

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 and sarcomas.

PATIENT
DISEASE Bone osteosarcoma
NAME
DATE OF BIRTH
SEX
MEDICAL RECORD #

PHYSICIAN
ORDERING PHYSICIAN
MEDICAL FACILITY
ADDITIONAL RECIPIENT
MEDICAL FACILITY ID
PATHOLOGIST

SPECIMEN
SPECIMEN SITE
SPECIMEN ID
SPECIMEN TYPE
DATE OF COLLECTION
SPECIMEN RECEIVED

Biomarker Findings

Microsatellite status - Cannot Be Determined^α
Tumor Mutational Burden - 7 Muts/Mb

Genomic Findings

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

- KIT** amplification
- PDGFRA** amplification
- CDK4** amplification
- FGFR1** amplification - equivocal[†]
- MDM2** amplification
- CCND3** amplification
- FRS2** amplification
- KDR** amplification
- RAD21** amplification
- TP53** R337H
- ZNF703** amplification - equivocal[†]

[†] See About the Test in appendix for details.
^α Patients with Microsatellite status of Cannot Be Determined should be re-tested with an orthogonal (alternative) method.

Report Highlights

- Variants with **diagnostic implications** that may indicate a specific cancer type: **CDK4 amplification** (p. 5), **MDM2 amplification** (p. 6)
- Targeted therapies with **NCCN categories of evidence** in this tumor type: **Sorafenib** (p. 12)
- Evidence-matched **clinical trial options** based on this patient's genomic findings: (p. 13)

BIOMARKER FINDINGS

Microsatellite status - Cannot Be Determined

Tumor Mutational Burden - 7 Muts/Mb

THERAPY AND CLINICAL TRIAL IMPLICATIONS

No therapies or clinical trials. see Biomarker Findings section

No therapies or clinical trials. see Biomarker Findings section

GENOMIC FINDINGS	THERAPIES WITH CLINICAL RELEVANCE (IN PATIENT'S TUMOR TYPE)	THERAPIES WITH CLINICAL RELEVANCE (IN OTHER TUMOR TYPE)
KIT - amplification 10 Trials see p. 17	none	Sorafenib <input type="checkbox"/> 2A Imatinib Nilotinib Sunitinib
PDGFRA - amplification 1 Trial see p. 20	none	Imatinib
CDK4 - amplification 10 Trials see p. 13	none	none
FGFR1 - amplification - equivocal 10 Trials see p. 15	none	none
MDM2 - amplification 1 Trial see p. 19	none	none

NCCN category

GENOMIC FINDINGS WITH NO REPORTABLE THERAPEUTIC OR CLINICAL TRIAL OPTIONS

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

CCND3 - amplification.....	p. 6	RAD21 - amplification.....	p. 8
FRS2 - amplification.....	p. 7	TP53 - R337H.....	p. 9
KDR - amplification.....	p. 7	ZNF703 - amplification - equivocal.....	p. 10

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.

ORDERED TEST #

BIOMARKER FINDINGS

BIOMARKER

Microsatellite status

RESULT

Cannot Be Determined

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

On the basis of prospective clinical evidence in multiple solid tumor types, microsatellite instability (MSI) and associated increased tumor mutational burden (TMB)¹⁻² may predict sensitivity to immune checkpoint inhibitors, including the approved PD-1-targeting agents cemiplimab, dostarlimab, nivolumab (alone or in combination with ipilimumab), and pembrolizumab³⁻⁸ and PD-L1-targeting agents atezolizumab, avelumab, and durvalumab⁹⁻¹¹. As the MSI status of this tumor is unknown, the relevance of these therapeutic approaches is

unclear.

FREQUENCY & PROGNOSIS

In osteosarcoma, MSI at any level has been reported in 14% (3/21) to 44% (8/18) of cases with high MSI observed in 11% (2/18) of cases¹²⁻¹³. However, other studies have reported an absence of MSI in osteosarcoma (0/7 and 0/68)¹⁴⁻¹⁵ and bone sarcoma (0/29)¹⁶. The prognostic significance of MSI in osteosarcoma or other bone mesenchymal tumors is unknown (PubMed, Sep 2021).

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 tumor¹⁷. 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 PMS2¹⁷⁻¹⁹. The level of MSI in this sample could not be determined with confidence. Depending on the clinical context, MSI testing of an alternate sample or by another methodology could be considered.

POTENTIAL GERMLINE IMPLICATIONS

While approximately 80% of MSI-H tumors arise due to somatic inactivation of an MMR pathway protein, about 20% arise due to germline mutations in one of the MMR genes¹⁷, which are associated with a condition known as Lynch syndrome (also known as hereditary nonpolyposis colorectal cancer or HNPCC)²⁰. Lynch syndrome leads to an increased risk of colorectal, endometrial, gastric, and other cancers²⁰⁻²² and has an estimated prevalence in the general population ranging from 1:600 to 1:2000²³⁻²⁵. Therefore, in the appropriate clinical context, germline testing of MLH1, MSH2, MSH6, and PMS2 is recommended.

BIOMARKER

Tumor Mutational Burden

RESULT

7 Muts/Mb

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

On the basis of clinical evidence in solid tumors, increased TMB may be associated with greater sensitivity to immunotherapeutic agents, including anti-PD-L1²⁶⁻²⁸, anti-PD-1 therapies²⁶⁻²⁹, and combination nivolumab and ipilimumab³⁰⁻³⁵. In multiple pan-tumor studies, higher TMB has been reported to be associated with increased ORR and OS from treatment with immune checkpoint inhibitors^{26-29,36}. Higher TMB was found to be significantly associated with improved OS upon immune checkpoint inhibitor treatment for patients with 9 types of advanced tumors²⁶. Analyses across several solid tumor types reported that patients with higher TMB (defined as $\geq 16-20$

Muts/Mb) achieved greater clinical benefit from PD-1 or PD-L1-targeting monotherapy, compared with patients with higher TMB treated with chemotherapy³⁷ or those with lower TMB treated with PD-1 or PD-L1-targeting agents²⁷. However, the KEYNOTE 158 trial of pembrolizumab monotherapy for patients with solid tumors found significant improvement in ORR for patients with TMB ≥ 10 Muts/Mb (based on this assay or others) compared to those with TMB < 10 Muts/Mb, in a large cohort that included multiple tumor types; similar findings were observed in the KEYNOTE 028 and 012 trials^{29,36}. Together, these studies suggest that patients with TMB ≥ 10 Muts/Mb may derive clinical benefit from PD-1 or PD-L1 inhibitors.

FREQUENCY & PROGNOSIS

Osteosarcoma harbors a median TMB of 2.5 mutations per megabase (mut/Mb), and 0.4% of cases have high TMB (> 20 muts/Mb)³⁸. Undifferentiated pleomorphic sarcomas reportedly have an increased mutation burden compared to Ewing sarcomas or rhabdomyosarcomas³⁹⁻⁴¹. The association of mutation burden with prognosis of bone sarcoma has not been studied extensively

(PubMed, Sep 2021); however, one study of 31 patients with high-grade osteosarcoma reported a significant association of high TMB and improved median OS by multivariate analysis (HR=0.05, p=0.03)⁴².

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 melanoma⁴³⁻⁴⁴ and cigarette smoke in lung cancer^{7,45}, treatment with temozolomide-based chemotherapy in glioma⁴⁶⁻⁴⁷, mutations in the proofreading domains of DNA polymerases encoded by the POLE and POLD1 genes⁴⁸⁻⁵², and microsatellite instability (MSI)^{48,51-52}. This sample harbors a TMB level associated with lower rates of clinical benefit from treatment with PD-1- or PD-L1-targeting immune checkpoint inhibitors compared with patients with tumors harboring higher TMB levels, based on several studies in multiple solid tumor types^{27-28,36}.

ORDERED TEST #

GENOMIC FINDINGS

GENE
KIT

ALTERATION
amplification

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

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, avapritinib, and ripretinib⁵³⁻⁶⁰. The use of mTOR inhibitors as an alternative therapeutic strategy has demonstrated limited success in KIT-mutated, imatinib-resistant melanoma, with 1 PR and 3 SD observed for 4 patients treated with everolimus⁶¹. However, no responses were observed for 10 patients with mastocytosis following everolimus monotherapy,

with 8/10 patients harboring the KIT D816V mutation⁶². The role of KIT amplification as a biomarker for response to mTOR inhibitors has not been investigated (PubMed, Mar 2021). Clinical benefit has been observed for patients with KIT amplified or overexpressing tumors following treatment with imatinib⁶³⁻⁷³, nilotinib⁷⁴, sorafenib⁷⁵⁻⁷⁸, and sunitinib⁷⁹⁻⁸⁰, suggesting that KIT amplification may be sensitive to these inhibitors. However, evidence demonstrating clinical benefit for regorafenib, dasatinib, pazopanib, or ponatinib in the context of KIT amplified or overexpressing tumors is limited.

FREQUENCY & PROGNOSIS

KIT amplification has been reported in 20% (13/66)⁸¹ to 35% (24/74)⁸² of osteosarcoma cases. Relative to all tumor types analyzed, co-amplification of KIT, KDR, and PDGFRA on the 4q12 locus has been reported to be enriched in osteosarcomas; although primarily bone-derived (6.4%; 27/420), 1 of 15 soft tissue osteosarcoma (extraskelatal) samples also harbored this amplicon⁸³. Studies have observed KIT

amplification and overexpression in pediatric osteosarcomas; KIT expression has been reported to correlate with a poor response to chemotherapy, but not with overall survival or disease-free survival, in pediatric osteosarcoma^{82,84}. KIT amplification and overexpression have also been reported in pediatric patients with osteosarcoma, and KIT expression has been reported to correlate with poor response to chemotherapy, but not with overall survival or disease-free survival^{82,84}.

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 pathways⁸⁵. KIT aberrations, including point mutations, translocations, amplification, and overexpression, have been associated with various malignancies, and KIT is considered an oncoprotein⁸⁶. KIT has been reported to be amplified in cancer⁸⁷ and may be biologically relevant in this context⁸⁸⁻⁸⁹.

GENE
PDGFRA

ALTERATION
amplification

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

On the basis of extensive clinical evidence in solid tumors and hematologic cancers, PDGFRA activating alterations are associated with sensitivity to imatinib⁹⁰⁻¹²⁷. Sorafenib has shown clinical and preclinical activity against the FIP1L1-PDGFR fusion in chronic eosinophilic leukemia (CEL) and mutations associated with clinical resistance to imatinib and sunitinib in both CEL and gastrointestinal stromal tumor (GIST)¹²⁸⁻¹³³. Complete responses to nilotinib have been reported in patients with CEL or hypereosinophilic syndrome with FIP1L1-PDGFR or activating mutations^{106,134-135};

preclinical evidence has reported efficacy of nilotinib in the context of PDGFRA mutations associated with GIST¹³⁶⁻¹³⁷. Patients with GIST harboring PDGFRA activating mutations have been reported to derive clinical benefit from treatment with sunitinib¹³⁸ or regorafenib¹³⁹⁻¹⁴⁰. Preclinical studies have reported sensitivity of activating PDGFRA mutations and FIP1L1-PDGFR fusion to dasatinib^{130,136}. PDGFRA D842 mutations were reported to be sensitive to avapritinib in clinical¹⁵³ and preclinical¹⁵³ studies of GIST, and demonstrated sensitivity to ripretinib for 1 patient¹⁴¹.

FREQUENCY & PROGNOSIS

Relative to all tumor types analyzed, co-amplification of KIT, KDR, and PDGFRA on the 4q12 locus has been reported to be enriched in osteosarcomas; although primarily bone-derived (6.4%; 27/420), 1 of 15 soft tissue osteosarcoma (extraskelatal) samples also harbored this amplicon⁸³. High PDGFRA expression was detected in 42% (40/96) of osteosarcoma samples

in one study; none of the 40 cases with high PDGFRA expression harbored mutation of PDGFRA exons 12 or 18¹⁴². PDGFRA mutation was not detected in any of the 243 osteosarcoma cases analyzed in COSMIC (Mar 2021)¹⁴³. PDGFRA expression has been reported in patients with osteosarcoma but was not correlated with overall or disease-free survival^{142,144-145}.

FINDING SUMMARY

PDGFRA encodes platelet-derived growth factor receptor alpha (PDGFR-alpha), a tyrosine kinase receptor that, upon binding of cognate ligands (PDGFA or PDGFB), activates several signaling pathways, including PI3K and MAPK¹⁴⁶. PDGFR aberrations, including point mutations, translocations, amplification, and/or overexpression, have been associated with various malignancies⁸⁶. Amplification of PDGFRA, frequently occurring with amplification of the genes KDR and KIT, has been associated with increased PDGFRA expression¹⁴⁷⁻¹⁵⁰ and poor prognosis^{147,151-153} in some subtypes of glioma.

ORDERED TEST #

GENOMIC FINDINGS

GENE
CDK4

ALTERATION
amplification

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

CDK4 amplification or activation may predict sensitivity to CDK4/6 inhibitors such as abemaciclib, palbociclib, and ribociclib¹⁵⁴⁻¹⁵⁷. Clinical benefit has been reported for limited tumor types including patients with CDK4-amplified liposarcoma and sarcoma in response to treatment with abemaciclib¹⁵⁸, palbociclib^{154,159}, and ribociclib¹⁶⁰.

FREQUENCY & PROGNOSIS

Amplification of CDK4 has been reported in a subset of osteosarcoma samples¹⁶¹⁻¹⁶⁵. CDK4 amplification has been reported to be relatively rare in conventional high-grade osteosarcoma but frequent in low-grade osteosarcomas, including parosteal osteosarcomas and low-grade central osteosarcomas, suggesting that CDK4 expression may help facilitate subclassification in osteosarcoma¹⁶³⁻¹⁶⁵. Deregulation of the G1/S checkpoint genes, including CDK4, and expression of the cyclin D1/CDK4 complex have been associated with poor prognosis in osteosarcoma patients¹⁶⁶⁻¹⁶⁷.

FINDING SUMMARY

CDK4 encodes the cyclin-dependent kinase 4, which regulates the cell cycle, senescence, and apoptosis¹⁶⁸. CDK4 and its functional homolog CDK6 are activated by D-type cyclins and promote

cell cycle progression by inactivating the tumor suppressor Rb¹⁶⁹⁻¹⁷⁰. Amplification of the chromosomal region that includes CDK4 has been reported in multiple cancer types, including lung cancer, glioblastoma, and liposarcoma, and has been associated with overexpression of CDK4 protein^{154,163,171-176}.

POTENTIAL DIAGNOSTIC IMPLICATIONS

Amplification of 12q13-q15, including CDK4 and MDM2, is characteristic of well-differentiated liposarcoma (WDLPS) and dedifferentiated (DDLPS) liposarcoma (NCCN Soft Tissue Sarcoma Guidelines, v2.2021)¹⁷⁶⁻¹⁷⁹, and low-grade osteosarcomas, including parosteal osteosarcomas and low-grade central osteosarcomas as opposed to conventional high-grade osteosarcoma (NCCN Soft Tissue Sarcoma Guidelines, v2.2021)^{163-165,180-181}.

GENE
FGFR1

ALTERATION
amplification - equivocal

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

Alterations that activate FGFR1 may predict sensitivity to selective FGFR inhibitors including erdafitinib¹⁸²⁻¹⁸⁴, pemigatinib¹⁸⁵, infigratinib¹⁸⁶⁻¹⁸⁷, rogaratinib¹⁸⁸, Debio 1347¹⁸⁹⁻¹⁹⁰, futibatinib¹⁹¹, and derazatinib¹⁹², or multikinase inhibitors such as pazopanib¹⁹³ and ponatinib¹⁹⁴⁻¹⁹⁶. The activity and efficacy of selective FGFR inhibitors for FGFR1-amplified tumors has been modest with limited responses reported in FGFR1-amplified lung squamous cell carcinoma (SCC) treated with infigratinib¹⁹⁷ or AZD457¹⁹⁸ and no responses reported among patients with FGFR1-amplified

breast cancer treated with infigratinib¹⁹⁷. Two case studies reported PRs in patients with FGFR1-amplified breast cancer treated with pazopanib¹⁹³.

— Potential Resistance —

Preclinical studies suggest that overexpression of FGFR1 may be a mechanism of acquired resistance to gefitinib; addition of an FGFR inhibitor restored gefitinib sensitivity in lung cancer cell lines¹⁹⁹⁻²⁰⁰.

FREQUENCY & PROGNOSIS

Putative amplification of FGFR1 has been detected in 4.4% of sarcoma cases, although this dataset only included soft tissue sarcoma (cBioPortal, Jun 2021)^{87,201}. FGFR1 amplification was reported in osteosarcoma cell lines and in 1/17 primary osteosarcoma samples²⁰². In one study, FGFR1 protein expression was observed in 82% (45/55) of phosphaturic mesenchymal tumors (PMTs)

analyzed, and expression was independent of FGFR1 fusion status; positive expression was also observed in a subset of PMT-mimicking tumors (10 samples per tumor type), such as solitary fibrous tumors (40%, 4/10), chondrosarcomas (40%), giant cell tumors of bone (38%), non-ossifying fibromas (20%), osteosarcomas (10%), and osteoblastomas (10%)²⁰³. Published data investigating the prognostic implications of FGFR1 alterations in osteosarcoma are limited (PubMed, Jun 2021).

FINDING SUMMARY

FGFR1 encodes the protein fibroblast growth factor receptor 1, which plays key roles in regulation of the cell cycle and angiogenesis and is an upstream regulator of the RAS, MAPK, and AKT signaling pathways²⁰⁴. Amplification of FGFR1 has been correlated with protein expression²⁰⁵⁻²⁰⁶ and may predict pathway activation and sensitivity to therapies targeting this pathway²⁰⁷⁻²⁰⁸.

ORDERED TEST #

GENOMIC FINDINGS

GENE
MDM2

ALTERATION
amplification

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

MDM2 antagonists disrupt the MDM2-p53 interaction, thereby stabilizing p53²⁰⁹. Preclinical studies have suggested that the amplification of MDM2, in the absence of concurrent TP53 mutations, may increase sensitivity to these agents²¹⁰⁻²¹¹. Preliminary Phase 1 studies of the MDM2-p53 antagonist alrizomadlin (APG-115) reported a PR in a patient with liposarcoma harboring an MDM2 amplification and wildtype for TP53 and SD in 21%-38% (6/28 and 5/13, respectively) of patients in genomically unselected solid tumors²¹²⁻²¹³. A Phase 2 trial of alrizomadlin in combination with pembrolizumab reported a PR in 1 of 3 patients with malignant peripheral nerve sheath tumor that had failed standard therapy, as well as PRs in patients with multiple types of solid tumors that had failed immunotherapy, including 1 out of 14 patients with non-small cell lung cancer; 1 out of 5 patients with urothelial carcinoma; and 2 out of 5, 1 out of 5, and 1 out of 11 patients with mucosal, uveal, and cutaneous melanoma, respectively²¹⁴. Phase 1b studies of the MDM2 inhibitor idasanutlin for

refractory AML in combination with cytarabine or venetoclax reported anti-leukemic response rates of 33% (25/75) and 37% (11/30), respectively²¹⁵⁻²¹⁶; clinical benefit (58% ORR, 7/12) with idasanutlin monotherapy has been reported for patients with polycythemia vera²¹⁷. The dual MDM2/MDM4 inhibitor ALRN-6924 led to an ORR of 27% (4/15) for patients with TP53 wildtype peripheral T-cell lymphoma in a Phase 2 study²¹⁸; responses have also been observed in TP53 wildtype AML, MDS, Merkel cell carcinoma, colorectal cancer, and liposarcoma²¹⁹⁻²²⁰.

FREQUENCY & PROGNOSIS

Amplification of MDM2 has been reported in 19-80% of osteosarcomas^{180-181,221}. MDM2 amplification has been reported to be relatively rare in conventional high-grade osteosarcoma but frequent in low-grade osteosarcomas, including parosteal osteosarcomas and low-grade central osteosarcomas, suggesting that MDM2 expression may help facilitate subclassification in osteosarcoma¹⁶³⁻¹⁶⁵. Deregulation of the G1/S checkpoint genes, including amplification of CDK4 and MDM2, has been found to be correlated with decreased survival and poor prognosis in osteosarcoma patients¹⁶⁶.

FINDING SUMMARY

MDM2 encodes an E3 ubiquitin protein ligase, which mediates the ubiquitination and subsequent degradation of p53, Rb1, and other proteins²²²⁻²²⁴.

MDM2 acts to prevent the activity of the tumor suppressor p53; therefore, overexpression or amplification of MDM2 may be oncogenic²²⁵⁻²²⁶. Overexpression or amplification of MDM2 is frequent in cancer⁸⁹. Although two retrospective clinical studies suggest that MDM2 amplification may predict a short time-to-treatment failure on anti-PD-1/PD-L1 immune checkpoint inhibitors, with 4/5 patients with MDM2 amplification²²⁷ and 2/3 patients with MDM2 or MDM4 amplification²²⁸ experiencing tumor hyperprogression, amplification of MDM2 or MDM4 was not associated with shorter progression-free survival (PFS) in a retrospective analysis of non-small cell lung cancer (NSCLC) outcomes with immune checkpoint inhibitors (hazard ratio of 1.4, p=0.44)²²⁹. The latter study reported PFS of >2 months for 5/8 patients with MDM2/MDM4 amplification²²⁹.

POTENTIAL DIAGNOSTIC IMPLICATIONS

Amplification of 12q13-q15, including CDK4 and MDM2, is characteristic of well-differentiated liposarcoma (WDLPS) and dedifferentiated (DDLPS) liposarcoma (NCCN Soft Tissue Sarcoma Guidelines, v2.2021)¹⁷⁶⁻¹⁷⁹, and low-grade osteosarcomas, including parosteal osteosarcomas and low-grade central osteosarcomas as opposed to conventional high-grade osteosarcoma (NCCN Soft Tissue Sarcoma Guidelines, v2.2021)^{163-165,180-181}.

GENE
CCND3

ALTERATION
amplification

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

Amplification or activation of CCND3 may predict sensitivity to CDK4/6 inhibitors²³⁰⁻²³², such as abemaciclib, palbociclib, and ribociclib^{154-157,233-234}. As supported by strong preclinical studies, tumors

with CCND3 activation depend on CDK4/6²³⁰⁻²³².

FREQUENCY & PROGNOSIS

One study reported frequent high-level amplification of CCND3 in 83% (5/6) of aggressive osteosarcomas studied²³⁵. Another study reports consistent overexpression of CCND3 in all 13 osteosarcoma patient samples analyzed and gene amplification in 2 out of 4 samples, although overexpression was not correlated with increased cyclin D3 protein levels²³⁶. Increased expression of Cyclin D3 has been reported in bone and soft tissue sarcomas compared with leiomyomas and normal tissue, suggesting a role for this protein in

bone and soft tissue tumors²³⁷. The prognostic significance of CCND3 alteration in osteosarcoma have not been extensively studied (PubMed, Sep 2021).

FINDING SUMMARY

CCND3 encodes cyclin D3, a G1/S-specific cell cycle regulator. Cyclin D3 interacts with and regulates the cyclin-dependent kinases CDK4 and CDK6, resulting in the phosphorylation and inactivation of Rb and the progression of the cell cycle²³⁸. CCND3 amplification has been associated with increased cyclin D3 expression²³⁹.

ORDERED TEST #

GENOMIC FINDINGS

GENE
FRS2

ALTERATION
amplification

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

There are no approved therapies that target alterations in FRS2. Amplification of FRS2 can lead to activation of the FGFR and MAPK-ERK pathways, and preliminary studies in liposarcoma cell lines have shown that cells with this alteration are sensitive to FGFR inhibitors^{175,240}.

FREQUENCY & PROGNOSIS

FRS2 amplification is particularly prevalent in liposarcoma, where amplification of the 12q13-15 region of chromosome 12 is considered to be a hallmark genetic alteration, although the effects of amplification of CDK4 and MDM2, also located in this region, have been studied in more detail than FRS2 in this context²⁴¹⁻²⁴². Amplification of FRS2 has been observed in 93%-100% of dedifferentiated liposarcoma, 32% of undifferentiated high-grade pleomorphic sarcoma, and 100% of well-differentiated liposarcoma^{175,243}. Amplification of the 12p15 chromosomal region containing FRS2, but not CDK4 or MDM2, was found in 12.5% of high-grade serous ovarian carcinomas, and knockdown of FRS2 in these cells resulted in apoptosis, indicating that cells with

12p15 amplification require FRS2²⁴⁰.

FINDING SUMMARY

FRS2 encodes the fibroblast growth factor receptor (FGFR) substrate 2, an adaptor protein involved in FGFR signaling, which may also mediate signaling through EGFR, NTRK, and VEGF receptors²⁴⁴⁻²⁴⁷. FRS2 amplification was found to correlate with overexpression in high-grade serous ovarian tumors, and FRS2 overexpression promoted tumorigenesis in a preclinical study²⁴⁰.

GENE
KDR

ALTERATION
amplification

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

On the basis of clinical benefit for patients with ccRCC²⁴⁸⁻²⁵² and a patient with breast angiosarcoma²⁵³, high VEGFR-2 expression has been associated with sensitivity to sunitinib. However, because data supporting concordance between VEGFR-2 expression and KDR genomic biomarkers are limited, it is unclear whether these therapeutic strategies would be beneficial in this case. On the basis of extensive clinical evidence across multiple tumor types, expression of plasma or tumor VEGFR-1 or VEGFR-2 has not been

established as a reliable biomarker to predict response to the VEGFA-targeted agent bevacizumab²⁵⁴⁻²⁷³.

FREQUENCY & PROGNOSIS

Relative to all tumor types analyzed, co-amplification of KIT, KDR, and PDGFRA on the 4q12 locus has been reported to be enriched in osteosarcomas; although primarily bone-derived (6.4%; 27/420), 1 of 15 soft tissue osteosarcoma (extraskeletal) samples also harbored this amplicon⁸³. KDR expression, mutation and amplification have been reported in various subtypes of sarcoma, most commonly in undifferentiated pleomorphic sarcoma of bone and angiosarcoma²⁷⁴. The Sarcoma Genome Project has reported KDR amplification in 2% of sarcoma samples¹⁷⁹. In the scientific literature, KDR mRNA expression has been reported in 67% of osteosarcoma specimens analyzed²⁷⁵. Another study reports frequent gain of KDR in

pleomorphic sarcoma of bone¹⁴⁵. The prognostic significance of KDR amplification in osteosarcoma has not been extensively studied (PubMed, Feb 2021). One retrospective IHC analysis of 30 pediatric osteosarcoma samples reported significantly poorer metastasis-free survival for patients with high versus low KDR protein expression²⁷⁶.

FINDING SUMMARY

KDR encodes vascular endothelial growth factor receptor 2 (VEGFR2), a member of the vascular endothelial growth factor receptor (VEGFR) family. It is a receptor tyrosine kinase that transmits signals from VEGFA and is involved in both tumor angiogenesis and vasculogenesis during development²⁷⁷. KDR amplification has been reported in many tumor types and may be oncogenic²⁷⁷.

ORDERED TEST #

GENOMIC FINDINGS

GENE
RAD21

ALTERATION
amplification

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

There are no therapies to target alterations in this gene.

FREQUENCY & PROGNOSIS

RAD21 amplifications, point mutations, and truncating mutations have been reported in various cancers²⁷⁸. In the context of breast cancer, increased RAD21 expression has been correlated with poor prognosis in multiple subtypes²⁷⁹⁻²⁸⁰, including sporadic Grade 3 but not Grade 1 cancers²⁷⁹, as well as hereditary BRCA2-mutant

and hereditary BRCA-wild-type but not hereditary BRCA1-mutant cancers²⁷⁹. Furthermore, SNPs in or near RAD21 have been linked with risk of breast cancer development²⁸¹⁻²⁸². RAD21 overexpression has also been correlated with poor prognosis in endometrial cancer²⁸³ and in colorectal cancer (CRC), especially in KRAS-mutant CRC²⁸⁴. Heterogeneity of RAD21 expression also correlated with aggressive tumor behavior and shorter survival in endometrial cancer²⁸⁵. RAD21 amplification has been more frequently reported in hormone-refractory than in treatment-naïve prostate cancer, but RAD21 amplification did not correlate with expression²⁸⁶. In the context of ovarian cancer, both RAD21 overexpression and downregulation have been observed, but RAD21 expression was not prognostic²⁸⁷. Downregulation of RAD21 expression resulted in sensitization of cultured breast^{280,288} and CRC²⁸⁴ cells to chemotherapy, thereby suggesting that RAD21 overexpression confers resistance to chemotherapy.

FINDING SUMMARY

RAD21 encodes a protein involved in DNA double-strand break repair and sister chromatid cohesion as a part of the cohesin complex²⁸⁹⁻²⁹². In preclinical studies, downregulation of RAD21 or other cohesin components leads to loss of expression from amplified genes, as well as amplifications themselves upon cell passaging²⁹³, but also leads to an increase in deletions, insertions, and other rearrangements²⁹⁴. High RAD21 expression has also been associated with increased genomic instability²⁷⁹. Cohesin complex also organizes chromatin domains and regulates gene expression²⁹⁵⁻²⁹⁶. Both overexpression and reduction of expression of RAD21 has been reported to alter gene expression²⁹⁷. RAD21 amplification has been correlated with increased expression in breast^{279-280,298} and endometrial²⁸³ cancers. Other RAD21 alterations, including truncating and point mutations, have been reported in the context of cancer, but the majority have not been characterized.

SAMPLE

ORDERED TEST #

GENOMIC FINDINGS

GENE
TP53

ALTERATION
R337H

TRANSCRIPT ID
NM_000546

CODING SEQUENCE EFFECT
1010G>A

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

There are no approved therapies to address TP53 mutation or loss. However, tumors with TP53 loss of function alterations may be sensitive to the WEE1 inhibitor adavosertib²⁹⁹⁻³⁰², or p53 gene therapy and immunotherapeutics such as SGT-53³⁰³⁻³⁰⁷ and ALT-801³⁰⁸. In a Phase 1 study, adavosertib in combination with gemcitabine, cisplatin, or carboplatin elicited PRs in 9.7% (17/176) and SDs in 53.4% (94/176) of patients with solid tumors; the response rate was 21.1% (4/19) for patients with TP53 mutations versus 12.1% (4/33) for patients who were TP53 wild-type³⁰⁹. A Phase 2 trial of adavosertib in combination with chemotherapy (gemcitabine, carboplatin, paclitaxel, or doxorubicin) reported a 31.9% (30/94, 3 CR) ORR and a 73.4% (69/94) DCR for patients with platinum-refractory TP53-mutated ovarian, Fallopian tube, or peritoneal cancer³¹⁰. A smaller Phase 2 trial of adavosertib in combination with carboplatin achieved a 42.9% (9/21, 1 CR) ORR and a 76.2% (16/21) DCR for patients with platinum-refractory TP53-mutated ovarian cancer³¹¹. The combination of adavosertib with paclitaxel and carboplatin for patients with TP53-mutated ovarian cancer also significantly increased PFS compared with paclitaxel and carboplatin alone³¹². In the Phase 2 VIKTORY trial, patients with TP53-mutated metastatic and/or recurrent gastric cancer experienced a 24.0% (6/25) ORR with adavosertib combined with paclitaxel³¹³. A Phase 1 trial of neoadjuvant adavosertib in combination with cisplatin and

docetaxel for head and neck squamous cell carcinoma (HNSCC) elicited a 71.4% (5/7) response rate for patients with TP53 alterations³¹⁴. In a Phase 1b clinical trial of SGT-53 in combination with docetaxel for patients with solid tumors, 75.0% (9/12) of evaluable patients experienced clinical benefit, including 2 confirmed and 1 unconfirmed PRs and 2 instances of SD with significant tumor shrinkage³⁰⁷. Additionally, the combination of a CHK1 inhibitor and irinotecan reportedly reduced tumor growth and prolonged survival in a TP53-mutated, but not TP53-wild-type, breast cancer xenotransplant mouse model³¹⁵. Missense mutations leading to TP53 inactivation may also be sensitive to therapies that reactivate mutated p53 such as APR-246³¹⁶⁻³¹⁸. In a Phase 1b trial for patients with p53-positive high-grade serous ovarian cancer, APR-246 combined with carboplatin and pegylated liposomal doxorubicin achieved a 52% (11/21) response rate and 100% DCR³¹⁹. ATR inhibitor treatment of chronic lymphocytic leukemia (CLL) cells with biallelic inactivation of TP53 suppressed cell viability, promoted DNA damage, and attenuated xenograft growth in preclinical studies³²⁰⁻³²¹; however, ATR inhibitors as monotherapy had little effect on these parameters in solid tumor models in other preclinical studies³²²⁻³²³. Therefore, it is unclear whether TP53 inactivation predicts sensitivity to ATR inhibition.

FREQUENCY & PROGNOSIS

Mutations of TP53 have been reported in 16% of bone tumors, including 25-50% of osteosarcomas (COSMIC, Sep 2021)^{143,324-325}. TP53 rearrangements affecting the 5' UTR have been identified as recurrent alterations in 11-55% of osteosarcomas (n = 11-215) and have been reported in the context of Li-Fraumeni syndrome³²⁶⁻³³⁰. Both TP53 alterations and increased expression of p53 have been reported to correlate with worse survival of patients with osteosarcoma^{324,331}.

FINDING SUMMARY

Functional loss of the tumor suppressor p53, which is encoded by the TP53 gene, is common in

aggressive advanced cancers²²⁵. Alterations such as seen here may disrupt TP53 function or expression³³²⁻³³⁶.

POTENTIAL GERMLINE IMPLICATIONS

One or more of the TP53 variants observed here has been described in the ClinVar database as a likely pathogenic or pathogenic germline mutation (by an expert panel or multiple submitters) associated with Li-Fraumeni syndrome (ClinVar, Sep 2021)³³⁷. Follow-up germline testing would be needed to distinguish whether the finding in this patient is somatic or germline. Germline mutations in TP53 are associated with the very rare autosomal dominant disorder Li-Fraumeni syndrome and the early onset of many cancers³³⁸⁻³⁴⁰, including sarcomas³⁴¹⁻³⁴². Estimates for the prevalence of germline TP53 mutations in the general population range from 1:5,000³⁴³ to 1:20,000³⁴². For pathogenic TP53 mutations identified during tumor sequencing, the rate of germline mutations was 1% in the overall population and 6% in tumors arising before age 30³⁴⁴. In the appropriate clinical context, germline testing of TP53 is recommended.

POTENTIAL CLONAL HEMATOPOIESIS IMPLICATIONS

Variants seen in this gene have been reported to occur in clonal hematopoiesis (CH), an age-related process in which hematopoietic stem cells acquire somatic mutations that allow for clonal expansion³⁴⁵⁻³⁵⁰. CH in this gene has been associated with increased mortality, risk of coronary heart disease, risk of ischemic stroke, and risk of secondary hematologic malignancy³⁴⁵⁻³⁴⁶. Clinical management of patients with CH in this gene may include monitoring for hematologic changes and reduction of controllable risk factors for cardiovascular disease³⁵¹. Comprehensive genomic profiling of solid tumors detects nontumor alterations that are due to CH^{349,352-353}. Patient-matched peripheral blood mononuclear cell sequencing is required to conclusively determine if this alteration is present in tumor or is secondary to CH.

ORDERED TEST #

GENOMIC FINDINGS

GENE
ZNF703

ALTERATION
amplification - equivocal

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

There are no available targeted therapies to directly address ZNF703 alterations in cancer. One preclinical study suggested that ZNF703 expression in breast cancer cell lines is associated with reduced sensitivity to tamoxifen through

AKT-mTOR activation³⁵⁴, although these findings have not been verified in the clinical setting.

FREQUENCY & PROGNOSIS

Amplification and high expression of ZNF703 has been observed in luminal B breast tumors, a subtype associated with aggressive disease progression and poor patient outcomes³⁵⁵⁻³⁵⁷. ZNF703 expression has also been linked with aggressive tumor characteristics in patients with gastric and colorectal cancers³⁵⁸⁻³⁵⁹. Putative high-level amplification of ZNF703 has been reported with the highest frequency in breast carcinoma, bladder urothelial carcinoma, uterine carcinosarcoma, lung squamous cell carcinoma

(SCC), esophageal carcinoma and head and neck SCC (5-13% of samples)(cBioPortal, 2021)^{87,201}.

FINDING SUMMARY

ZNF703 encodes a transcriptional repressor that plays roles in stem cell proliferation, cell cycle progression, and other key cellular functions^{356,360}. Amplification of ZNF703 has been correlated with protein expression³⁵⁵⁻³⁵⁶. ZNF703 was established as a breast cancer oncoprotein by studies showing that ZNF703 expression resulted in transformation and increased proliferation of cultured cells^{355-356,361}, as well as increased lung metastases in a breast cancer xenograft model³⁶¹.



ORDERED TEST #

THERAPIES WITH CLINICAL BENEFIT

IN OTHER TUMOR TYPE

Imatinib

Assay findings association

KIT
amplification

PDGFRA
amplification

AREAS OF THERAPEUTIC USE

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. Please see the drug label for full prescribing information.

GENE ASSOCIATION

On the basis of clinical and preclinical data in KIT-

mutated^{64-65,102,362}, KIT-amplified⁶³⁻⁶⁶, or KIT-expressing tumors^{68-73,363-364}, KIT activating alterations may confer sensitivity to imatinib. PDGFRA amplification may predict sensitivity to tyrosine kinase inhibitors such as imatinib; a patient with Merkel cell carcinoma expressing PDGFRA achieved a complete response to imatinib¹⁰⁰.

SUPPORTING DATA

A Phase 2 study of imatinib for children with refractory or relapsed solid tumors did not report any objective responses for the included osteosarcoma cases³⁶⁵. Genomically unselected patients with osteosarcoma experienced a 4-month progression-free survival rate of 18% (3/17) on imatinib³⁶⁶.

Nilotinib

Assay findings association

KIT
amplification

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. Please see the drug label for full prescribing information.

GENE ASSOCIATION

On the basis of clinical and preclinical data in KIT-mutated^{74,367-370}, KIT-amplified⁷⁴, or KIT-expressing tumors³⁷¹, KIT activating alterations may confer sensitivity to nilotinib.

SUPPORTING DATA

Clinical data on the efficacy of nilotinib for the treatment of bone sarcoma are limited (PubMed, Jun 2021). Nilotinib

has been primarily investigated as a therapeutic option for the treatment of CML or gastrointestinal stromal tumors (GIST). In the context of CML, a Phase 3 clinical trial of Ph+ patients treated with imatinib or nilotinib (300 mg or 400 mg) reported progression-free survival (PFS) rates of 93% and 97-98% and overall survival (OS) rates of 93% and 94-97%, respectively, at 4 years³⁷². For imatinib-resistant Japanese patients with CML, a Phase 2 trial reported a 47.8% major medical response rate to treatment with nilotinib at 12 months³⁷³. A Phase 3 clinical trial of single-agent nilotinib in 240 patients with advanced GIST who failed prior treatment with imatinib or sunitinib reported no significant difference in progression-free survival between nilotinib and the best supportive care, but did report increased overall survival for nilotinib-treated patients³⁷⁴. A Phase 2 trial has shown that nilotinib was well tolerated and suggested it may be particularly useful for treating patients with GIST harboring mutations in KIT exon 17³⁷⁵. Preclinical, cell-based assays have reported efficacy for nilotinib alone and in combination with additional therapies in the context of leiomyosarcoma and synovial sarcoma³⁷⁶.

ORDERED TEST #

THERAPIES WITH CLINICAL BENEFIT

IN OTHER TUMOR TYPE

Sorafenib

Assay findings association

KIT
amplification

AREAS OF THERAPEUTIC USE

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. Please see the drug label for full prescribing information.

GENE ASSOCIATION

On the basis of clinical and preclinical data in KIT-mutated³⁷⁷⁻³⁸⁴ or KIT-expressing tumors⁷⁵⁻⁷⁸, KIT activating alterations may predict sensitivity to sorafenib.

SUPPORTING DATA

A retrospective study of osteosarcoma reported 3 SD in 4 patients treated with sorafenib³⁸⁵. Phase 2 studies in non-small cell lung cancer (NSCLC) report that single-agent sorafenib improved disease control rates and that the addition of sorafenib to erlotinib increased survival in EGFR wild-type patients³⁸⁶⁻³⁸⁷. A Phase 1 trial of everolimus combined with sorafenib reported 11% partial

responses and 77% stable disease³⁸⁸. In the context of small cell lung carcinoma, sorafenib combined with cisplatin plus etoposide was highly toxic and ineffective³⁸⁹. In HER2-negative breast cancer, Phase 2b trials found improved progression-free survival for sorafenib added to capecitabine, but not when added to paclitaxel³⁹⁰⁻³⁹¹. Phase 2 studies of sorafenib in biliary tract cancer reported disease control rates of 33-39%³⁹². Three patients with cholangiocarcinoma derived clinical benefit from sorafenib³⁹³⁻³⁹⁴. However, the addition of sorafenib to gemcitabine did not improve outcome in patients with biliary tract tumors compared with gemcitabine alone³⁹⁵. A Phase 2 study of sorafenib and bicalutamide in castration-resistant prostate cancer (CRPC) observed a PSA response or stable disease (>6 months) in 47% (18/39) of patients³⁹⁶. Single-agent sorafenib was moderately active as second-line treatment for CRPC (3.7 months PFS and 18.0 months OS)³⁹⁷. For the treatment of glioblastoma or high-grade gliomas, sorafenib alone or combined with temozolomide/radiotherapy or erlotinib did not show efficacy³⁹⁸⁻⁴⁰¹.

Sunitinib

Assay findings association

KIT
amplification

AREAS OF THERAPEUTIC USE

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. Please see the drug label for full prescribing information.

GENE ASSOCIATION

On the basis of clinical and preclinical data in KIT-mutated^{79,402-406} or KIT-expressing tumors⁷⁹⁻⁸⁰, KIT activating alterations may predict sensitivity to sunitinib.

SUPPORTING DATA

A retrospective study of osteosarcoma reported 1 partial response and 1 stable disease in 5 patients treated with sunitinib³⁸⁵ and a patient with breast osteosarcoma did not report clinical benefit from sunitinib⁴⁰⁷. A preclinical study of osteosarcoma reported a significant reduction of tumor growth and vasculature in response to sunitinib⁴⁰⁸.

Phase 1 and 2 studies of sunitinib, alone or in combination with capecitabine, reported partial responses in patients with cancer of the biliary tract (6 responses), pancreas (3 responses), breast, thyroid, neuroendocrine, bladder, and large intestine (1 response each)⁴⁰⁹⁻⁴¹⁰. Partial responses have also been observed in patients with melanoma (3/36)⁴¹¹ and were more frequent in melanomas with KIT mutation (3/4) than in melanomas with KIT amplification (1/6)⁷⁹. Sunitinib has been evaluated as maintenance therapy for non-small cell lung cancer (NSCLC)⁴¹². A Phase 3 study in NSCLC reported that sunitinib plus erlotinib was associated with better response rate and progression-free survival, as compared with erlotinib alone⁴¹³. Sunitinib has shown preliminary activity against soft tissue sarcomas⁴¹⁴⁻⁴¹⁵ and sunitinib-involving regimens have provided significant benefit to at least 5 patients with angiosarcoma, including one complete response^{253,416-419}. In a Phase 2 study of patients with cervical carcinoma, no objective response to sunitinib was observed in 19 patients, but 84% (16/19) patients had stable disease⁴²⁰. In several Phase 2 trials of patients with glioblastoma, sunitinib did not achieve objective responses or improve clinical outcome⁴²¹⁻⁴²⁴.

NOTE Genomic alterations detected may be associated with activity of certain FDA approved drugs, however, the agents listed in this report may have varied evidence in the patient's tumor type.

CLINICAL TRIALS

ORDERED TEST #

NOTE 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](https://www.foundationmedicine.com/genomic-testing#support-services). Or visit <https://www.foundationmedicine.com/genomic-testing#support-services>.

GENE
CDK4

RATIONALE
CDK4 amplification may predict sensitivity to

CDK4/6 inhibitors.

ALTERATION
amplification

NCT04801966

Safety and Oversight of the Individually Tailored Treatment Approach: A Novel Pilot Study

LOCATIONS: Melbourne (Australia)

PHASE NULL

TARGETS
CDK4, CDK6, PI3K-alpha, PD-L1, MEK, PARP, PD-1, BRAF

NCT02693535

TAPUR: Testing the Use of Food and Drug Administration (FDA) Approved Drugs That Target a Specific Abnormality in a Tumor Gene in People With Advanced Stage Cancer

LOCATIONS: Florida, North Carolina, Georgia, Virginia, Alabama, Pennsylvania, New Hampshire, Maine

PHASE 2

TARGETS
VEGFRs, ABL, SRC, ALK, AXL, MET, ROS1, TRKA, TRKC, CDK4, CDK6, CSF1R, FLT3, KIT, RET, mTOR, EGFR, ERBB2, ERBB3, MEK, BRAF, SMO, DDR2, PARP, PD-1, CTLA-4, ERBB4

NCT03994796

Genetic Testing in Guiding Treatment for Patients With Brain Metastases

LOCATIONS: Florida, North Carolina

PHASE 2

TARGETS
ALK, ROS1, TRKA, TRKB, TRKC, CDK4, CDK6, PI3K, mTOR

NCT03310879

Study of the CDK4/6 Inhibitor Abemaciclib in Solid Tumors Harboring Genetic Alterations in Genes Encoding D-type Cyclins or Amplification of CDK4 or CDK6

LOCATIONS: Massachusetts

PHASE 2

TARGETS
CDK4, CDK6

CLINICAL TRIALS

ORDERED TEST #

NCT02896335	PHASE 2
Palbociclib In Progressive Brain Metastases	TARGETS CDK4, CDK6
LOCATIONS: Massachusetts	
NCT03242382	PHASE 2
Phase II Multicenter Trial of Palbociclib in Second Line of Advanced Sarcomas With CDK4 Overexpression.	TARGETS CDK4, CDK6
LOCATIONS: Sevilla (Spain)	
NCT03297606	PHASE 2
Canadian Profiling and Targeted Agent Utilization Trial (CAPTUR)	TARGETS VEGFRs, ABL, SRC, ALK, AXL, MET, ROS1, TRKA, TRKC, DDR2, KIT, EGFR, PD-1, CTLA-4, PARP, CDK4, CDK6, CSF1R, FLT3, RET, mTOR, ERBB2, ERBB3, MEK, BRAF, SMO
LOCATIONS: Kingston (Canada), Montreal (Canada), Toronto (Canada), London (Canada), Ottawa (Canada), Regina (Canada), Saskatoon (Canada), Edmonton (Canada), Vancouver (Canada)	
NCT04040205	PHASE 2
Abemaciclib for Treatment of Advanced Bone and Soft Tissue Sarcoma Identified as Having CDK Pathway Alteration	TARGETS CDK4, CDK6
LOCATIONS: Missouri, Wisconsin, Iowa	
NCT04594005	PHASE 1/2
CDK4/6 Tumor, Abemaciclib, Paclitaxel	TARGETS CDK4, CDK6
LOCATIONS: Seoul (Korea, Republic of)	
NCT03239015	PHASE 2
Efficacy and Safety of Targeted Precision Therapy in Refractory Tumor With Druggable Molecular Event	TARGETS EGFR, ERBB2, ERBB4, PARP, mTOR, MET, RET, ROS1, VEGFRs, BRAF, CDK4, CDK6
LOCATIONS: Shanghai (China)	

ORDERED TEST #

CLINICAL TRIALS

GENE	RATIONALE	
FGFR1	FGFR inhibitors may be relevant in tumors with alterations that activate FGFR1.	
ALTERATION amplification - equivocal		
NCT02549937		PHASE 1/2
A Multi-Center, Open-Label Study of Sulfatinib(HMPL-012) in Patients With Advanced Solid Tumors		TARGETS CSF1R, FGFR1, VEGFRs
LOCATIONS: Florida, Virginia, New York, Tennessee, Texas, Wisconsin, Colorado, Milano (Italy), California		
NCT04042116		PHASE 1/2
A Study to Evaluate Lucitanib in Combination With Nivolumab in Patients With a Solid Tumor		TARGETS FGFRs, VEGFRs, PD-1
LOCATIONS: Florida, North Carolina, New York, Massachusetts, Tennessee, Pennsylvania, Ohio, Cordoba (Spain), Madrid (Spain), Oklahoma		
NCT04565275		PHASE 1/2
A Study of ICP-192 in Patients With Advanced Solid Tumors		TARGETS FGFR1, FGFR2, FGFR3, FGFR4
LOCATIONS: Florida, Minnesota, Arizona, Colorado		
NCT04784247		PHASE 2
Lenvatinib and Pembrolizumab in People With Advanced Soft Tissue Sarcoma		TARGETS FGFRs, KIT, PD-1, PDGFRA, RET, VEGFRs
LOCATIONS: New Jersey, New York		
NCT04729348		PHASE 2
Pembrolizumab And Lenvatinib In Leptomeningeal Metastases		TARGETS FGFRs, KIT, PD-1, PDGFRA, RET, VEGFRs
LOCATIONS: Massachusetts		
NCT02272998		PHASE 2
Ponatinib for Patients Whose Advanced Solid Tumor Cancer Has Activating Mutations Involving the Following Genes: FGFR1, FGFR2, FGFR3, FGFR4, RET, KIT.		TARGETS ABL, FGFRs, FLT3, KIT, RET, VEGFRs
LOCATIONS: Ohio		

ORDERED TEST #

CLINICAL TRIALS

<p>NCT04169672</p> <p>Study of Surufatinib Combined With Toripalimab in Patients With Advanced Solid Tumors</p> <p>LOCATIONS: Beijing (China), Shanghai (China)</p>	<p>PHASE 2</p> <p>TARGETS CSF1R, FGFR1, VEGFRs, PD-1</p>
<p>NCT04803318</p> <p>Trametinib Combined With Everolimus and Lenvatinib for Recurrent/Refractory Advanced Solid Tumors</p> <p>LOCATIONS: Guangzhou (China)</p>	<p>PHASE 2</p> <p>TARGETS mTOR, FGFRs, KIT, PDGFRA, RET, VEGFRs, MEK</p>
<p>NCT03564691</p> <p>Study of MK-4830 as Monotherapy and in Combination With Pembrolizumab (MK-3475) in Participants With Advanced Solid Tumors (MK-4830-001)</p> <p>LOCATIONS: Cape Town (South Africa), Johannesburg (South Africa), Durban (South Africa), New York, New Jersey, Ohio, Texas, Hamilton (Canada), Toronto (Canada), Ottawa (Canada)</p>	<p>PHASE 1</p> <p>TARGETS ITL4, FGFRs, KIT, PDGFRA, RET, VEGFRs, PD-1</p>
<p>NCT02856425</p> <p>Trial Of Pembrolizumab And Nintedanib</p> <p>LOCATIONS: Villejuif (France)</p>	<p>PHASE 1</p> <p>TARGETS FGFR1, FGFR2, FGFR3, FLT3, LCK, LYN, SRC, VEGFRs, PD-1</p>

ORDERED TEST #

CLINICAL TRIALS

GENE
KIT

ALTERATION
amplification

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 pathway inhibitors may be relevant in a tumor with KIT activation.

NCT03277924

PHASE 1/2

Trial of Sunitinib Plus Nivolumab After Standard Treatment in Advanced Soft Tissue and Bone Sarcomas

TARGETS
PD-1, CSF1R, FLT3, KIT, RET, VEGFRs

LOCATIONS: La Laguna (Spain), Sevilla (Spain), Madrid (Spain), Valencia (Spain), Zaragoza (Spain), Barcelona (Spain), Candiolo (Italy), Milano (Italy)

NCT04449549

PHASE 2

Rapid Analysis and Response Evaluation of Combination Anti-Neoplastic Agents in Rare Tumors (RARE CANCER) Trial: RARE 1 Nilotinib and Paclitaxel

TARGETS
ABL, KIT

LOCATIONS: Maryland

NCT03711058

PHASE 1/2

Study of PI3Kinase Inhibition (Copanlisib) and Anti-PD-1 Antibody Nivolumab in Relapsed/Refractory Solid Tumors With Expansions in Mismatch-repair Proficient (MSS) Colorectal Cancer

TARGETS
PD-1, PI3K

LOCATIONS: Maryland

NCT04784247

PHASE 2

Lenvatinib and Pembrolizumab in People With Advanced Soft Tissue Sarcoma

TARGETS
FGFRs, KIT, PD-1, PDGFRA, RET, VEGFRs

LOCATIONS: New Jersey, New York

NCT04729348

PHASE 2

Pembrolizumab And Lenvatinib In Leptomeningeal Metastases

TARGETS
FGFRs, KIT, PD-1, PDGFRA, RET, VEGFRs

LOCATIONS: Massachusetts

ORDERED TEST #

CLINICAL TRIALS

NCT03297606

PHASE 2

Canadian Profiling and Targeted Agent Utilization Trial (CAPTUR)

TARGETS
VEGFRs, ABL, SRC, ALK, AXL, MET, ROS1, TRKA, TRKC, DDR2, KIT, EGFR, PD-1, CTLA-4, PARP, CDK4, CDK6, CSF1R, FLT3, RET, mTOR, ERBB2, ERBB3, MEK, BRAF, SMO

LOCATIONS: Kingston (Canada), Montreal (Canada), Toronto (Canada), London (Canada), Ottawa (Canada), Regina (Canada), Saskatoon (Canada), Edmonton (Canada), Vancouver (Canada)

NCT04803318

PHASE 2

Trametinib Combined With Everolimus and Lenvatinib for Recurrent/Refractory Advanced Solid Tumors

TARGETS
mTOR, FGFRs, KIT, PDGFRA, RET, VEGFRs, MEK

LOCATIONS: Guangzhou (China)

NCT02461849

PHASE 2

Patients With Refractory, Metastatic Cancer Harboring KIT Mutation or Amplification to Investigate the Clinical Efficacy and Safety of Imatinib Therapy

TARGETS
KIT, ABL

LOCATIONS: Seoul (Korea, Republic of)

NCT03564691

PHASE 1

Study of MK-4830 as Monotherapy and in Combination With Pembrolizumab (MK-3475) in Participants With Advanced Solid Tumors (MK-4830-001)

TARGETS
ITL4, FGFRs, KIT, PDGFRA, RET, VEGFRs, PD-1

LOCATIONS: Cape Town (South Africa), Johannesburg (South Africa), Durban (South Africa), New York, New Jersey, Ohio, Texas, Hamilton (Canada), Toronto (Canada), Ottawa (Canada)

NCT03502733

PHASE 1

Copanlisib and Nivolumab in Treating Patients With Metastatic Solid Tumors or Lymphoma

TARGETS
PI3K, PD-1

LOCATIONS: Maryland, Texas

ORDERED TEST #

CLINICAL TRIALS

GENE
MDM2

RATIONALE
Inhibitors of the MDM2-p53 interaction are being tested in clinical trials. Overexpression or

amplification of MDM2 may increase sensitivity to these agents, but more data are required.

ALTERATION
amplification

NCT03611868 **PHASE 1/2**

A Study of APG-115 in Combination With Pembrolizumab in Patients With Metastatic Melanomas or Advanced Solid Tumors

TARGETS
MDM2, PD-1

LOCATIONS: Florida, Virginia, District of Columbia, Pennsylvania, New York, Tennessee, Texas, Missouri

SAMPLE

ORDERED TEST #

CLINICAL TRIALS

GENE
PDGFRA

RATIONALE
PDGFRA amplification may predict sensitivity to imatinib and to anti-PDGFR α antibodies.

ALTERATION
amplification

NCT01738139

Ipilimumab and Imatinib Mesylate in Advanced Cancer

PHASE 1

TARGETS
KIT, ABL, CTLA-4

LOCATIONS: Texas

SAMPLE

ORDERED TEST #

APPENDIX Variants of Unknown Significance

NOTE One or more variants of unknown significance (VUS) were detected in this patient's tumor. These variants may not have been adequately characterized in the scientific literature at the time this report was issued, and/or the genomic context of these alterations makes their significance unclear. We choose to include them here in the event that they become clinically meaningful in the future.

FLCN
T22M

HDAC7
amplification

INPP4B
V594A

KDM5A
P1237H

NCOR2
Q510_P511insQQQQ

RICTOR
R1202fs*4

SOX10
E359D

WT1
amplification

SAMPLE

ORDERED TEST #

APPENDIX

Genes Assayed in FoundationOne®Heme

FoundationOne Heme is designed to include genes known to be somatically altered in human hematologic malignancies and sarcomas 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	B2M
BAP1	BARD1	BCL10	BCL11B	BCL2	BCL2L2	BCL6	BCL7A
BCORL1	BIRC3	BLM	BRAF	BRCA1	BRCA2	BRD4	BRIP1
BTG2	BTK	BTLA	C11orf30 (EMSY)	CAD	CALR*	CARD11	CBFB
CCND1	CCND2	CCND3	CCNE1	CCT6B	CD22	CD274 (PD-L1)	CD36
CD70	CD79A	CD79B	CDC73	CDH1	CDK12	CDK4	CDK6
CDKN1B	CDKN2A	CDKN2B	CDKN2C	CEBPA	CHD2	CHEK1	CHEK2
CIITA	CKS1B	CPS1	CREBBP	CRKL	CRLF2	CSF1R	CSF3R
CTNNA1	CTNNB1	CUX1	CXCR4	DAXX	DDR2	DDX3X	DNM2
DOT1L	DTX1	DUSP2	DUSP9	EBF1	ECT2L	EED	EGFR
EP300	EPHA3	EPHA5	EPHA7	EPHB1	ERBB2	ERBB3	ERBB4
ESR1	ETS1	ETV6	EXOSC6	EZH2	FAF1	FAM46C	FANCA
FANCD2	FANCE	FANCF	FANCG	FANCL	FAS (TNFRSF6)	FBXO11	FBXO31
FGF10	FGF14	FGF19	FGF23	FGF3	FGF4	FGF6	FGFR1
FGFR3	FGFR4	FHIT	FLCN	FLT1	FLT3	FLT4	FLYWCH1
FOXO1	FOXO3	FOXP1	FRS2	GADD45B	GATA1	GATA2	GATA3
GNA11	GNA12	GNA13	GNAQ	GNAS	GPR124	GRIN2A	GSK3B
HDAC1	HDAC4	HDAC7	HGF	HIST1H1C	HIST1H1D	HIST1H1E	HIST1H2AC
HIST1H2AL	HIST1H2AM	HIST1H2BC	HIST1H2BJ	HIST1H2BK	HIST1H2BO	HIST1H3B	HNF1A
HSP90AA1	ICK	ID3	IDH1	IDH2	IGF1R	IKBKE	IKZF1
IKZF3	IL7R	INHBA	INPP4B	INPP5D (SHIP)	IRF1	IRF4	IRF8
JAK1	JAK2	JAK3	JARID2	JUN	KAT6A (MYST3)	KDM2B	KDM4C
KDM5C	KDM6A	KDR	KEAP1	KIT	KLHL6	KMT2A (MLL)	KMT2C (MLL3)
KRAS	LEF1	LRP1B	LRRK2	MAF	MAFB	MAGED1	MALT1
MAP2K2	MAP2K4	MAP3K1	MAP3K14	MAP3K6	MAP3K7	MAPK1	MCL1
MDM4	MED12	MEF2B	MEF2C	MEN1	MET	MIB1	MITF
MLH1	MPL	MRE11A	MSH2	MSH3	MSH6	MTOR	MUTYH
MYCL (MYCL1)	MYCN	MYD88	MYO18A	NCOR2	NCSTN	NF1	NF2
NFKBIA	NKX2-1	NOD1	NOTCH1	NOTCH2	NPM1	NRAS	NT5C2
NTRK2	NTRK3	NUP93	NUP98	P2RY8	PAG1	PAK3	PALB2
PAX5	PBRM1	PC	PCBP1	PCLO	PDCD1	PDCD11	PDCD1LG2 (PD-L2)
PDGFRB	PDK1	PHF6	PIK3CA	PIK3CG	PIK3R1	PIK3R2	PIM1
POT1	PPP2R1A	PRDM1	PRKAR1A	PRKDC	PRSS8	PTCH1	PTEN
PTPN2	PTPN6 (SHP-1)	PTPRO	RAD21	RAD50	RAD51	RAF1	RARA
RB1	RELN	RET	RHOA	RICTOR	RNF43	ROS1	RPTOR
S1PR2	SDHA	SDHB	SDHC	SDHD	SERP2	SETBP1	SETD2
SGK1	SMAD2	SMAD4	SMARCA1	SMARCA4	SMARCB1	SMC1A	SMC3
SOCS1	SOCS2	SOCS3	SOX10	SOX2	SPEN	SPOP	SRC
STAG2	STAT3	STAT4	STAT5A	STAT5B	STAT6	STK11	SUFU
TAF1	TBL1XR1	TCF3 (E2A)	TCL1A (TCL1)	TET2	TGFBR2	TLL2	TMEM30A
TMSB4XP8 (TMSL3)		TNFAIP3	TNFRSF11A	TNFRSF14	TNFRSF17	TOPI1	TP53
TRAF2	TRAF3	TRAF5	TSC1	TSC2	TSHR	TUSC3	TYK2
U2AF2	VHL	WDR90	WHSC1 (MMSET or NSD2)		WISP3	WT1	XBPI
YYIAP1	ZMYM3	ZNF217	ZNF24 (ZSCAN3)	ZNF703	ZRSR2		XPO1

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APPENDIX

Genes Assayed in FoundationOne®Heme

*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	CRLF2	EGFR	EPOR
ETV1	ETV4	ETV5	ETV6	EWSR1	FGFR2	IGH	IGK	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
FGFR10P	FGFR2	FGFR3	FLI1	FBNP1	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	PFAFH1B2	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)

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APPENDIX Performance Specifications

The median exon coverage for this sample is 664x

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 Instability-High (MSI-H) status	Positive Predictive Agreement (PPA)	100.0% (87.54%-100.00%)*
Sensitivity: Microsatellite Stable (MSS) status	Positive Predictive Agreement (PPA)	89.66% (81.50%, 94.46%)*
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 Instability-High (MSI-H) status	Negative Predictive Agreement (NPA)	97.44% (91.12%-99.29%)*
Specificity: Microsatellite Stable (MSS) status	Negative Predictive Agreement (NPA)	94.44% (86.57%, 97.82%)*
Accuracy: Tumor Mutation 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	

*95% Confidence Interval

Assay specifications were determined for pical 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).

In the fractional-based MSI algorithm, a tumor specimen will be categorized as MSI-H, MSS, or MS-Equivocal according to the fraction of microsatellite loci determined to be altered or unstable (i.e., the fraction unstable loci score). In the FoundationOne Heme assay, MSI is evaluated based on a genome-wide analysis across >2000 microsatellite loci. For a given microsatellite locus, non-somatic alleles are discarded, and the microsatellite is categorized as unstable if remaining alleles differ from the reference genome. The final fraction unstable loci score is calculated as the number of unstable microsatellite loci divided by the number of evaluable microsatellite loci. The MSI-H and MSS cut-off thresholds were determined by analytical concordance to a PCR comparator

assay using a pan-tumor sample set. Patients with results categorized as "MS-Stable" with median exon coverage <300X, "MS-Equivocal," or "Cannot Be Determined" should receive confirmatory testing using a validated orthogonal (alternative) method.

Tumor Mutational Burden (TMB) is determined by measuring the number of somatic mutations in sequenced genes on the FoundationOne Heme test and extrapolating to the genome as a whole. TMB is assayed for all FoundationOne Heme samples and is reported as the number of mutations per megabase (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.

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APPENDIX About FoundationOne®Heme

ABOUT FOUNDATIONONE HEME

FoundationOne®Heme is a comprehensive genomic profiling test for hematologic malignancies and sarcomas. 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.

FoundationOne Heme was developed and its performance characteristics determined by Foundation Medicine, Inc. (Foundation Medicine). FoundationOne Heme 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 Therapies and Clinical Trials

Ranking of Therapies in Summary Table
Therapies are ranked based on the following criteria: Therapies with clinical benefit (ranked alphabetically within each evidence category), followed by therapies associated with resistance (when applicable).

Ranking of Clinical Trials

Pediatric trial qualification → Geographical proximity → Later trial phase.

NATIONAL COMPREHENSIVE CANCER NETWORK® (NCCN®) CATEGORIZATION

Biomarker and genomic findings detected may be associated with certain entries within the NCCN Drugs & Biologics Compendium® (NCCN Compendium®) (www.nccn.org). The NCCN Categories of Evidence and Consensus indicated reflect the highest possible category for a given therapy in association with each biomarker or genomic finding. Please note, however, that the accuracy and applicability of these NCCN categories within a report may be impacted by the patient's clinical history, additional biomarker information, age, and/or co-occurring alterations. For additional information on the NCCN categories, please refer to the NCCN Compendium®. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®). © National Comprehensive Cancer Network, Inc. 2021. All rights reserved. To view the most recent and complete version of the guidelines, go online to NCCN.org. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way.

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 fulfills the requirements of the European Directive 98/79 EC for in vitro diagnostic medical devices and is registered as a CE-IVD product by Foundation Medicine's EU Authorized Representative, Qarad b.v.b.a, Ciplalstraat 3, 2440 Geel, Belgium.

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APPENDIX

About FoundationOne®Heme

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REPORT HIGHLIGHTS

The Report Highlights includes select genomic and therapeutic information with potential impact on patient care and treatment that is specific to the genomics and tumor type of the sample analyzed. This section may highlight information including targeted therapies with potential sensitivity or resistance; evidence-matched clinical trials; and variants with potential diagnostic, prognostic, nontargeted treatment, germline, or clonal hematopoiesis implications. Information included in the Report Highlights is expected to evolve with advances in scientific and clinical research. Findings included in the Report Highlights should be considered in the context of all other information in this report and other relevant patient information. Decisions on patient care and treatment are the responsibility of the treating physician.

VARIANTS TO CONSIDER FOR FOLLOW-UP GERMLINE TESTING

The variants indicated for consideration of follow-up germline testing are 1) limited to reportable short variants with a protein effect listed in the ClinVar genomic database (Landrum et al., 2018; 29165669) as Pathogenic, Pathogenic/Likely Pathogenic, or Likely Pathogenic (by an expert panel or multiple submitters), 2) associated with hereditary cancer-predisposing disorder(s), 3) detected at an allele frequency of >10%, and 4) in select genes reported by the ESMO Precision Medicine Working Group (Mandelker et al., 2019; 31050713) to have a greater than 10% probability of germline origin if identified during tumor sequencing. The selected genes are *ATM*, *BAP1*, *BRCA1*, *BRCA2*, *BRIP1*, *CHEK2*, *FLCN*, *MLH1*, *MSH2*, *MSH6*, *MUTYH*, *PALB2*, *RET*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, *TSC2*, and *VHL*, and are not inclusive of all cancer susceptibility genes. The content in this report should not substitute for genetic counseling or follow-up germline testing, which is needed to distinguish whether a finding in this patient's tumor sequencing is germline or somatic. Interpretation should be based on clinical context.

VARIANTS THAT MAY REPRESENT CLONAL HEMATOPOIESIS

Variants that may represent clonal hematopoiesis (CH) are limited to select reportable short variants in defined genes identified in solid tumors only. Variant selection was determined based on gene tumor-suppressor or oncogene status, known role in solid tumors versus hematological malignancies, and literature prevalence. The defined genes are *ASXL1*, *CBL*, *DNMT3A*, *IDH2*, *JAK2*, *KMT2D* (*MLL2*),

MPL, *MYD88*, *SF3B1*, *TET2*, and *U2AF1* and are not inclusive of all CH genes. The content in this report should not substitute for dedicated hematological workup. Comprehensive genomic profiling of solid tumors detects nontumor alterations that are due to CH. Patient-matched peripheral blood mononuclear cell sequencing is required to conclusively determine if this alteration is present in tumor or is secondary to CH. Interpretation should be based on clinical context.

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

MR Suite Version 5.2.0

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