| | | | |
|---|---|---|---|
| **Date of Birth** | | **Medical Facility** | |
| **Sex** | | **Ordering Physician** | **Specimen Received** |
| **FMI Case #** | SRF201616 | **Additional Recipient** | **Date of Collection** |
| **Medical Record #** | | **Medical Facility ID #** | **Specimen Type** |
| **Specimen ID** | | **Pathologist** | |

## ABOUT THE TEST:

FoundationACT™ (Assay for Circulating Tumor DNA) is a next-generation sequencing (NGS) assay that identifies clinically relevant genomic alterations in circulating tumor DNA and matches them to targeted therapies and clinical trials.

## PATIENT RESULTS

**4 genomic alterations**

**16 therapies associated with potential clinical benefit**

**0 therapies associated with lack of response**

**12 clinical trials**

## TUMOR TYPE: UNKNOWN PRIMARY CANCER (NOS)

**Genomic Alterations Identified**†

*EGFR* exon 19 deletion (L747_A750>P)
*PTEN* splice site 489_492+1delAAAGG
*RET* amplification – equivocal#
*TP53* M237I – equivocal#, splice site 672+1G>T

† 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 | Allele Frequency | FDA-Approved Therapies (in patient's tumor type) | FDA-Approved Therapies (in another tumor type) | Potential Clinical Trials |
|---|---|---|---|---|
| ***EGFR*** exon 19 deletion (L747_A750>P) | 31.9% | None | Afatinib<br>Cetuximab<br>Erlotinib<br>Gefitinib<br>Lapatinib<br>Osimertinib<br>Panitumumab | Yes, see clinical trials section |
| ***PTEN*** splice site 489_492+1delAAAGG | 27.8% | None | Everolimus<br>Temsirolimus | Yes, see clinical trials section |
| ***RET*** amplification - equivocal | N/A | None | Cabozantinib<br>Lenvatinib<br>Ponatinib<br>Regorafenib<br>Sorafenib<br>Sunitinib<br>Vandetanib | Yes, see clinical trials section |

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| Genomic Alterations Detected | Allele Frequency | FDA-Approved Therapies (in patient's tumor type) | FDA-Approved Therapies (in another tumor type) | Potential Clinical Trials |
|---|---|---|---|---|
| ***TP53*** M237I - equivocal, splice site 672+1G>T | 0.36%, 27.8% | 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.

N/A= Not Applicable; Allele Frequency is not applicable for copy number amplifications or rearrangements.

## GENOMIC ALTERATIONS

| GENE ALTERATION | INTERPRETATION |
|---|---|
| ● ***EGFR*** exon 19 deletion (L747_A750>P) | **Gene and Alteration:** EGFR encodes the epidermal growth factor receptor, which belongs to a class of proteins called receptor tyrosine kinases. In response to signals from the environment, EGFR passes biochemical messages to the cell that stimulate it to grow and divide[1]. The EGFR mutation seen here is a deletion in exon 19, encoding a portion of the kinase domain of EGFR; such mutations have been shown to activate the tyrosine kinase activity of EGFR and to confer sensitivity to EGFR tyrosine kinase inhibitors such as erlotinib and gefitinib[2,3,4].<br><br>**Frequency and Prognosis:** EGFR mutations are particularly frequent in lung adenocarcinomas (35%), and have also been observed in colorectal (3%), stomach (4%), and prostate (3%) adenocarcinomas (COSMIC, Nov 2015). The Cancer Genome Atlas project reports EGFR amplification in a number of adenocarcinomas, with the highest incidence in lung (10%), stomach (6%), and cervical (2%) adenocarcinomas, and in <1% of prostate and colorectal adenocarcinomas and ovarian serous cystadenocarcinomas (cBioPortal, Nov 2015).<br><br>**Potential Treatment Strategies:** EGFR activating mutations or amplification may predict sensitivity to EGFR inhibitors including erlotinib, gefitinib, afatinib, osimertinib, cetuximab, panitumumab, and lapatinib[5,6,7,8,9]. Other EGFR-targeted therapies are also in clinical trials. A Phase 2 trial of the pan-ERBB inhibitor dacomitinib in patients with lung adenocarcinoma reported 98% (44/45) disease control [partial response (PR) or stable disease], including a 76% PR rate, in patients with EGFR exon 19 deletions or the L858R mutation; lower disease control and PR rates were reported in patients with other EGFR mutations, wild-type EGFR, or unknown EGFR status[10]. Irreversible EGFR inhibitors, as well as HSP90 inhibitors, may be appropriate for patients with de novo or acquired resistance to (prior) EGFR-targeted therapy[11,12,13,14]. Necitumumab is an anti-EGFR antibody that is approved to treat metastatic squamous NSCLC in combination with gemcitabine and cisplatin[15,16]. The reovirus Reolysin, which targets cells that harbor activated RAS signaling due to alterations in RAS genes or upstream activators such as EGFR[17,18,19], is also in clinical trials in some tumor types. Reolysin has demonstrated mixed clinical efficacy, with the highest rate of response reported for head and neck cancer[20,21,22,23,24,25,26,27,28]. |
| ● ***PTEN*** splice site 489_492+1delAAAG G | **Gene and Alteration:** PTEN encodes an inositol phosphatase that functions as a tumor suppressor by negatively regulating the PI3K-AKT-mTOR pathway; loss of PTEN can lead to uncontrolled cell growth and suppression of apoptosis[29]. PTEN alterations that disrupt the N-terminal PIP2 binding motif[30], the phosphatase domain (amino acids 14-185)[31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56], the C2 domain (amino acids 190-350)[31,33,43,57,58,59,60,61,62,63] and/or C-terminal region[64,65], such as observed here, are predicted to cause a loss of function. Mutations in PTEN underlie several inherited disorders collectively termed PTEN hamartoma tumor syndrome (PHTS), which includes Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome (BRRS), PTEN-related Proteus syndrome (PS), and Proteus-like syndrome[66,67]. The mutation rate for PTEN in these disorders ranges from 20-85% of patients. The estimated incidence of Cowden syndrome is approximately 1:200,000, but it is widely believed that this may be an underestimate[66,68]. Given the association between PTEN and these inherited syndromes, in the appropriate clinical context, germline testing for mutations affecting PTEN is recommended. |



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| GENE ALTERATION | INTERPRETATION |
|---|---|
| | **Frequency and Prognosis:** In the TCGA datasets, PTEN mutation has been observed in endometrial carcinoma (65%)[69], glioblastoma (23%)[70], kidney chromophobe carcinoma (9%)[71], stomach adenocarcinoma (8%)[72], lung squamous cell carcinoma (8%)[73], renal clear cell carcinoma[74], colorectal adenocarcinoma (4%)[75] and breast invasive carcinoma (3.6%)[76]. Studies in the literature have reported loss of PTEN protein expression in 14% of primary esophageal adenocarcinoma, 18% of pulmonary adenocarcinoma, 26% of pancreatic ductal adenocarcinoma, and 46% of endometrioid adenocarcinoma samples analyzed[77,78,79,80]. Loss of PTEN, either by mutation or deletion, has been correlated with decreased survival in several tumor types[77,81].<br><br>**Potential Treatment Strategies:** PTEN loss or mutation leads to activation of the PI3K-AKT-mTOR pathway and may predict sensitivity to inhibitors of this pathway[29,82,83]. The mTOR inhibitors temsirolimus and everolimus are FDA approved for use in other indications and are being studied in clinical trials in multiple tumor types. Other mTOR inhibitors, as well as inhibitors of PI3K and AKT, are also being investigated in clinical trials, alone or in combination with other therapies. Preclinical studies suggest that PTEN-deficient cancers, in the absence of other oncogenic mutations, depend primarily on the beta isoform of PI3K (PI3K-beta)[84,85,86]; PI3K-beta-specific inhibitors are in clinical trials for PTEN-deficient tumors. In the context of concurrent PIK3CA mutation, PTEN loss may predict resistance to PI3K-alpha-specific inhibitors[87,88]. In addition, limited preclinical data suggest that PTEN mutations may predict sensitivity to PARP inhibitors[89] and two patients with PTEN mutations in their tumors have benefited from the PARP inhibitor olaparib (Dougherty et al., 2014; ASCO Abstract 5536)[90]. Olaparib is FDA approved for the treatment of ovarian cancer. Loss of PTEN expression may also contribute to trastuzumab resistance in breast cancer[91,92]. |
| ● ***RET***<br>amplification - equivocal | **Gene and Alteration:** RET (rearranged during transfection) is a receptor tyrosine kinase, primarily expressed in cells of the nervous system. It has been identified as a proto-oncogene that results in transformation of cells upon recombination with a partner gene[93]. RET amplification has been reported in cancer[94], and has been associated with responses to therapies that target RET[95,96].<br><br>**Frequency and Prognosis:** In the TCGA datasets, RET amplification as observed in 2.8% of cholangiocarcinomas, 1.1-1.8% of lung squamous cell carcinomas (SCC), 1.5-2.3% of bladder urothelial carcinomas, 1.6% of esophageal carcinomas, and 1.0% of ovarian serous cystadenocarcinomas and head and neck SCC (HNSCC) (cBioPortal, Mar 2016)[73,97]. RET mutations have been reported with highest frequency in medullary thyroid carcinoma (41.3%), pheochromocytoma (6.3%), colorectal carcinoma (3.7%), and lung carcinoma (2.1%) (COSMIC, Mar 2016). In one study of 116 lung tumors, RET copy number gain and amplification was observed in 52% and 12% of samples, respectively, with amplification more prevalent in adenocarcinomas than other subtypes[98]. RET amplification is associated with high-grade tumors and poorer outcome in thyroid carcinomas[99,100]. Increased RET expression in breast cancer tumors is associated with a worse prognosis and shorter survival times, as compared to breast cancer samples with low or no RET expression[101]. RET signaling in breast cancer has been associated with estrogen independence and with tamoxifen independence, and RET expression has been shown to be regulated by both estrogen and retinoic acid in breast cancer[102]. RET has been suggested to be a tumor suppressor in colorectal cancer; this study identified inactivating mutations and methylation in the RET gene in colorectal cancer and correlated methylation of RET with decreased Ret protein expression[103]. |

| GENE ALTERATION | INTERPRETATION |
|---|---|
| | **Potential Treatment Strategies:** Several RET inhibitors are approved by the FDA in various indications, including ponatinib, sunitinib, vandetanib, cabozantinib, lenvatinib, regorafenib, and sorafenib. Twenty three patients with RET-rearranged NSCLC treated with cabozantinib achieved 7 partial responses, 2 unconfirmed partial responses and 12 additional stable disease outcomes (Drilon et al., 2015; ASCO Abstract 8007)[104,105,106,107] and four patients with tumors harboring RET fusions (three with lung cancer and one with papillary thyroid carcinoma) benefited from vandetanib-containing therapy[108,109,110,111]. Additionally, a case study reported an exceptional response to sunitinib in a patient with a RET-amplified germ cell tumor[95]. Treatment of RET-amplified tongue adenocarcinoma with sorafenib resulted in disease stabilization lasting four months; additional treatment with sorafenib and sulindac provided disease stabilization for an additional three months[96]. Lenvatinib is FDA approved for the treatment of locally recurrent or metastatic, progressive, radioiodine-refractory differentiated thyroid cancer (DTC)[112] and, in combination with everolimus, for the treatment of advanced renal cell carcinoma following prior antiangiogenic therapy[113]. In a Phase 2 trial, lenvatinib benefited patients with medullary thyroid carcinoma and RET mutations (6 PR, 8 SD, 1 PD), as well as those without detected RET mutations (3 PR, 2 SD, 2 PD), with a trend for higher progression-free survival for patients with RET mutations (Schlumberger et al., 2012; ASCO Abstract 5591). Preclinical studies demonstrated that cells transformed by KIF5B-RET are sensitive to treatment with vandetanib, sorafenib, ponatinib, sunitinib, cabozantinib, and lenvatinib (Gozgit et al., 2013; AACR Abstract 2084)[104,114,115,116,117]. Additional preclinical studies reported that lung cancer cell lines containing a CCDC6-RET fusion were sensitive to vandetanib[118] and lenvatinib[117]. These agents and other drugs targeting RET are in clinical trials for patients with various solid tumors (Hellerstedt, 2012; ASCO Abstract 7514). |
| ● ***TP53*** M237I - equivocal, splice site 672+1G>T | **Gene and Alteration:** Functional loss of the tumor suppressor p53, which is encoded by the TP53 gene, is common in aggressive advanced cancers[119]. Mutations affecting the DNA binding domain (aa 100-292), the tetramerization domain (aa 325-356), or the C-terminal regulatory domain (aa 356-393), such as observed here, are thought to disrupt the transactivation of p53-dependent genes and are predicted to promote tumorigenesis[120,121,122,123]. Germline mutations in TP53 are associated with the very rare disorder Li-Fraumeni syndrome and the early onset of many cancers[124,125,126,127,128,129]. Estimates for the prevalence of germline TP53 mutations in the general population range from 1:5,000[130] to 1:20,000[129], and in the appropriate clinical context, germline testing of TP53 is recommended.<br><br>**Frequency and Prognosis:** Pan-cancer analysis of the TCGA datasets across 12 cancer types identified TP53 as the most frequently mutated gene, with 42% of more than 3,000 tumors harboring a TP53 mutation; in this study TP53 mutation occurred most frequently in ovarian serous carcinoma (95%), lung squamous cell carcinoma (79%), head and neck squamous cell carcinoma (70%), colorectal adenocarcinoma (59%), lung adenocarcinoma (52%), and bladder urothelial carcinoma (50%)[131]. TP53 loss of heterozygosity (LOH) is frequently seen in tumors and often occurs when one copy of TP53 harbors a mutation; in some tumors, LOH is correlated with progression[132,133,134,135]. |

| GENE ALTERATION | INTERPRETATION |
|---|---|

**Potential Treatment Strategies:** There are no approved therapies to address TP53 mutation or loss. However, tumors with TP53 loss of function alterations may be sensitive to the WEE1 inhibitor AZD1775[136,137,138,139], therapies that reactivate mutant p53 such as APR-246[140], or p53 gene therapy and immunotherapeutics such as SGT-53[141,142,143,144] and ALT-801 (Hajdenberg et al., 2012; ASCO Abstract e15010). Combination of AZD1775 with paclitaxel and carboplatin achieved significantly longer progression-free survival than paclitaxel and carboplatin alone in patients with TP53-mutant ovarian cancer (Oza et al., 2015; ASCO Abstract 5506). Furthermore, AZD1775 in combination with carboplatin achieved a 27% (6/22) response rate and 41% (9/22) stable disease rate in patients with TP53-mutant ovarian cancer refractory or resistant to carboplatin plus paclitaxel (Leijen et al., 2015; ASCO Abstract 2507). In a Phase 1 clinical trial, 8 of 11 evaluable patients receiving SGT-53 as a single agent exhibited stable disease[145]. Clinical trials of SGT-53 in combination with chemotherapy are underway. Additionally, the combination of a CHK1 inhibitor and irinotecan reportedly reduced tumor growth and prolonged survival in a TP53 mutant, but not TP53 wild-type, breast cancer xenotransplant mouse model[146]. Kevetrin has also been reported to activate p53 in preclinical studies and might be relevant in the context of mutant p53 (Kumar et al., 2012; AACR Abstract 2874). Clinical trials of these agents are under way for some tumor types for patients with a TP53 mutation.

## THERAPIES

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 |
|---|---|
| Afatinib | **Approved Indications:** Afatinib is an irreversible kinase inhibitor that targets the kinase domains of EGFR, ERBB2/HER2, and ERBB4. It is FDA approved to treat metastatic non-small cell lung cancer (NSCLC) in patients with EGFR exon 19 deletions or exon 21 (L858R) missense mutations.<br>**Gene Association:** EGFR activating mutations or amplification may indicate sensitivity to afatinib. Afatinib, compared with chemotherapy, improved progression-free survival (PFS) in patients with EGFR-mutant NSCLC (11.1 months vs. 6.9 month)[7].<br>**Supporting Data:** Patients with EGFR-mutant NSCLC treated with afatinib exhibited significant improvement in progression free survival (PFS) vs. chemotherapy treatments[7,147]. A Phase 2 trial of afatinib in patients with either EGFR or ERBB2 amplification and esophagogastric, biliary tract, urothelial tract, or gynecologic cancer reported a 5% (1/20) objective response, with complete response achieved in one patient and stable disease achieved in 8 patients; the authors concluded that afatinib activity as a single agent was encouraging[148]. A Phase 1 trial of afatinib in advanced cancer reported disease stabilization in 14/31 patients[149]. A Phase 1 study of afatinib combined with pemetrexed in patients with advanced solid tumors reported confirmed partial response in 3% (1/30) of patients and stable disease in 33% (10/30) of patients (Chu et al., 2013; ASCO Abstract 2523). A Phase 1 trial of volasertib and afatinib in patients with advanced solid tumors reported partial response in 7% (2/29) of patients (Peeters et al., 2013; ASCO Abstract 2521). Outcomes of partial response and/or stable disease have been reported in various clinical trials involving multiple cancer types , including HER2-positive breast cancer, NSCLC, colorectal cancer, and esophageal cancer[7,147,150,151,152]. |
| Cetuximab | **Approved Indications:** Cetuximab is a monoclonal antibody that targets EGFR. It is FDA approved for the treatment of head and neck squamous cell carcinoma (HNSCC) and KRAS wild-type metastatic colorectal cancer (CRC).<br>**Gene Association:** EGFR amplification or activating alterations may confer sensitivity to anti-EGFR antibodies such as cetuximab.<br>**Supporting Data:** A Phase 3 trial of combined cetuximab and platinum/5-FU in HNSCC demonstrated improved response compared to platinum/5-FU alone, but EGFR amplification was not shown to predict response to this treatment[153]. A Phase 3 study of 745 patients with pancreatic adenocarcinoma did not report any improved outcome in patients treated with a combination of cetuximab plus gemcitabine vs gemcitabine alone[154]. In a Phase 1/2 trial of 36 patients with metastatic castration-resistant prostate cancer treated with cetuximab in combination with doxorubicin, stable disease was reported in 63% of patients[155]. A Phase 1 study of the combination therapy of cetuximab, erlotinib, and bevacizumab reported stable disease in 21% (7/34) of patients with non-small cell lung cancer (NSCLC)[156]. |
| Erlotinib | **Approved Indications:** Erlotinib is a small molecule inhibitor of EGFR. It is FDA approved for the treatment of non-small cell lung cancer (NSCLC) and pancreatic cancer.<br>**Gene Association:** EGFR amplification or activating alterations may predict sensitivity to erlotinib. |

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| | **Supporting Data:** The approval of erlotinib in NSCLC is based on a Phase 3 randomized trial demonstrating prolonged overall survival for unselected NSCLC patients treated with erlotinib compared to standard chemotherapy[157]. Furthermore, several randomized Phase 3 trials have shown a significant improvement in response and progression-free survival for this class of medications compared with combination chemotherapy in patients with known EGFR mutations, including the EURTAC trial of erlotinib vs. platinum-based chemotherapy[5]. A Phase 2 clinical trial of erlotinib plus bevacizumab in hepatocellular carcinoma reported a median progression-free survival of 2.9 months and a median overall survival of 10.7 months[158]. |
| Gefitinib | **Approved Indications:** Gefitinib targets the tyrosine kinase EGFR and is FDA approved to treat non-small cell lung cancer (NSCLC) harboring exon 19 deletions or exon 21 (L858R) substitution mutations in EGFR.<br>**Gene Association:** Amplification or activation of EGFR may predict sensitivity to therapies such as gefitinib. Clinical studies have consistently shown significant improvement in response rates and progression-free survival for patients with EGFR-mutated NSCLC treated with gefitinib, compared to chemotherapy[159,160,161,162,163,164].<br>**Supporting Data:** Investigations into the efficacy of gefitinib have primarily been in the context of lung cancer. Gefitinib achieved an objective response rate of 69.8% and an overall survival of 19.2 months as first-line treatment of Caucasian patients with NSCLC and EGFR sensitizing mutations, which were mostly EGFR exon 19 deletions and EGFR L858R[6]. In the retrospective analysis of a Phase 3 study in Asia, gefitinib increased progression-free survival in a subgroup of patients with EGFR mutation-positive NSCLC as compared with carboplatin/paclitaxel doublet chemotherapy (hazard ratio for progression 0.48)[162,165]. In a Phase 2 trial, gefitinib resulted in a best response of stable disease that was observed in 38% of patients with renal cell carcinoma[166]. A preclinical study of erlotinib and gefitinib in platinum-resistant cancers reports a poor response rate for both agents in platinum pretreated ovarian cancer cells[167]. A preclinical study of erlotinib and gefitinib in platinum-resistant cancers reports a poor response rate for both agents in platinum-pretreated ovarian cancer cells[167]. |
| Lapatinib | **Approved Indications:** Lapatinib is a tyrosine kinase inhibitor that targets EGFR, ERBB2/HER2, and to a lesser degree, ERBB4. It is FDA approved in combination with capecitabine or letrozole for the treatment of HER2-overexpressing (HER2+) metastatic breast cancer.<br>**Gene Association:** EGFR amplification or activating alterations may confer sensitivity to EGFR/multi-tyrosine kinase inhibitors such as lapatinib.<br>**Supporting Data:** Investigations into the efficacy of lapatinib have primarily been in the context of breast cancer[168,169,170,171,172,173]. As first-line therapy for HER2+ metastatic breast cancer, lapatinib plus taxane resulted in shorter median PFS compared with trastuzumab plus taxane (9.0 vs. 11.3 months, hazard ratio of 1.37)[174]. For patients who have progressed on trastuzumab plus taxane, ado-trastuzumab emtansine (T-DM1) was superior to lapatinib plus capecitabine (OS of 30.9 vs. 25.1 months)[175]. In postmenopausal patients with hormone receptor-positive (HR+) HER2+ metastatic breast cancer, lapatinib combined with letrozole increased median PFS compared to letrozole alone (8.2 vs. 3.0 months)[176]. A Phase 2 study selecting patients with ERBB2-amplified solid tumors reported one complete response in a patient with esophageal adenocarcinoma[177]. Phase 1 studies evaluating lapatinib alone or in combination with chemotherapy agents reported partial responses in patients with various solid tumors and one complete response in a patient with EGFR-overexpressing head and neck squamous cell carcinoma[178,179,180,181]. In a Phase 1 trial of lapatinib plus pazopanib, one patient with a salivary gland tumor experienced a partial response[182]. |

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| Osimertinib | **Approved Indications:** Osimertinib is an irreversible EGFR tyrosine kinase inhibitor (TKI) that is selective for EGFR TKI-sensitizing mutations and the EGFR T790M mutation. It is FDA approved to treat patients with metastatic EGFR T790M-positive non-small cell lung cancer (NSCLC) and disease progression on or after EGFR TKI therapy.<br>**Gene Association:** EGFR TKI-sensitizing mutations and/or the EGFR T790M mutation may predict sensitivity to osimertinib[8,183]. T790M-positive patients showed higher response rates than T790M-negative cases in a Phase 1 study for patients with acquired EGFR TKI resistance (61% vs. 21%)[8].<br>**Supporting Data:** Osimertinib has been studied primarily for the treatment of EGFR-mutant NSCLC. Phase 2 studies of osimertinib demonstrated objective response rates (ORR) of 57-61% and disease control rates (DCR) of 90-92% for patients with T790M-positive advanced NSCLC who had progressed on prior EGFR TKI therapy; most objective responses (96%) were ongoing at the median 4-month follow-up (Yang et. al., 2015; WCLC Abstract 943, Mitsudomi et al., 2015; WCLC Abstract 1406). In the Phase 1 expansion cohort with the approved dose of osimertinib (80 mg), the ORR was 54% (32/59), the median duration of response was 12.4 months, and the median progression-free survival (PFS) was 13.5 months for patients with T790M-positive NSCLC (Janne et al., 2015; DOI: 10.1093/annonc/mdv128.05). This trial reported an ORR of 21% and median PFS of 2.8 months for T790M-negative cases with acquired EGFR TKI resistance[8]. Treatment-naïve patients with EGFR-mutant NSCLC achieved an ORR of 60% (18/30) and a DCR of 93% (28/30) (Ramalingnam et al., 2015; ASCO Abstract 8000). A Phase 1b study combined osimertinib with the investigational immunotherapy durvalumab, MEK inhibitor selumetinib, or MET inhibitor savolitinib, and observed partial responses (PR) for each of the combinations (9/14 PR with durvalumab, 9/23 PR with selumetinib, 6/11 PR with savolitinib) (Ramalingam et al., 2015; ASCO Abstract 2509). Osimertinib is being compared with erlotinib or gefitinib as first-line treatment for EGFR-mutant NSCLC (NCT02296125). |
| Panitumumab | **Approved Indications:** Panitumumab is a monoclonal antibody that targets EGFR. It is FDA approved for the treatment of KRAS wild-type metastatic colorectal cancer (CRC).<br>**Gene Association:** EGFR amplification or activating alterations may confer sensitivity to EGFR inhibitory antibodies such as panitumumab.<br>**Supporting Data:** A Phase 3 clinical trial of panitumumab with FOLFOX4 in colorectal carcinoma reported significantly better progression-free survival, as compared with FOLFOX 4 alone; this finding only held true for KRAS wild-type patients[184]. A randomized Phase 2 trial of 93 patients with metastatic pancreatic cancer reported an increase in progression-free survival in patients treated with a combination of panitumumab, erlotinib, and gemcitabine versus an erlotinib-gemcitabine combination (Kim et al. 2011; ASCO Abstract 38). Two Phase 2 studies of panitumumab and chemotherapy in biliary tract cancer, including cholangiocarcinoma, reported encouraging efficacy and manageable toxicity[185,186]. In a Phase 2 trial of advanced NSCLC, the addition of panitumumab to paclitaxel/carboplatin did not result in improved clinical benefit[187]. A Phase 1 study of panitumumab for patients with metastatic renal cell carcinoma resulted in a response rate of 6% and stable disease in 50% of patients[188]. |
| Everolimus | **Approved Indications:** Everolimus is an orally available mTOR inhibitor that is FDA approved to treat renal cell carcinoma (RCC) following antiangiogenic therapy; pancreatic neuroendocrine tumors and well-differentiated non-functional neuroendocrine tumors of the lung or gastrointestinal tract; and, in association with tuberous sclerosis complex (TSC), renal angiomyolipoma and subependymal giant cell astrocytoma. Everolimus is also approved to treat hormone receptor-positive, HER2-negative advanced breast cancer in combination with exemestane following prior therapy with letrozole or anastrozole, as well as in combination with the multikinase inhibitor lenvatinib to treat advanced RCC following prior antiangiogenic therapy. |

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| | **Gene Association:** PTEN inactivation may predict benefit from mTOR inhibitors, such as everolimus, based on clinical data in various tumor types. For patients with prostate cancer, PTEN loss correlated with response to single-agent everolimus[189]. Retrospective clinical data suggest that patients with advanced breast cancer and PTEN inactivation, particularly in the context of HER2-positive disease, may benefit from everolimus combined with targeted therapy and/or chemotherapy[190,191,192].<br>**Supporting Data:** In a Phase 1 study to assess the safety and pharmacokinetics of everolimus and paclitaxel in patients with advanced solid tumors, 6/16 patients exhibited stable disease for more than 4 months[193]. A Phase 1 study to assess the safety and pharmacokinetics of everolimus, bevacizumab, and panobinostat (LBH-589) in patients with advanced solid tumors reported a partial response in 1/9 patients and stable disease in 2/9 patients, but the safety and tolerability profiles were not acceptable and the authors of the study did not recommend further study[194]. In a Phase 1 study of the combination of everolimus and cetuximab in patients with advanced cancer, 5/16 evaluable patients exhibited stable disease for at least four months (4-19 months)[195]. A Phase 1 study of the combination of everolimus and lapatinib reported 17% (13/78) of patients exhibited partial response or stable disease for at least four months[196]. A Phase 1b study of everolimus with lenvatinib in patients with metastatic renal cell carcinoma identified partial response in 6/18 and stable disease in 9/18 patients[197]. A Phase 2 study of everolimus in combination with bevacizumab in advanced colorectal cancer reported modest activity for the combination[198]. A Phase 1b trial of a combination of everolimus and the MEK inhibitor trametinib in patients with solid tumors reported frequent adverse events, and the study was unable to identify a recommended Phase 2 dose and schedule for the combination[199]. |
| Temsirolimus | **Approved Indications:** Temsirolimus is an intravenous mTOR inhibitor that is FDA approved for the treatment of advanced renal cell carcinoma.<br>**Gene Association:** PTEN inactivation may predict benefit from mTOR inhibitors, such as temsirolimus, based on clinical data in various tumor types. Out of 10 patients with metaplastic breast cancer and PTEN alterations, 2 cases responded to temsirolimus or everolimus plus doxorubicin and bevacizumab (Basho et al., 2015; SABCS Abstract P3-14-02)[200]. Temsirolimus achieved stable disease for 6 of 7 patients with PTEN-deficient cervical carcinoma[201]. Clinical studies in renal cell carcinoma[202,203], glioblastoma[204,205], or endometrial cancer[206,207,208,209] did not observe a correlation of PTEN deficiency with response to temsirolimus, although several patients with those tumor types and PTEN loss have benefited from mTOR inhibitors.<br>**Supporting Data:** A Phase 1 trial of bevacizumab and temsirolimus plus liposomal doxorubicin in patients with advanced solid tumors showed that the combination was well tolerated and resulted in six-month stable disease in 21% of patients, with a 21% rate of partial or complete remission[210]. In a Phase 2 clinical trial in non-small cell lung cancer (NSCLC), temsirolimus showed clinical benefit, but further studies are warranted[211]. A Phase 2 study of temsirolimus in patients with KRAS-mutant colorectal cancer reported limited efficacy; however, all patients who exhibited tumor reduction were found to have low levels of mutated KRAS in plasma samples[212]. A Phase 2 clinical trial in patients with pancreatic cancer reported that temsirolimus monotherapy had limited efficacy, and may have contributed to disease progression[213]. A study examining the efficacy of temsirolimus-involving regimens in 24 patients with mesenchymal/metaplastic breast cancer (MpBCs) reported 2 complete responses, 4 partial responses, 2 instances of stable disease longer than 6 months, and 4 instances of stable disease shorter than 6 months[200]. |
| Cabozantinib | **Approved Indications:** Cabozantinib is a kinase inhibitor that targets MET, RET, VEGFRs, KIT, FLT-3, TIE-2, AXL, and TrkB. It is FDA approved for the treatment of medullary thyroid cancer.<br>**Gene Association:** Alterations that are expected to increase signaling through these kinases, in particular MET, RET and VEGFR-2 (KDR), may predict sensitivity to treatment with cabozantinib. |

**Supporting Data:** A Phase 1 ascending dose study of cabozantinib in patients with advanced solid tumors reported early indications of drug response and prolonged stable disease, with no dose-limiting toxicities or serious adverse events (Yamamoto et al., 2011; AACR-EORTC Abstract C26). Phase 2 studies of cabozantinib observed antitumor activity in renal cell carcinoma, non-small cell lung cancer, and breast cancer and a safety profile similar to that of other tyrosine kinase inhibitors (Hellerstedt et al., 2012; ASCO Abstract 7514, Winer et al., 2012; ASCO Abstract 535)[214].

Lenvatinib

**Approved Indications:** Lenvatinib targets several kinases, including the VEGFRs, FGFRs, PDGFRs, RET, and KIT. It is FDA approved to treat locally recurrent or metastatic, radioiodine-refractory differentiated thyroid cancer and, in combination with everolimus, for the treatment of advanced renal cell carcinoma following prior antiangiogenic therapy.

**Gene Association:** Activating mutations, fusions, or amplification of RET can increase signaling through this receptor and may predict sensitivity to lenvatinib[117,215]. Lenvatinib benefited patients with medullary thyroid carcinoma and RET mutations (6 PR, 8 SD, 1 PD), as well as patients without detected RET mutations (3 PR, 2 SD, 2 PD), with a trend for higher progression-free survival (PFS) in patients with RET mutations (Schlumberger et al., 2012; ASCO Abstract 5591).

**Supporting Data:** Lenvatinib has primarily been evaluated for the treatment of iodine-131-refractory, differentiated thyroid carcinoma (RR-DTC)[112] or for patients with advanced renal cell carcinoma[113]. In the Phase 3 SELECT trial, which enrolled patients with RR-DTC, lenvatinib treatment increased PFS to 18.3 months, compared with 3.6 months for the placebo arm[112]. Lenvatinib treatment elicited a response rate of 65%, including 4 complete responses (CR) and 165 partial responses (PRs; 165/216; 63%)[112]. Lenvatinib benefited patients with medullary thyroid carcinoma (MTC) and RET mutations (6 PR, 8 SD, 1 PD) as well as patients without detected RET mutations (3 PR, 2 SD, 2 PD), with a trend for higher PFS in patients with RET mutations (Schlumberger et al., 2012; ASCO Abstract 5591); 2 patients with MTC and dual PIK3CA and RET mutations experienced PRs in response to lenvatinib[216]. Several Phase 1 and 2 trials have reported PRs in approximately 10% of patients and SD in 30-50% of patients in response to lenvatinib (Hong et al., 2010; ASCO Abstract 2540, Nemunaitis et al., 2008; ASCO Abstract 14583, Yamamoto et al., 2013; JMSO Abstract O2–029)[217,218]. PRs have been observed in patients with melanoma (O'Day et al., 2013; ASCO Abstract 9026)[218], sarcoma (Yamamoto et al., 2013; JMSO Abstract O2–029)[217,219], NSCLC (Havel et al., 2014; ASCO Abstract 8043, Calvo et al., 2011; Intl Melanoma Conf Abstract SMR-P43)[220], and a variety of other solid tumors (Hong et al., 2010; ASCO Abstract 2540, Nemunaitis et al., 2008; ASCO Abstract 14583, Yamamoto et al., 2013; JMSO Abstract O2–029, Vergote et al., 2013; ASCO Abstract 5520, Mitsunaga et al., 2013; ASCO Abstract 231, Okita et al., 2012; ASCO Abstract 320, Nemunaitis et al., 2011; ASCO Abstract 8595, Sherman et al., 2011; ASCO Abstract 5503)[217,218]. Trials investigating lenvatinib in combination with other antineoplastic agents have reported even higher PR rates. The combination of lenvatinib and everolimus for the treatment of metastatic renal cell carcinoma led to PRs for 30% of patients (12/38) and SD for an additional 50% (19/38)[197], whereas the combination of lenvatinib and golvatinib for the treatment of advanced solid tumors resulted in PRs for 5/14 patients; an additional 13/24 patients experienced SD (Kwak et al., 2014; ANE Abstract 484).

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 for the treatment of 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:** Ponatinib has been shown to potently inhibit activated forms of RET kinase, both point mutations and fusions, in in vitro studies of medullary thyroid carcinoma and non-small cell lung cancer (NSCLC) cell lines (Gozgit et al., 2013; AACR Abstract 2084)[221]; RET activating mutation or amplification may therefore predict sensitivity to ponatinib.

| | |
|---|---|
| | **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)[222]. |
| Regorafenib | **Approved Indications:** Regorafenib is a small-molecule inhibitor of multiple kinases, including RET, VEGFRs, PDGFRs, KIT, and RAF family proteins[223]. It is FDA approved for the treatment of metastatic colorectal cancer (CRC) or advanced gastrointestinal stromal tumors (GIST)[224,225,226].<br>**Gene Association:** RET activating mutation or amplification may predict sensitivity to regorafenib.<br>**Supporting Data:** Regorafenib has primarily been studied as a treatment for CRC and GIST, and data are limited for other tumor types. Regorafenib improved overall survival in patients with CRC and progression-free survival in patients with imatinib/sunitinib-refractory GIST as compared with placebo[224,225]. A Phase 1 trial of regorafenib in 47 patients with solid tumors reported 3 (6%) partial responses in patients with CRC, renal cell carcinoma, or osteosarcoma[227]. A Phase 1 trial of regorafenib in 17 patients with refractory non-small cell lung cancer (NSCLC) reported stable disease lasting longer than 12 weeks in 4 (23%) patients (Kies et al., 2010; ASCO Abstract 7585). Regorafenib use has been linked to the development of intestinal perforation in 2 patients[228]. |
| 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.<br>**Gene Association:** Activating mutations or fusions of RET may predict sensitivity to sorafenib. A preclinical study demonstrated that cells transformed by KIF5B-RET were sensitive to treatment with sorafenib[114]. Treatment of a patient with RET-amplified tongue adenocarcinoma with sorafenib resulted in disease stabilization lasting four months; additional treatment with sorafenib and sulindac provided disease stabilization for an additional three months[96].<br>**Supporting Data:** 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 patients[229,230]. The combination of sorafenib and everolimus achieved partial response or stable disease in two patients with lung adenocarcinoma[231]. In the context of small cell lung carcinoma, sorafenib combined with cisplatin plus etoposide was highly toxic and ineffective[232]. In HER2-negative breast cancer, Phase 2b trials found improved progression-free survival for sorafenib added to capecitabine, but not when added to paclitaxel[233,234]. Phase 2 studies of sorafenib in biliary tract cancer reported disease control rates of 33-39%[235]. Three patients with cholangiocarcinoma derived clinical benefit from sorafenib[236,237]. However, the addition of sorafenib to gemcitabine did not improve outcome in patients with biliary tract tumors compared with gemcitabine alone[238]. 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 patients[239]. Single-agent sorafenib was moderately active as second-line treatment for CRPC (3.7 months PFS and 18.0 months OS)[240]. For the treatment of glioblastoma or high-grade gliomas, sorafenib alone or combined with temozolomide/radiotherapy or erlotinib did not show efficacy[241,242,243,244]. |
| 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 GIST after progression on imatinib.<br>**Gene Association:** Kinase inhibitors targeting RET, such as sunitinib, may be relevant in a tumor bearing a RET activating mutation or amplification. |

| | |
|---|---|
| | **Supporting Data:** Sunitinib is under clinical investigation in multiple tumor types, although large-scale trials have focused on the indications listed above. 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)[245,246]. Partial responses have also been observed in patients with melanoma (3/36) (Decoster et al., 2010; ASCO Abstract 8518) and were more frequent in melanomas with KIT mutation (3/4) than in melanomas with KIT amplification (1/6)[247]. Sunitinib has been evaluated as maintenance therapy for non-small cell lung cancer (NSCLC)[248]. 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 alone[249]. Sunitinib has shown preliminary activity against soft-tissue sarcomas[250,251] and sunitinib-involving regimens have provided significant benefit to at least 5 patients with angiosarcoma, including one complete response[252,253,254,255,256]. In several Phase 2 trials of patients with glioblastoma, sunitinib did not achieve objective responses or improve clinical outcome[257,258,259,260]. |
| Vandetanib | **Approved Indications:** Vandetanib is a multikinase inhibitor that targets RET, VEGFRs, SRC family kinases, and EGFR. It is FDA approved for the treatment of medullary thyroid cancer.<br>**Gene Association:** Kinase inhibitors targeting RET, such as vandetanib, may be relevant in a tumor bearing a RET activating mutation or amplification.<br>**Supporting Data:** A Phase 1 trial of vandetanib in combination with cyclophosphamide and methotrexate in patients with advanced breast cancer reported partial responses in 2/20 patients and stable disease in three patients[261]. A Phase 1 clinical trial of vandetanib with capecitabine and oxaliplatin as first line treatment of metastatic colorectal cancer reported a 46% response rate[262]. A Phase 3 clinical trial of vandetanib after EGFR inhibitors in advanced non-small cell lung cancer (NSCLC) reported no improvement in overall survival compared with placebo after EGFR inhibitors; some improvement was reported in progression-free survival and response rate[263]. Another Phase 3 clinical trial comparing vandetanib with erlotinib in unselected patients with NSCLC reported similar efficacy between the two[264]. |

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 |
|---|---|
| ● ***EGFR*** exon 19 deletion (L747_A750>P) | EGFR activating mutations or amplification may predict sensitivity to EGFR-targeted therapies, including inhibitors of multiple ERBB family members, or to PI3K/Akt/mTOR inhibitors, either alone or in combination with EGFR-targeted therapies.<br><br>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 "EGFR", "cetuximab", "panitumumab", "erlotinib", "gefitinib", "lapatinib", "afatinib", "osimertinib", "necitumumab", "BIBW 2992", "CO-1686", "AZD9291", "PF-00299804", "HSP90", "reolysin", "solid tumor", and/or "advanced cancer". |

| TITLE | PHASE | TARGETS | LOCATIONS | NCT ID |
|---|---|---|---|---|
| A Phase I/II, Multicenter, Open-label Study of EGFRmut-TKI EGF816, Administered Orally in Adult Patients With EGFRmut Solid Malignancies | Phase 1/Phase 2 | EGFR | Massachusetts, New York, Catalunya (Spain), Fukuoka (Japan), Koeln (Germany), Korea (Korea, Republic of), Madrid (Spain), Ontario (Canada), Singapore (Singapore), Taiwan ROC (Taiwan) | NCT02108964 |
| A Phase Ib/II Study of Pembrolizumab and Monoclonal Antibody Therapy in Patients With Advanced Cancer (PembroMab | Phase 1/Phase 2 | PD-1, EGFR, ERBB2 | Arizona | NCT02318901 |
| The First-in-human Phase I Trial of PU-H71 in Patients With Advanced Malignancies | Phase 1 | HSP90 | New York | NCT01393509 |
| An Open-label, Multicenter, Multinational, Phase 2 Study Exploring the Efficacy and Safety of Neratinib Therapy in Patients With Solid Tumors With Activating HER2, HER3 or EGFR Mutations or With EGFR Gene Amplification. | Phase 2 | EGFR, ERBB2, ERBB4 | California, Massachusetts, Missouri, New Jersey, New York, Tennessee, Texas, Barcelona (Spain), Cremona (Italy), Helsinki (Finland), Madrid (Spain), Petch Tiqwa (Israel), Rehovot (Israel), Torino (Italy), Valencia (Spain), Victoria (Australia) | NCT01953926 |
| A Phase I Trial of the IGF-1R Antibody AMG 479 in Combination With Everolimus (RAD001) and Panitumumab in Patients With Advanced Cancer (The RAP Trial) | Phase 1 | IGF1R, mTOR, EGFR | North Carolina | NCT01061788 |



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## CLINICAL TRIALS TO CONSIDER (cont.)

| GENE | RATIONALE FOR POTENTIAL CLINICAL TRIALS |
|---|---|
| ***PTEN*** splice site 489_492+1delAAAGG | PTEN loss or inactivating mutation may predict sensitivity to PI3K-AKT-mTOR pathway inhibitors or PARP inhibitors.<br><br>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 "PTEN", "PI3K", "AKT", "mTOR", "everolimus", "temsirolimus", "PARP", "olaparib", "rucaparib", "BMN 673", "ABT-888", "veliparib", "E7449", "niraparib", "carcinoma", "solid tumor", and/or "advanced cancer". |

| TITLE | PHASE | TARGETS | LOCATIONS | NCT ID |
|---|---|---|---|---|
| A Phase I Trial of the IGF-1R Antibody AMG 479 in Combination With Everolimus (RAD001) and Panitumumab in Patients With Advanced Cancer (The RAP Trial) | Phase 1 | IGF1R, mTOR, EGFR | North Carolina | NCT01061788 |
| A Phase I, First-in-Human, Dose Escalation Trial of MSC2363318A, a Dual p70S6K/Akt Inhibitor, in Subjects With Advanced Malignancies | Phase 1 | AKT, p70S6K | California, Michigan, Texas, Vermont | NCT01971515 |
| A Phase I Study of BKM120 and Everolimus in Advanced Solid Malignancies | Phase 1 | mTOR, PI3K | Georgia | NCT01470209 |
| A Phase I Multi-centre Trial of the Combination of Olaparib (PARP Inhibitor) and AZD5363 (AKT Inhibitor) in Patients With Advanced Solid Tumours | Phase 1 | PARP, AKT | Newcastle upon Tyne (United Kingdom), Surrey (United Kingdom) | NCT02338622 |

## CLINICAL TRIALS TO CONSIDER (cont.)

| GENE | RATIONALE FOR POTENTIAL CLINICAL TRIALS |
|---|---|
| ● ***RET*** amplification - equivocal | RET amplification, activating mutations, or fusions may predict sensitivity to kinase inhibitors targeting RET.<br><br>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 "RET", "vandetanib", "sorafenib", "sunitinib", "ponatinib", "regorafenib", "cabozantinib", "lenvatinib", "XL184", "carcinoma", "solid tumor", and/or "advanced cancer". |

| TITLE | PHASE | TARGETS | LOCATIONS | NCT ID |
|---|---|---|---|---|
| Phase II Trial of Cabozantinib (XL184) in Patients With Advanced Solid (Non-breast, Non-prostate) Malignancies and Bony Metastases | Phase 2 | AXL, FLT3, KIT, MET, RET, VEGFRs, TIE2, TRKB | Massachusetts | NCT01588821 |
| A Phase 1b, Multi-center, Non-randomized, Open Label, Dose Escalation Design Study of Regorafenib (BAY73-4506) in Combination With Cetuximab in Subjects With Locally Advanced or Metastatic Solid Tumors Who Are Not Candidates for Standard Therapy or in Whom Regorafenib or Cetuximab is Considered as a Standard Treatment | Phase 1 | RAFs, EGFR, KIT, PDGFRs, RET, VEGFRs | California, Colorado, Missouri, Pennsylvania | NCT01973868 |
| An Open-Label, Phase 1/1b, Single-Agent Study of RXDX-105 in Patients With Advanced Solid Tumors | Phase 1 | BRAF, RET, EGFR | California, Florida, Michigan, Missouri, Pennsylvania | NCT01877811 |
| A Phase 1/1b Study of MGCD516 in Patients With Advanced Solid Tumor Malignancies | Phase 1 | TRKs, MET, AXL, VEGFRs, PDGFRs, DDR2, EPHs | Alabama, California, Massachusetts, Michigan, Missouri, New Mexico, New York, South Carolina, Tennessee, Texas, Utah, Washington, Wisconsin | NCT02219711 |

# 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.

***BRAF***
E533Q,amplification

***CD274***
H233L

***CDK6***
amplification

***EGFR***
amplification

***MET***
amplification

***NF1***
S2040F

***PTEN***
K163N

***SMO***
amplification

# APPENDIX

## GENES ASSAYED IN FOUNDATIONACT™

FoundationACT interrogates the complete exonic sequence of 27 genes, introns of 6 genes involved in rearrangements, and select exons of an additional 34 genes. The assay will be updated periodically to reflect new knowledge about cancer biology.

**DNA Gene List: Entire Exonic Sequence for the Detection of Base Substitutions, Insertions/Deletions, and Copy Number Alterations**

| | | | | | |
|---|---|---|---|---|---|
| *BRCA1* | *CDK4* | *ERBB2* | *KRAS* | *MYCN* | *PTPN11* |
| *BRCA2* | *CDK6* | *ERRFI1* | *MDM2* | *NF1* | *SMO* |
| *CCND1* | *CDKN2A* | *FGFR1* | *MET* | *PDCD1LG2 (PD-L2)* | *TP53* |
| *CD274 (PD-L1)* | *CRKL* | *FGFR2* | *MYC* | *PTEN* | *VEGFA* |
| *CDH1* | *EGFR* | *FOXL2* | | | |

**DNA Gene List: For the Detection of Select Rearrangements**

| | | | | | |
|---|---|---|---|---|---|
| *ALK* | *EGFR* | *FGFR3* | *PDGFRA* | *RET* | *ROS1* |

**DNA Gene List: Select Exonic Sequence for the Detection of Base Substitutions, Insertions/Deletions, and Copy Number Alterations**

| | | | | | |
|---|---|---|---|---|---|
| *ABL1*<br>Exons 4-9 | *CTNNB1*<br>Exon 3 | *GNA11*<br>Exons 4,5 | *JAK2*<br>Exon 14 | *MTOR*<br>Exons 19,30,39,40, 43-45,47,48,53,56 | *PIK3CA*<br>Exons 2,3,5-8,10,14, 19,21 |
| *AKT1*<br>Exon 3 | *DDR2*<br>Exons 5,17,18 | *GNAQ*<br>Exons 4,5 | *JAK3*<br>Exons 5,11-13,15,16 | *MYD88*<br>Exon 4 | *RAF1*<br>Exons 3,4,6,7,10,14, 15,17 |
| *ALK*<br>Exons 20-29 | *ESR1*<br>Exons 4-8 | *GNAS*<br>Exon 1 | *KIT*<br>Exons 8,11,12,17 | *NPM1*<br>Exons 4-6,8,10 | *RET*<br>Exons 11,13-16 |
| *ARAF*<br>Exons 4,5,7,11,13, 15,16 | *EZH2*<br>Exon 16 | *HRAS*<br>Exons 2,3 | *MAP2K1 (MEK1)*<br>Exons 2,3 | *NRAS*<br>Exons 2,3 | *TERT*<br>(Promoter only) |
| *BRAF*<br>Exons 11-18 | *FGFR3*<br>Exons 7,9,14 | *IDH1*<br>Exon 4 | *MAP2K2 (MEK2)*<br>Exons 2-4,6,7 | *PDGFRA*<br>Exons 12,18 | |
| *BTK*<br>Exons 2,15 | *FLT3*<br>Exons 14,15,20 | *IDH2*<br>Exon 4 | *MPL*<br>Exon 10 | *PDGFRB*<br>Exons 12-21,23 | |



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# APPENDIX

## FOUNDATIONACT™ PERFORMANCE SPECIFICATIONS

| | Mutant Allele Frequency (MAF) / Tumor Fraction† | Sensitivity | Positive Predictive Value (PPV) |
|---|---|---|---|
| **Base Substitutions** | ≥0.5% | >98.9% (99.6%-100%)* | > 99.9% (99.6%-100%)* |
| | 0.1% - 0.5% | 67.3% (61.7%-72.5%)* | 93.6% (89.2%-96.3%)* |
| **Insertions/Deletions (1-40 bp)** | ≥1% | >99% (97.2%-100%)* | 98.8% (95.3%-99.8%)* |
| **Rearrangements**** | ≥1% | >99% (90.8%-100%)* | 98.0% (87.8%-99.9%)* |
| | <1% | 86.8% (71.1%-95.1%)* | > 99% (87.0%-100%)* |
| **Copy Number Amplifications‡** | ≥20% | 95.3% (82.9%-99.2%)* | 97.6% (85.9%-99.9%)* |
| **REPRODUCIBILITY** (average concordance between replicates) | | 96.8% inter-batch precision<br>100% intra-batch precision | |

*95% Confidence Interval

** Performance for gene fusions within targeted introns only. Sensitivity for gene fusions occurring outside targeted introns or in highly repetitive intronic sequence contexts is reduced.

† Copy Number Amplifications were calculated using Tumor Fraction.

‡ Copy-number ≥8 in genes with at least four targets.

Assay specifications are based on a minimum unique median exon coverage of 5,000x. For cell-free DNA input of ≥ 50 ng, the unique median exon coverage is typically 6,000-10,000x.

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

# APPENDIX

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## ABOUT FOUNDATIONACT™

**FoundationACT™:** FoundationACT was developed and its performance characteristics determined by Foundation Medicine, Inc. (Foundation Medicine). FoundationACT 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. FoundationACT 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:** FoundationACT identifies alterations to select cancer-associated genes or portions of genes (biomarkers).

**Qualified Alteration Calls (equivocal):** All equivocal calls, regardless of alteration type, imply that there is adequate evidence to call the alteration with confidence. However, the repeatability of equivocal calls may be lower than non-equivocal calls. The threshold used in FoundationACT for identifying a copy number amplification is five (5) for ERBB2 and six (6) for all other genes. For copy number amplifications, the equivocal status may be applied to calls in samples with calculated tumor fraction <30% but above the noise threshold. In addition, copy number amplifications in genes with three (3) baited exons are also marked as equivocal. For substitutions, the equivocal status is applied to calls with allele frequency between 0.1% and 0.5%.

**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 FoundationACT.

**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 of variant characteristics may result in reduced sensitivity. These include: low sample quality, deletions and insertions >40bp, or repetitive/high homology sequences. FoundationACT is performed using cell-free DNA, and as such germline events may not be reported. The following target typically has low coverage resulting in a reduction in sensitivity: *TP53* exon 1 and *PDGFRA* exon 12.