oncoExTra™

EXACT SCIENCES

Report Date: MM/DD/YYYY

| | | | |
|---|---|---|---|
| Patient: | Sample Patient | Ordering Client: | Medical Center |
| Sex at Birth: | Female | Specimen Type: | FFPE Block |
| DOB: | MM/DD/YYYY | Specimen Site: | Bladder |
| Medical Record #: | MR 000000 | Tumor Collection Date: | MM/DD/YYYY |
| Client Accession #: | CA 000000 | Normal Collection Date: | MM/DD/YYYY |
| Ordering Physician: | Sample Physician | Received Date: | MM/DD/YYYY |

**Results Snapshot**

Analytes sequenced: DNA+RNA
Actionable Targets: 4
TMB: Low
MSI: Stable
Clinical Trials: Yes

Diagnosis: **Bladder cancer**



## KEY BIOMARKER FINDINGS

| KEY BIOMARKERS | FDA-APPROVED DRUGS -for patient's cancer[1] | FDA-APPROVED DRUGS -for another cancer[1] | DRUGS PREDICTED NON-BENEFICIAL/ REDUCED BENEFIT | POTENTIAL CLINICAL TRIALS |
|---|---|---|---|---|
| ARID1A (E2250fs) | | | | Yes |
| FGFR3/TACC3 (Fusion) | erdafitinib | lenvatinib, pazopanib, ponatinib | | Yes |
| PIK3CA (E542K) | | alpelisib, copanlisib, everolimus, temsirolimus | | Yes |
| TP53 (R273fs) | | | | Yes |
| **TUMOR MUTATION BURDEN (TMB)** | | | | |
| LOW (1 mut/Mb) | | | | No |
| **MICROSATELLITE STATUS (MSI)** | | | | |
| STABLE | | | | No |

## HIGH INTEREST BIOMARKERS

*As part of the OncoExTra test, key biomarkers relevant in the patient's tumor type have been assessed:* ***NTRK1, NTRK2, NTRK3, RET, BRAF, FGFR2, FGFR3****. If clinically pertinent event(s) in these biomarkers have been identified, the biomarker(s) will appear within the 'Key Biomarker Findings' section of the report. If Biomarkers from this list do not appear, clinically pertinent event(s) have not been identified or fell outside of the OncoExTra reporting thresholds (please see Disclaimer Limitations information).*

## ADDITIONAL SIGNIFICANT ALTERATIONS

| | |
|---|---|
| TERT (c.-124C>T) | No |

*NOTE: The FGFR3/TACC3 fusion was detected at both the RNA level and as a structural duplication at the DNA level in the sample. The FGFR3/TACC3 fusion event is reported in the Key Biomarker Findings section of the report, and the structural duplication at the DNA level of the same is listed in the VUS section to avoid repetition of contents related to therapy and clinical trials.

[1]The prescribing information for the FDA-approved therapeutic option may not include the associated Key Biomarker.

# Genomic Alterations Detail

| Genomic Alteration | | Therapeutic Implication | |
|---|---|---|---|
| Alteration: | ARID1A (E2250fs) | **Drug** | **Status** |
| Alteration Type: | Frameshift | See Clinical Trials Section | |
| Coordinate: | chr1:27107135 | | |
| Allele Frequency: | 35% | | |
| Origin: | DNA | | |
| Read Depth: | 335 | | |
| Location: | 20/20 | | |

## Biomarker Summary

The AT Rich Interactive Domain 1A (ARID1A) gene encodes BAF250A/ARID1A protein, a member of SWI/SNF ATP-dependent chromatin remodeling complex (Reisman D et al., 2009; PMID: 19234488). ARID1A functions as a tumor suppressor and gate keeper gene; it regulates several biological processes, including cell cycle progression, DNA replication, methylation, and DNA repair, as well as plays a critical role in preventing genomic instability (Clapier CR and Bradley RC., 2009; PMID: 19355820). Somatic mutations in ARID1A, which lead to loss of protein expression, including deletions, insertions, and point mutations, have been reported in several cancer types (Aso T et al., 2015; PMID: 26637902, Lee SY et al., 2015; PMID: 25503393, Cajuso T et al., 2014; PMID: 24382590, Ross JS et al., 2015; PMID: 26182302). Pre-clinical studies have shown that ARID1A-deficient cells show an increased sensitivity to treatment with PARP inhibitors and inhibitors of the PI3K/AKT pathway (Shen J et al., 2015; PMID: 26069190, Samartzis EP et al., 2014; PMID: 24979463). EZH2 inhibition is another therapeutic option for ARID1A-mutated tumors, as it exploits the PRC2 (polycomb repressive complex 2) dependency in these malignancies, leading to synthetic lethality and tumor cell death (Bitler BG et al., 2015; PMID: 25686104, Alldredge JK and Eskander RN, 2017; PMID: 29093822, Kim KH et al., 2015; PMID: 26552009). Although ARID1A truncating mutations, potentially translating into ARID1A protein loss in a subset of high-grade bladder cancers, are the most common SWI/SNF genetic alterations in bladder cancer, one study suggested that ARID1A-deficiency is not a predictive biomarker for EZH2-inhibitor treatment response in bladder cancer (Garczyk S et al., 2018; PMID: 30138427). Lastly, the multi-kinase inhibitor dasatinib has also been shown to cause synthetic lethality in ARID1A-deficient tumors in vitro and in vivo, via the p21/RB1 pathway, and is being evaluated in clinical trials (Miller RE et al., 2016; PMID: 27364904).

## Molecular Function

The tumor sample harbors a frameshift mutation which is predicted to result in premature truncation of the protein, leading to loss of function (Weigert O et al., 2011; PMID: 22585168). Loss of ARID1A function has been correlated with increased cell proliferation, tumor infiltration, higher tumor grade, and poor overall patient survival (Abe H et al., 2012; PMID: 22915242).





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| Genomic Alteration | |
|---|---|
| Alteration: | FGFR3/TACC3 (Fusion) |
| Alteration Type: | Fused Genes |
| Coordinate: | chr4:1808661; chr4:1741429 |
| Transcript ID: | ENST00000340107.4; ENST00000313288.4 |
| Origin: | RNA |
| Location: | E17; E11 |

| Therapeutic Implication | |
|---|---|
| **Drug** | **Status** |
| erdafitinib (Balversa) | PREDICTED BENEFICIAL |
| lenvatinib (Lenvima) | PREDICTED BENEFICIAL |
| pazopanib (Votrient) | PREDICTED BENEFICIAL |
| ponatinib (Iclusig) | PREDICTED BENEFICIAL |

## Biomarker Summary

FGFR3/TACC3 fusions result from in-frame oncogenic chromosomal rearrangements involving tyrosine kinase coding domains of fibroblast growth factor receptor 3 (FGFR3), and the transforming acidic coiled-coil (TACC) coding domains of TACC3 (Singh D et al., 2012; PMID: 22837387). FGFR3 constitutive expression due to FGFR3-TACC3 fusion has been reported in different malignancies (Granberg KJ et al., 2017; PMID: 28379477, Costa R et al., 2016; PMID: 27409839, Helsten T et al. 2016; PMID: 26373574). The FGFR/TACC fusion proteins demonstrated oncogenic activity when introduced into astrocytes in brain tissue of pre-clinical models. The fusion protein had a constitutively active tyrosine kinase domain and promoted aneuploidy. Inhibition of FGFR kinase corrected the aneuploidy, and oral administration of FGFR inhibitors prolonged the survival of animal models harboring intracranial FGFR3/TACC3-initiated glioma. Patients with FGFR/TACC fusions could potentially benefit from targeted FGFR kinase inhibition (Singh D et al., 2012; PMID: 22837387). Currently, several multi TKI inhibitors that also target FGFRs have been approved by the FDA, including lenvatinib, pazopanib, ponatinib, and erdafitinib. Erdafitinib is currently approved for advanced or metastatic urothelial carcinoma that has susceptible FGFR2 or FGFR3 genetic alterations and progressed during or following at least one line of prior platinum containing chemotherapy including within 12 months of neoadjuvant or adjuvant platinum-containing chemotherapy. Clinical trials are underway investigating the efficacy of FGFR inhibitors in FGFR altered tumors.

## Molecular Function

FGFR3/TACC3 is a fusion oncogene detected in the tumor sample with breakpoints in FGFR3 exon 17 and exon 11 of TACC3 (Qin A et al., 2019; PMID: 30267839). Reports of non-small cell lung cancer (NSCLC) have shown fusion rearrangements involving FGFR3 and TACC3 with breakpoints in intron 17, exon 17, and exon 18 of FGFR3, which maintain the kinase domain, fused to multiple different introns and exons of TACC3, while retaining the coiled-coil domain (Qin A et al., 2019; PMID: 30267839, Zhou Z et al., 2021; PMID: 33710807). The FGFR3/TACC3 chimeric protein has been reported to localize to mitotic spindle poles, displaying constitutive kinase activity and inducing mitotic and chromosomal segregation defects, and triggering aneuploidy (Singh D et al., 2012; PMID: 22837387). In vitro, the fusion was also seen to result in IL3-independent growth of tumor cells, which were also sensitive to pan-FGFR inhibitors (Capelletti M et al., 2014; PMID: 25294908). FGFR3/TACC3 gene fusion in NSCLC has been suggested as an acquired resistance mechanism, which can bypass EGFR blockade by all generations of EGFR tyrosine kinase inhibitors (TKIs), including osimertinib (Ou SI et al., 2017; PMID: 28838400).





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| Genomic Alteration | |
|---|---|
| Alteration: | PIK3CA (E542K) |
| Alteration Type: | Missense |
| Coordinate: | chr3:178936082 |
| Allele Frequency: | 36% |
| Origin: | DNA |
| Read Depth: | 535 |
| Location: | 10/21 |

| Therapeutic Implication | |
|---|---|
| **Drug** | **Status** |
| alpelisib (Piqray) | PREDICTED BENEFICIAL |
| copanlisib (Aliqopa) | PREDICTED BENEFICIAL |
| everolimus (Afinitor) | PREDICTED BENEFICIAL |
| temsirolimus (Torisel) | PREDICTED BENEFICIAL |

## Biomarker Summary

The phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) gene encodes the protein p110-alpha, which is the catalytic subunit of phosphatidylinositol 3-kinase (PI3K). The PI3K pathway is involved in cell signaling that regulates several critical cellular functions, including cell growth, proliferation, differentiation, motility, and survival (Samuels Y et al., 2005; PMID: 15950905, Engelman JA et al., 2009; PMID: 19629070). In bladder cancer, activating mutations of PIK3CA are found in bladder tumors of all grades and stages. Although these are more common in cancers of low grade and stage (26–34 %), they are also found at significant frequency (12–20 %) in advanced urothelial cancer (> stage T2) (López-Knowles E et al., 2006; PMID: 16885334, Platt FM et al., 2009; PMID: 19789314, Weinstein JN et al., 2014; PMID: 24476821, Ross et al., 2016; PMID: 27465249). Mutations that activate the PI3K/Akt/mTOR pathway may predict sensitivity to PI3K or Akt inhibitors, which are under investigation in clinical trials, or to mTOR inhibitors, which are approved in some tumor types and in clinical trials for other solid tumors (Janku F et al., 2011; PMID: 21216929, Massacesi C et al., 2013; PMID: 23551097). Even though activating PIK3CA mutations may predict sensitivity to PI3K/Akt/mTOR pathway inhibitors, there is contradictory data about the role of PIK3CA in predicting drug response to mTOR inhibitors (Kim A et al., 2013; PMID: 23475782). It is to be noted that response to mTOR inhibitors in certain cancers appears to be independent of molecular markers of PI3K/Akt pathway activation (Oza AM et al., 2011; PMID: 21788564, Trédan O et al., 2013; PMID: 23238879). There are also conflicting reports on the role of PIK3CA mutations in causing resistance to anti-EGFR therapy (de Roock W et al., 2010; PMID: 20619739, Sartore-Bianchi A et al., 2009; PMID: 19223544, Prenen H et al., 2009; PMID: 19366826). The FDA approved alpelisib in combination with fulvestrant for postmenopausal women, and men, with hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative, PIK3CA-mutated, advanced or metastatic breast cancer as detected by an FDA-approved test following progression on or after an endocrine-based regimen (André F et al., 2019; PMID: 31091374).

## Molecular Function

PIK3CA (E542K) is a hotspot mutation which lies within the PIK helical domain of the Pik3ca protein (Cheung LW et al., 2011; PMID: 21984976). In vitro studies have shown that E542K results in increased phosphorylation of Akt, growth factor-independent cell survival, and is transforming (Qiu W et al., 2006; PMID: 16533766, Dogruluk T et al., 2015; PMID: 26627007, Ng PK et al., 2018; PMID: 29533785). In cell culture, expression of PIK3CA E542K confered sensitivity to inhibition of PI3Kα (alpelisib), PI3K/MTOR (BEZ235), and AKT (MK2206) as well as MEK1/2 inhibition (trametinib) (Dogruluk T et al., 2015; PMID: 26627007), but in a different context was associated with resistance to doxorubicin and the PIK3CA inhibitor BKM120 (Chen L et al., 2018; PMID: 29636477). PIK3CA-mutant cells were capable of proliferation-independent of EGF and mTOR activation, suggesting that these cells adopt additional pathways that lead to tumor progression (Gustin JP et al., 2009; PMID: 19196980). In a Phase III trial (SOLAR-1) that supported FDA approval, alpelisib and fulvestrant combination treatment resulted in improved median progression-free survival (11 vs 5.7 mo, HR=0.65, p<0.001) compared to fulvestrant plus placebo in patients with advanced hormone-receptor positive, Erbb2 (Her2) negative breast cancer who had prior endocrine therapy and harboring PIK3CA mutations as detected by an FDA-approved test, including PIK3CA E542K (André F et al., 2019; PMID: 31091374).





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| Genomic Alteration | |
|---|---|
| Alteration: | TP53 (R273fs) |
| Alteration Type: | Frameshift |
| Coordinate: | chr17:7577122 |
| Allele Frequency: | 37% |
| Origin: | DNA |
| Read Depth: | 353 |
| Location: | 8/11 |

| Therapeutic Implication | |
|---|---|
| **Drug** | **Status** |
| See Clinical Trials Section | |

## Biomarker Summary

The tumor protein p53 (TP53) gene encodes a tumor suppressor protein and oncogene with three main domains: transcriptional activation, DNA binding (DBD) and oligomerization, which keeps the cells under control by inducing cell cycle arrest, apoptosis, senescence, DNA repair or changes in metabolism when cells are stressed or damaged (Wang LH et al., 2018; PMID: 30562755, Yamamoto S and Iwakuma T., 2018; PMID: 30577483). TP53 mutations have been reported in 50% of bladder cancer patients and in 76% of muscle-invasive bladder cancer (MIBC) cases in association with poor disease outcome (Cazier J-B et al., 2014; PMID: 24777035, Weinstein JN et al., 2014; PMID: 24476821, Rentsch CA et al., 2017; PMID: 28688612, Wu G et al., 2019; PMID: 31871844). Clinically, patients with muscle-invasive bladder cancer (MIBC) have more TP53 mutations than patients with non-muscle-invasive bladder cancer (NMIBC) (Wu G et al., 2019; PMID: 31871844). At present, there are no approved therapies targeting TP53 alterations, despite their high prevalence in cancer. Tumors with TP53 mutations may be sensitive to the Wee1 inhibitor MK-1775; clinical trials are currently underway for patients with solid tumors (Hirai H et al., 2010; PMID: 20107315, Bridges KA et al., 2011; PMID: 21799033). A phase II study of WEE1 inhibitor AZD1775 plus carboplatin in patients with TP53-mutated refractory ovarian cancer showed median PFS and OS of 5.3 months and 12.6 months, with 2 patients demonstrating ongoing response for more than 31 and 42 months at data cutoff (Leijen S et al., 2016; PMID: 27998224). According to a report, TP53 mutations predict sensitivity to VEGF/VEGFR inhibitors in the clinic (Wheler JJ et al., 2016; PMID: 27466356).

## Molecular Function

The tumor sample harbors a frameshift mutation which is predicted to result in loss of function of TP53. Loss of function mutations within TP53 functional domains are predicted to be deleterious (Joerger AC and Fersht AR., 2008; PMID: 18410249, Kato S et al., 2003; PMID: 12826609).





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# Additional Significant Alterations Detail

| Additional Significant Alteration | | Therapeutic Implication |
|---|---|---|
| Alteration: | TERT (c.-124C>T) | **Status** |
| Alteration Type: | Upstream Gene Variant | See Clinical Trials Section |
| Coordinate: | chr5:1295228 | |
| Allele Frequency: | 34% | |
| Origin: | DNA | |

## Biomarker Summary

The telomerase reverse transcriptase (TERT) gene encodes one of the subunits of telomerase, thereby playing a major role in the maintenance of telomere length via reverse transcriptase activity (Autexier C and Lue NF., 2006; PMID: 16756500, Roake CM and Artandi SE., 2020; PMID: 32242127). The protein component possesses reverse transcriptase activity, while the RNA component serves as a template for the telomere repeat. TERT plays a critical role in cellular aging and is repressed in somatic cells resulting in progressive shortening of telomeres (Patel PL et al., 2016; PMID: 27503890). TERT may also play a role in chromosomal repair synthesis of telomere repeats. Deregulation of TERT function because of TERT promoter mutations (TERTpm) is associated with oncogenic events in diverse tumor types (Heidenreich B et al., 2014; PMID: 24657534, Pezzuto F et al., 2017; PMID: 28529542). TERTpm are independent of other gene mutations in bladder cancer (Nickerson ML et al., 2014; PMID: 25225064). According to one report, about 65.4% of bladder tumors carried a defined TERTpm (Rachakonda PS et al., 2013; PMID: 24101484). TERT mutation in urine samples has been reported as a predictor of urothelial bladder cancer recurrence. TERT-positive status after initial surgery increased risk of recurrence by 5.34-fold, and TERT-positive status was associated with recurrence in the subset of patients with negative cystoscopy (Descotes F et al., 2017; PMID: 28683471). Several TERT-related therapies are currently being evaluated in clinical settings. Investigational agents, like imetelstat (GRN163L), telomestatin, and telomelysin, have been found to be effective in GBM cells in vitro and in clinical studies (Bollam SR et al., 2018; PMID: 29525892).

## Molecular Function

TERT (c.-124C>T) (also known as C228T and C124T) is a mutation in the GC-rich promoter sequence of the TERT gene (Panebianco F et al., 2019; PMID: 31408918, Huang D-S et al., 2015; PMID: 25843513). This mutation creates a de novo binding motif for ETS transcription factors, and it has been shown that GA-binding proteins (GABPA and GABPB1) are specifically recruited to the mutant rather than wt TERT promoter in cancer cells, thereby causing sustained telomerase activity and cancer progression (Huang FW et al., 2013; PMID: 23348506, Bell RJ et al., 2015; PMID: 25977370, Mancini A et al., 2018; PMID: 30205050, Li Y et al., 2015; PMID: 26389665). In vitro studies have shown that the selective inhibition of GABPA or GABPB1 expression rather than other ETS members leads to diminished TERT expression in cancer cells bearing a mutant TERT promoter (Bell RJ et al., 2015; PMID: 25977370, Mancini A et al., 2018; PMID: 30205050). TERT mutations are associated with shorter overall survival (Mosrati MA et al., 2015; PMID: 26143636, Nonoguchi N et al., 2013; PMID: 23955565). In HCC cell lines, it has been demonstrated that TERT promoter mutations (TPMs) mark regions where RNA pol II is preferentially recruited to open chromatin, rather than on the wild-type promoter allele (Stern JL et al., 2015; PMID: 26515115). The C228T is a recurrent hotspot mutation (Cerami E et al., 2012; PMID: 22588877, Gao J et al., 2013; PMID: 23550210, Tate JG et al., 2019; PMID: 30371878).





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# Drug Evidence Detail

## Literature Supporting Therapeutic Implication

| Drug | Biomarker | Therapeutic Implication |
|---|---|---|
| **erdafitinib (Balversa)** | FGFR3/TACC3 (Fusion) | PREDICTED BENEFICIAL |

*In an analysis of therapeutic efficacy of erdafitinib in GBM and grade II and III glioma patients , 795 patients (584 GBM, 85 grades II and III with wild-type and 126 with IDH1/2 mutation) were screened for FGFR-TACC fusions. Three of 85 IDH1/2 wild-type (3.5%) but none of 126 IDH1/2-mutant grade II and III gliomas harbored FGFR3-TACC3 fusions. FGFR-TACC rearrangements were present in 17 of 584 GBM (2.9%). Two patients with FGFR3-TACC3 rearrangements who received JNJ-42756493 manifested clinical improvement with stable disease and minor response, respectively.*

https://www.ncbi.nlm.nih.gov/pubmed/25609060 *(Di Stefano AL et al., Clin Cancer Res. 2015 Jul 15;21(14):3307-17)*

*In an open-label, Phase II study, 99 enrolled patients who had locally advanced and unresectable or metastatic urothelial carcinoma with prespecified FGFR alterations to evaluate the FGFR1-4 tyrosine kinase inhibitor erdafitinib. The primary end point was the objective response rate. A total of 99 patients in the selected-regimen group received a median of five cycles of erdafitinib. Of these patients, 43% had received at least two previous courses of treatment, 79% had visceral metastases, and 53% had a creatinine clearance of less than 60 ml per minute. The rate of confirmed response to erdafitinib therapy was 40% (3% with a complete response and 37% with a partial response). Among the 22 patients who had undergone previous immunotherapy, the confirmed response rate was 59%. The median duration of progression-free survival was 5.5 months, and the median duration of overall survival was 13.8 months.*

https://www.ncbi.nlm.nih.gov/pubmed/31340094 *(Loriot Y et al., N Engl J Med 2019; 381:338-348)*

| Drug | Biomarker | Therapeutic Implication |
|---|---|---|
| **lenvatinib (Lenvima)** | FGFR3/TACC3 (Fusion) | PREDICTED BENEFICIAL |

*A 57 y old, post-menopausal woman with platinum resistant metastatic endometrioid endometrial cancer received chemo- and radiation therapy, 9 months after which she presented with disease recurrence. Tumor genomic profiling identified FGFR3-TACC3 fusion, PIK3CA T1025S and a rearrangement involving TSC2. She was treated with an FGFR3 inhibitor in clinical trial settings, following which SD was achieved for nearly 2 months, but treatment was stopped due to toxicity. Based on the presence of PIK3CA mutation, she was subsequently treated with temsirolimus for 8 months, and in addition to it she received letrozole for the following 9 months. Temsirolimus was discontinued after >17 mo because of disease progression.*

https://pubmed.ncbi.nlm.nih.gov/29588307/ *(Dhami J et al., Cold Spring Harb Mol Case Stud. 2018 Apr 2;42)*

*A phase Ib/II trial of lenvatinib plus pembrolizumab in patients with advanced urothelial cancer showed an objective response rate of 25% and a median progression-free survival of 5.4 months. Of the 20 patients enrolled on study, 1 remained in complete remission.*

https://meetinglibrary.asco.org/record/170580/abstract *(Vogelzang NJ et al., J Clin Oncol 37, 2019 (suppl 8; abstr 11))*





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| Drug | Biomarker | Therapeutic Implication |
|---|---|---|
| **pazopanib (Votrient)** | FGFR3/TACC3 (Fusion) | PREDICTED BENEFICIAL |

*In a Phase I clinical trial of metastatic urothelial carcinoma, the combination therapy of everolimus wit pazopanib was investigated. Overall, 23 patients were enrolled who also had molecular profiling as part of the study. The primary end point was objective response rate (ORR); secondary end points were safety, duration of response (DOR), progression-free survival (PFS) and overall survival (OS). One patient had complete response, 3 partial, and 8 had stable disease. Overall ORR was 21%. DOR, PFS, and OS were 6.5, 3.6, and 9.1 months, respectively. Four patients with clinical benefit (one CR, two PR, one SD) had mutations in TSC1/TSC2 or mTOR and a 5th patient with PR had a FGFR3-TACC3 fusion.*

https://pubmed.ncbi.nlm.nih.gov/30220708/ *(Bellmunt J et al., Br J Cancer. 2018 Sep;119(6):707-712)*

*A 57 y old, post-menopausal woman with platinum resistant metastatic endometrioid endometrial cancer received chemo- and radiation therapy, 9 months after which she presented with disease recurrence. Tumor genomic profiling identified FGFR3-TACC3 fusion, PIK3CA T1025S and a rearrangement involving TSC2. She was treated with an FGFR3 inhibitor in clinical trial settings, following which SD was achieved for nearly 2 months, but treatment was stopped due to toxicity. Based on the presence of PIK3CA mutation, she was subsequently treated with temsirolimus for 8 months, and in addition to it she received letrozole for the following 9 months. Temsirolimus was discontinued after >17 mo because of disease progression.*

https://pubmed.ncbi.nlm.nih.gov/29588307/ *(Dhami J et al., Cold Spring Harb Mol Case Stud. 2018 Apr 2;42)*

| Drug | Biomarker | Therapeutic Implication |
|---|---|---|
| **ponatinib (Iclusig)** | FGFR3/TACC3 (Fusion) | PREDICTED BENEFICIAL |

*A 57 y old, post-menopausal woman with platinum resistant metastatic endometrioid endometrial cancer received chemo- and radiation therapy, 9 months after which she presented with disease recurrence. Tumor genomic profiling identified FGFR3-TACC3 fusion, PIK3CA T1025S and a rearrangement involving TSC2. She was treated with an FGFR3 inhibitor in clinical trial settings, following which SD was achieved for nearly 2 months, but treatment was stopped due to toxicity. Based on the presence of PIK3CA mutation, she was subsequently treated with temsirolimus for 8 months, and in addition to it she received letrozole for the following 9 months. Temsirolimus was discontinued after >17 mo because of disease progression.*

https://pubmed.ncbi.nlm.nih.gov/29588307/ *(Dhami J et al., Cold Spring Harb Mol Case Stud. 2018 Apr 2;42)*

*A multicenter retrospective analysis of cholangiocarcinoma (CCA) patients with FGFR genetic aberrations (GAs) was conducted. Of the 377 patients with CCA that were identified, 95 had FGFR1 (2 mutations, FGFR2 (62 fusions, 1 amplification, 7 mutations), FGFR3 (1 fusion, 6 amplifications, 1 mutation), FGFR4 (2 mutations), or FGF19 (11 amplifications). In patients with CCA, FGFR GAs were associated with a longer OS compared with patients without FGFR GAs (37 v 20 months, respectively; P < .001). A total of 36 patients treated with FGFR inhibitors (23 patients received BGJ398 (infigratinib), 8 received ponatinib, 3 received TAS-120, and one patient each received dovitinib and PRN1371). There was no OS difference between CCA with FGFR2 fusions (n = 63) versus other FGFR GAs (n = 29) (P = .60). Patients with FGFR GAs had a better OS with FGFR-targeted therapy compared with standard treatment (P = .01).*

https://ascopubs.org/doi/full/10.1200/PO.17.00080?journalCode=po *(Jain A et al, JCO Precision Oncology, January 17, 2018)*





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| Drug | Biomarker | Therapeutic Implication |
|---|---|---|
| **alpelisib (Piqray)** | PIK3CA (E542K) | PREDICTED BENEFICIAL |

*In a randomized, phase 3 trial, alpelisib plus fulvestrant with placebo plus fulvestrant was compared in patients with HR-positive, HER2-negative advanced breast cancer who had received endocrine therapy previously. Patients were enrolled into two cohorts on the basis of tumor-tissue PIK3CA mutation status. A total of 572 patients underwent randomization, including 341 patients with confirmed tumor-tissue PIK3CA mutations. In the cohort of patients with PIK3CA-mutated cancer, progression-free survival at a median follow-up of 20 months was 11.0 months (95% confidence interval [CI], 7.5 to 14.5) in the alpelisib-fulvestrant group, as compared with 5.7 months (95% CI, 3.7 to 7.4) in the placebo-fulvestrant group (hazard ratio for progression or death, 0.65; 95% CI, 0.50 to 0.85; P<0.001); in the cohort without PIK3CA-mutated cancer, the hazard ratio was 0.85 (95% CI, 0.58 to 1.25; posterior probability of hazard ratio <1.00, 79.4%). Overall response among all the patients in the cohort without PIK3CA-mutated cancer was greater with alpelisib-fulvestrant than with placebo-fulvestrant (26.6% vs. 12.8%); among patients with measurable disease in this cohort, the percentages were 35.7% and 16.2%, respectively.*

https://pubmed.ncbi.nlm.nih.gov/31091374/ *(Andre F et al., N Engl J Med. 2019 May 16;380(20):1929-1940)*

*A phase Ib study evaluated alpelisib plus letrozole's safety, tolerability, and preliminary activity in patients with metastatic ER+ breast cancer refractory to endocrine therapy. Twenty-six patients received letrozole and alpelisib daily. The clinical benefit rate (lack of progression ≥6 months) was 35% (44% in patients with PIK3CA-mutated and 20% in PIK3CA wild-type tumors; 95% CI, 17%-56%), including five objective responses. Of eight patients remaining on treatment ≥12 months, six had tumors with a PIK3CA mutation. Among evaluable tumors, those with FGFR1/2 amplification and KRAS and TP53 mutations did not derive clinical benefit. Overexpression of FGFR1 in ER+/PIK3CA mutant breast cancer cells attenuated the response to alpelisib in vitro.*

https://pubmed.ncbi.nlm.nih.gov/27126994/ *(Mayer IA et al., Clin Cancer Res. 2017 Jan 1;23(1):26-34)*

| Drug | Biomarker | Therapeutic Implication |
|---|---|---|
| **copanlisib (Aliqopa)** | PIK3CA (E542K) | PREDICTED BENEFICIAL |

*A phase I dose escalation study evaluated copanlisib in advanced solid tumors and NHL. Of the 48 patients with solid tumors, clinical responses were observed only in patients from the MTD expansion cohort. An endometrial carcinoma with mutations in PIK3CA and PTEN, and complete PTEN protein loss, achieved a CR after 10 cycles.*

https://pubmed.ncbi.nlm.nih.gov/27672108/ *(Patnaik A et al., Ann Oncol. 2016 Oct;27(10):1928-40)*

*A phase I dose-escalation study of copanlisib in combination with gemcitabine or cisplatin plus gemcitabine in patients with advanced cancer showed promising clinical response. Response rates from the 50 treated patients were as follows: copanlisib plus gemcitabine, 6.3% (one partial response in a patient with peritoneal carcinoma); copanlisib plus CisGem, 12% (one complete response and three partial responses all in patients with Biliary Tract Cancer (response rate 17.4% in patients with Biliary Tract Cancer). Mutations were detected in PIK3CA (1 out of 43), KRAS (10 out of 43) and BRAF (2 out of 22), with PTEN loss in 41% (12 out of 29).*

https://pubmed.ncbi.nlm.nih.gov/29348486/ *(Kim RD et al., Br J Cancer. 2018 Feb 20;118(4):462-470)*





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| Drug | Biomarker | Therapeutic Implication |
|---|---|---|
| **everolimus (Afinitor)** | PIK3CA (E542K) | PREDICTED BENEFICIAL |

*A Phase 2 trial of everolimus in 37 patients with locally advanced or metastatic transitional cell carcinoma of the urothelial tract reported PR in 2 patients and SD in 8; PI3K (E542K) mutation was reported in a patient with objective response.*

https://pubmed.ncbi.nlm.nih.gov/22473592/ *(Seront E et al., Ann Oncol, 2012; 23(10): 2663-70)*

*Next generation sequencing analyzing somatic mutations in patients with exceptional response to everolimus reported PR with PFS of 23.9 months in a renal carcinoma patient harboring PIK3CA (E542K) mutation.*

https://pubmed.ncbi.nlm.nih.gov/26859683/ *(Lim SM et al., Oncotarget. 2016 Mar 1;7(9):10547-56)*

| Drug | Biomarker | Therapeutic Implication |
|---|---|---|
| **temsirolimus (Torisel)** | PIK3CA (E542K) | PREDICTED BENEFICIAL |

*In a retrospective analysis of patients with advanced solid tumors, 35% (6/17) of patients harboring PIK3CA mutations achieved a partial response when treated with PI3K/AKT/mTOR pathway inhibitor based therapies, while only 6% (15/241) of patients without documented PIK3CA mutations responded to similar regiments. One patient with high grade ovarian carcinoma harboring a PIK3CA mutation at the E542 residue had a best overall response of stable disease when treated with temsirolimus and time to progression was longer than 17.9 weeks, with the tumor not progressing at the time of data cutoff.*

https://pubmed.ncbi.nlm.nih.gov/21216929/ *(Janku F et al., Mol Cancer Ther. 2011 Mar;10(3):558-65)*

*In a phase II study to evaluate the benefit of adding temsirolimus to low-dose weekly carboplatin and paclitaxel for patients with recurrent and/or metastatic (R/M) head and neck squamous cell carcinoma (HNSCC), 15 of 36 (41.7%) patients experienced an objective response, all were partial responses (PR), and 19 (52.3%) patients had stable disease (SD) as best response. The median duration on study was 5.3 months and the median progression-free survival and overall survival were 5.9 months and 12.8 months, respectively. PIK3CA E545K and PIK3CA E542K were identified in three patients and one patient, respectively. One patient with a PIK3CA E545K mutation had a PR, with a CR in the target lesion. The other three patients with PIK3CA mutations had SD as best response, with two of these patients experiencing tumor regression. Responses were also seen in patients with other genetic alterations in the PI3K/mTOR pathway.*

https://pubmed.ncbi.nlm.nih.gov/28961834/ *(Dunn LA et al., Ann Oncol . 2017 Oct 1;28(10):2533-2538)*





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# Clinical Trials Report

**Potential trials based on genomic targets indicated in the OncoExTra™ Report**

| Genomic Alterations | Targeted Investigational Agents | Trial IDs |
|---|---|---|
| ARID1A (E2250fs) | AKT inhibitors: (Afuresertib [GSK2110183], Capivasertib [AZD-5363], Ipatasertib [GDC-0068, RG-7440], Miransertib [ARQ092], ARQ751, MK-2206, Triciribine [TCN-P]),<br>ATR inhibitors: (Berzosertib [M 6620, M6620, VX 970, VX970, VE-822], M4344 [VX-803], Ceralasertib [AZD6738], Elimusertib [BAY1895344], RP-3500),<br>ATM kinase inhibitor: (M4076),<br>BET/BRD4 inhibitors: (AZD5153, ZEN-3694 [BETi ZEN-3694], BMS-986158, CPI-0610, PLX51107),<br>EZH2 or EED/PRC2 inhibitors: (Tazemetostat, Valemetostat [DS-3201b, DS-3201], PF-06821497, CPI-0209, EED/PRC2 inhibitor MAK683),<br>mTOR inhibitors: (Everolimus, Temsirolimus, Sapanisertib [INK0128, MLN0128, TAK-228], Vistusertib [AZD2014]),<br>PARP inhibitors: (Niraparib, Olaparib, Rucaparib, Talazoparib, Veliparib [ABT-888], Pamiparib [BGB-290], RBN-2397),<br>Pan-PI3K inhibitors: (Apitolisib [GDC-0980, RG7422]),<br>PI3K/mTOR dual kinase inhibitors: (BEZ235, Gedatolisib [PKI-587, PF-05212384], LY3023414),<br>PIK3Cα/PIK3Cδ inhibitors: (Copanlisib),<br>PIK3Cα inhibitors: (Serabelisib [INK1117, MLN1117, TAK-117]),<br>PIK3Cα/PIK3Cδ/PIK3Cγ inhibitors: (Taselisib [GDC-0032, RG7604]),<br>Src inhibitors: (Dasatinib) | NCT05023655<br>NCT02484404<br>NCT05053971<br>NCT01582191<br>NCT03065062<br>NCT03842228<br>NCT04840589 |
| FGFR3/TACC3 (Fusion) | pan-FGFR inhibitors: (AZD4547, Erdafitinib, INCB054828, LY2874455, PRN1371, BAY1163877, Rogaratinib [BAY1163877], AZD4547, KIN-3248, Gunagratinib [ICP-192]),<br>FGFR inhibitors: (Futibatinib [TAS-120]),<br>FGFR1-3: (Pemigatinib [INCB054828], debio 1347 [CH5183284, FF284], Derazantinib [ARQ 087]),<br>FGFR/VEGFR/KIT/PDGFR/RET multi-kinase inhibitors: (Lenvatinib, Sunitinib),<br>FGFR1-3/PDGFR/VEGFR1-3 multi-kinase inhibitors: (Nintedanib), | NCT03390504<br>NCT05007106<br>NCT04963153<br>NCT05052372 |





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| Genomic Alterations | Targeted Investigational Agents | Trial IDs |
|---|---|---|
| | FGFR1-3/VEGFR1-3/KIT/PDGFR/FLT3 multi-kinase inhibitors: (Pazopanib), FGFR1-3/VEGFR1-3/TIE2/FLT3/BCR-ABL multi-kinase inhibitors: (Ponatinib), FGFR/VEGFR dual kinase inhibitors: (Lucitanib [AL3810, E-3810, E-3810]), FGFR3-selective inhibitors : (TYRA-300) | |
| PIK3CA (E542K) | Pan-PI3K inhibitors: (Apitolisib [GDC-0980, RG7422]), PI3K/mTOR dual kinase inhibitors: (Gedatolisib [PKI-587, PF05212384], Samotolisib [LY3023414]), PIK3Cα inhibitors: (Alpelisib, Taselisib [GDC-0032, RG7604], INK1117 [MLN1117, TAK-117], Inavolisib [GDC-0077, RG6114, RO 7113755], XL147 [SAR245408], CYH33, MEN1611, RLY-2608), PIK3Cα/PIK3Cδ inhibitors: (Copanlisib), AKT inhibitors: (Afuresertib [GSK2110183], Capivasertib [AZD5363, AZD-5363], Ipatasertib [GDC-0068, RG-7440], Miransertib [ARQ092, ARQ 092], ARQ751, MK-2206, Triciribine [TCN-P]), mTOR inhibitors: (Everolimus, Temsirolimus, Sapanisertib [INK0128, MLN0128, TAK-228], Ridaforolimus [AP23573, MK8669, Deforolimus], Vistusertib [AZD2014]) | NCT01582191 NCT03065062 NCT03842228 NCT03006172 NCT03878524 NCT05216432 NCT05300048 |
| TERT (c.-124C>T) | TERT inhibitors: (Telomelysin [OBP-301], Imetelstat [GRN163L]) | Not recruiting for tumor type |
| TP53 (R273fs) | ATR inhibitors: (Berzosertib [M6620, VX-970, VE-822]), Small molecule inhibitor: (AMG 650), TP53 reactivator: (SGT-53), WEE1 inhibitors: (Adavosertib [AZD-1775, MK-1775]) | NCT04802174 NCT02595931 |



## Disclaimer:

These clinical trial results were procured by keyword search on www.ClinicalTrials.gov, last updated on MM/DD/YYYY. The information contained in this site changes frequently and may be out of date. Search terms were based on alterations identified in the OncoExTra Report, drugs indicated in the OncoExTra Report, and the reported cancer type of the patient. The search strategy was not exhaustive and may not have retrieved every relevant trial for this patient. Healthcare professionals are encouraged to investigate other possibilities through additional searches at this site. The identified trials may have specific inclusion or exclusion criteria that would make a trial inappropriate for the patient. Consideration of any listed option should be made in the context of the patient's complete medical history.



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## Variants of Unknown Significance

| Alteration | Alteration Type | Allele Freq |
|---|---|---|
| DUSP4 (L131P) | Missense | 11 |
| ILDR2 (P511_S515del) | Inframe Deletion | 13 |
| KIFC3 (K624*) | Stop Gain | 18 |
| LRRIQ1 (S1711fs) | Frameshift | 12 |
| NCAPG2 (V392I) | Missense | 14 |
| PLCE1 (E1131del) | Inframe Deletion | 14 |
| SLCO5A1 (L236fs) | Frameshift | 14 |
| TACC3_FGFR3 | Breakpoint: Duplication | . |
| UFL1 (E22*) | Stop Gain | 14 |





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# General Information

**Methodology:**
OncoExTra Test is a Next Generation Sequencing tumor/normal exome and tumor RNA Seq assay that provides for the detection of substitutions, insertions, deletions, copy number events, and fusions in tumor tissue. MET exon 14 skipping, AR-v7, and EGFRvIII variants are also detected in RNA. Genomic DNA is extracted from the patient's normal and tumor samples. The isolated DNA is then prepared using a custom xGen target capture (IDT). This library preparation includes shearing, purification, adaptor ligation and PCR amplification. Total RNA is extracted from the patient's tumor sample. The isolated RNA is then prepared using KAPA HyperPrep with Riboerase (Kapa Biosystems). Libraries are then clustered on a flow cell and sequenced using the Illumina NovaSeq 6000.
Sequence data are analyzed using various validated bioinformatics tools and custom Next Generation Sequencing pipeline NG2-LDT 1.1.2. The reference genome assembly used for alignment is NCBI GRCh37. Each tumor's cancer-specific mutations are then queried against a proprietary gene-drug database based on peer reviewed literature to identify potential therapeutic associations.
Copy number events (amplifications/deletions) reported are focal in nature (<25mb).
Allele frequency is dependent on tumor purity. Tumor purity is not taken into account when reporting allele frequencies.
Tumor Mutation Burden (TMB) is determined by measuring the number of somatic mutations occurring in sequenced genes, counting all mutations expected to change the amino acid sequence of the impacted protein. TMB results are rounded to the nearest integer and are classified as follows: TMB-High: ≥ 20 mutations per megabase (mut/Mb); TMB-Intermediate: 6-19 mut/Mb inclusive; TMB-Low: ≤ 5 mut/Mb. "Indeterminate" results may be due to poor sample quality or sequencing coverage. MSI is calculated by scanning certain indels indicative of microsatellite instability. If the number of these, exome wide, is ≥5, then the sample is declared to be "MSI-High". Otherwise, the sample is labelled "MSI-Stable".
Mean target coverage for tumor sample DNA averages 440x (unique reads). Tumor sample RNA averages 121 million reads.

**Immunohistochemistry:**
IHC testing is performed on formalin fixed paraffin-embedded tissue (FFPE) utilizing the detection method of avidin-biotin free polymer and is employed according to an optimized protocol. HER2 testing meets the 2018 ASCO-CAP HER2 testing guidelines in breast cancer and results are reported using the ASCO/CAP scoring criteria as defined as defined in the IHC Thresholds table appearing at the end of the report. For ER and PR, historical cut-offs for all non-breast tissues are followed.
The following are the antibody clones for each test: Anti HER2/neu (4B5); ER (SP1); PR (1E2).
These assays have not been validated on decalcified specimens.
External tissue controls are performed and reviewed on all stains for appropriate positive and negative immunoreactivity and found to be acceptable.
If HER2 by FISH is required, it is currently being performed by PhenoPath: 1737 Airport Way S, Ste 201 Seattle, WA 98134. HER2 FISH testing and scoring by PhenoPath is being completed according to the 2018 ASCO-CAP Guidelines, with its methodology listed in their final report. A copy of the final FISH report is stored and can be provided by Exact Sciences/GHI upon request.

**Limitations:**
Samples with a tumor content of less than 20% may have reduced sensitivity and lead to false negative results. It is also possible that the sample contains a mutation below our established limit of detection (1% allele frequency in hotspots, 5% in other regions), or in a region excluded by our assay.
Alterations present in repetitive or high GC content region or non-coding areas may not be detected. Indels larger than 40bp may not be detected. Copy number signal relative to background noise inherent in DNA from FFPE samples may affect sensitivity of reporting amplifications/deletions. Some gene rearrangements like internal tandem duplications (ITD) involving FLT3 and BCOR may not be reliably detected by the test.
The lack of a variant call does not necessarily indicate the absence of a variant since technical limitations to acquire data in some genetic regions may limit assay detection.
Given the nature of RNA isolated from FFPE, sequencing failures may be seen with highly degraded samples, as they may produce sequence reads too short to align informatically.
Previously unspecified fusions cannot be called by the informatics pipeline if the partner genes occur between two closely adjacent genes on the same strand of the same chromosome. In addition, some fusions that are important in hematolymphoid malignancies, including those involving IGH, are difficult to detect with short read sequencing and may be better detected by other modalities.

**Disclaimer:**
This report does not make any promise or guarantee that a particular drug or treatment regimen will be effective or helpful in the treatment of disease in any patient. This report also makes no promise or guarantee that a drug with a potential clinical benefit will in fact provide a clinical benefit or that a drug with potential lack of clinical benefit will in fact provide no clinical benefit. Exact Sciences expressly disclaims and makes no representation or warranties whatsoever relating, directly or indirectly, to this review of evidence or identified scientific literature, the conclusions drawn from it or any of the information set forth in this report that is derived from such review, including information and conclusions relating to therapeutic agents that are included or omitted from this report. This assay has not been validated on decalcified tissues. Results should be interpreted with caution given the possibility of false negative results on decalcified specimens.

The tests included in this report were developed, and their performance characteristics determined by Exact Sciences. They have not been cleared or approved by the US Food and Drug Administration. The test has been validated as a Laboratory Developed Test per institutional and applicable CLIA regulation (CLIA# 03D2048606) and College of American Pathology (CAP# 8869063) as qualified to perform high complexity clinical laboratory testing. Data interpretations are based on our current understanding of genes and variants as of the report date. Alterations are listed alphabetically and not in order of strength of evidence or appropriateness for the patient's disease. When the report does identify variants with therapeutic implications, this does not promise or guarantee that a particular drug or treatment regimen will be effective or helpful in the treatment of disease in any patient, and the selection of any drug for patient treatment is done at the discretion of the treating physician.

General genomic alterations should be considered in the context of the patient's history, risk factors and any previous genomic testing. Consideration of Variants of Unknown Significance (VUS) may associate with potential therapies in the future. Exact Sciences does not update reports or send notification regarding reclassification of these alterations.

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