", "PKC-412", "quizartinib", "AC220", "pexidartinib", "PLX3397", "gilteritinib", "ASP-2215", "lestaurtinib", "CEP-701", "dovitinib", "TKI-258", "AML", "leukemia", and/or "advanced cancer".

TITLE	PHASE	TARGETS	LOCATIONS	NCT ID
A Single-arm Phase II Trial to Assess the Efficacy of Midostaurin (PKC412) Added to Standard Primary Therapy in Patients With Newly Diagnosed c-KIT or FLT3-ITD Mutated t(8;21) AML	Phase 2	FLT3	Chemnitz (Germany), Dresden (Germany), Düsseldorf (Germany), Erlangen (Germany), Frankfurt Main (Germany), Heidelberg (Germany), Jena (Germany), Marburg (Germany), Münster (Germany), Nürnberg (Germany), Regensburg (Germany)	NCT01830361
Phase I/II Study of the Combination of Quizartinib (AC220) With 5-Azacytidine or Low-Dose Cytarabine for the Treatment of Patients With Acute Myeloid Leukemia (AML) and Myelodysplastic Syndrome (MDS)	Phase 1/Phase 2	FLT3, CSF1R, KIT, PDGFRs, DNMT	Texas	NCT01892371
Phase II Study of Sorafenib Plus 5-Azacitidine for the Initial Therapy of Patients With Acute Myeloid Leukemia and High Risk Myelodysplastic Syndrome With FLT3-ITD Mutation	Phase 2	RAFs, KIT, FLT3, RET, VEGFRs, PDGFRs, CSF1R, DNMT	Texas	NCT02196857
Individualized Treatment for Relapsed/Refractory Acute Leukemia Based on Chemosensitivity and Genomics/Gene Expression Data	N/A	MEK, Others	Washington	NCT02551718

CLINICAL TRIALS TO CONSIDER (cont.)

GENE	RATIONALE FOR POTENTIAL CLINICAL TRIALS
● ***NF1*** L2149fs*20	On the basis of clinical evidence and strong preclinical evidence, NF1 inactivation may predict sensitivity to MEK inhibitors. Limited clinical data and strong preclinical data indicate that loss or inactivation of NF1 may also predict sensitivity to mTOR inhibitors. However, it is not known whether these therapeutic approaches would be relevant in the context of alterations that have not been fully characterized, as seen here. 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 'NF1', 'RAS', 'mTOR', 'PI3K', 'MEK', 'everolimus', 'temsirolimus', 'trametinib', 'cobimetinib', 'AML', and/or 'leukemia'.

TITLE	PHASE	TARGETS	LOCATIONS	NCT ID
A Phase Ib Open-label, Multi-center, Dose Escalation and Expansion Study of Orally Administered MEK162 Plus BYL719 in Adult Patients With Selected Advanced Solid Tumors	Phase 1/Phase 2	MEK, PI3K-alpha	Illinois, Massachusetts, New York	NCT01449058
A Phase 1b Study Evaluating the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of AMG 232 Alone and in Combination With Trametinib in Adult Subjects With Relapsed/Refractory Acute Myeloid Leukemia	Phase 1	MEK, MDM2	Alabama, New York, North Carolina, Utah, Washington	NCT02016729
A Phase II Study of Azacitidine and Sirolimus for the Treatment of High Risk Myelodysplastic Syndrome or Acute Myeloid Leukemia Refractory to or Not Eligible for Intensive Chemotherapy	Phase 2	DNMT, mTOR	Pennsylvania	NCT01869114
A Pilot, Pharmacodynamic Correlate Trial of Sirolimus in Combination With Chemotherapy (Idarubicin, Cytarabine) for the Treatment of Newly Diagnosed Acute Myelogenous Leukemia	N/A	mTOR	Pennsylvania	NCT01822015

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.

ABL1 E197K	***ARID1A*** P158S	***ATR*** R635Q	***FAM123B*** G89V	***HNF1A*** D546A	***MED12*** Q2120_Q2121>HQQQQQ
NSD1 M455T	***PCLO*** A433T	***PRKAR1A*** V348I			

APPENDIX

GENES ASSAYED IN FOUNDATIONONE HEME

FoundationOne Heme is designed to include all 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 405 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.

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*	*APC*	*APH1A*	*AR*	*ARAF*	*APFRP1*	*ARHGAP26*
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*	*CAD*	*CARD11*	*CBFB*	*CBL*	*CCND1*
CCND2	*CCND3*	*CCNE1*	*CCT6B*	*CD22*	*CD274*	*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*	*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*	*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*	*IRF1*	*IRF4*	*IRF8*	*IRS2*	*JAK1*	*JAK2*	*JAK3*	*JARID2*
JUN	*KAT6A*	*KDM2B*	*KDM4C*	*KDM5A*	*KDM5C*	*KDM6A*	*KDR*	*KEAP1*	*KIT*	*KLHL6*	*KMT2A*	*KMT2B*
KMT2C	*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*	*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*	*PDGFRA*	*PDGFRB*	*PDK1*	*PHF6*	*PIK3CA*	*PIK3CG*
PIK3R1	*PIK3R2*	*PIM1*	*PLCG2*	*POT1*	*PPP2R1A*	*PRDM1*	*PRKAR1A*	*PRKDC*	*PRSS8*	*PTCH1*	*PTEN*	*PTPN11*
PTPN2	*PTPN6*	*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*	*TCL1A*	*TET2*	*TGFBR2*	*TLL2*	*TMEM30A*	*TMSB4XP8*	*TNFAIP3*	*TNFRSF11A*
TNFRSF14	*TNFRSF17*	*TOP1*	*TP53*	*TP63*	*TRAF2*	*TRAF3*	*TRAF5*	*TSC1*	*TSC2*	*TSHR*	*TUSC3*	*TYK2*
U2AF1	*U2AF2*	*VHL*	*WDR90*	*WHSC1*	*WISP3*	*WT1*	*XBP1*	*XPO1*	*YY1AP1*	*ZMYM3*	*ZNF217*	*ZNF24*
ZNF703	*ZRSR2*											

DNA Gene List: For the Detection Select Rearrangements

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

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RNA Gene List: For the Detection of Select Gene Fusions

ABI1	*ABL1*	*ABL2*	*ACSL6*	*AFF1*	*AFF4*	*ALK*	*ARHGAP26*	*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	*CDK6*	*CDX2*	*CHIC2*	*CHN1*	*CIC*	*CIITA*	*CLP1*	*CLTC*	*CLTCL1*	*CNTRL*	*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*	*KDSR*	*KIF5B*	*KMT2A*	*LASP1*	*LCP1*	*LMO1*	*LMO2*
LPP	*LYL1*	*MAF*	*MAFB*	*MALT1*	*MDS2*	*MECOM*	*MKL1*	*MLF1*	*MLLT1*	*MLLT10*	*MLLT3*
MLLT4	*MLLT6*	*MN1*	*MNX1*	*MSI2*	*MSN*	*MUC1*	*MYB*	*MYC*	*MYH11*	*MYH9*	*NACA*
NBEAP1	*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*	*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*
RUNX2	*SEC31A*	*SEPT5*	*SEPT6*	*SEPT9*	*SET*	*SH3GL1*	*SLC1A2*	*SNX29*	*SRSF3*	*SS18*	*SSX1*
SSX2	*SSX4*	*STAT6*	*STL*	*SYK*	*TAF15*	*TAL1*	*TAL2*	*TBL1XR1*	*TCF3*	*TCL1A*	*TEC*
TET1	*TFE3*	*TFG*	*TFPT*	*TFRC*	*TLX1*	*TLX3*	*TMPRSS2*	*TNFRSF11A*	*TOP1*	*TP63*	*TPM3*
TPM4	*TRIM24*	*TRIP11*	*TTL*	*TYK2*	*USP6*	*WHSC1*	*WHSC1L1*	*YPEL5*	*ZBTB16*	*ZMYM2*	*ZNF384*
ZNF521											

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FOUNDATIONONE HEME PERFORMANCE SPECIFICATIONS

SENSITIVITY	Base Substitutions at ≥5% Minor Allele Frequency	>99%
	Insertions/Deletions (1-40 base pairs) at ≥10% Minor Allele Frequency	98%
	Focal Copy Number Alterations (homozygous deletions or amplifications ≥8 copies)	>95%
	Known Gene Fusions	>95%
SPECIFICITY	Positive Predictive Value (PPV) for Base Substitutions, Insertions/Deletions, and Focal Copy Number Alterations	>99%
	Positive Predictive Value (PPV) for Known Gene Fusions	>95%
REPRODUCIBILITY	Concordance between replicates inter-batch	97%
	Concordance between replicates intra-batch	97%

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.

[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 or the receipt of only a pre-extracted DNA sample, precluding RNA sequencing.

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APPENDIX

ABOUT FOUNDATIONONE HEME™

FoundationOne Heme™: FoundationOne Heme (the Test) was developed and its performance characteristics determined by Foundation Medicine, Inc. (Foundation Medicine). The Test 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. The Test 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 Heme 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 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.

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 the Test.

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