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:	Breast
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: 2
TMB: Low
MSI: Stable
Clinical Trials: Yes

Diagnosis: **Triple Negative Breast 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
ETV6/NTRK3 (Fusion)	entrectinib, larotrectinib	crizotinib		Yes
TP53 (H179R)				Yes
TUMOR MUTATION BURDEN (TMB)				
LOW (2 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, BRCA1, BRCA2, ERBB2, ESR1, PIK3CA****. 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

PEG3 (S640*)	No
TERT (c.-124C>T)	No

*NOTE: The ETV6/NTRK3 fusion was detected at both the RNA level and as a structural translocation at the DNA level in the sample. The ETV6/NTRK3 fusion event is reported in the Key Biomarker Findings section of the report, and the structural translocation 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	
Alteration:	ETV6/NTRK3 (Fusion)
Alteration Type:	Fused Genes
Coordinate:	chr12:12006495; chr15:88576276
Transcript ID:	ENST00000396373.4; ENST00000360948.2
Origin:	RNA
Location:	E4; E13

Therapeutic Implication	
Drug	**Status**
crizotinib (Xalkori)	PREDICTED BENEFICIAL
entrectinib (Rozlytrek)	PREDICTED BENEFICIAL
larotrectinib (Vitrakvi)	PREDICTED BENEFICIAL

Biomarker Summary

The neurotrophic receptor tyrosine kinase 3 (NTRK3) gene, also known as TRKC, encodes a member of the tropomyosin receptor kinase (TRK) family, which is a membrane receptor tyrosine kinase that binds neurotrophins, initiating signaling cascades that lead to cell growth and differentiation (Grady WM., 2013; PMID: 23396845). The TRK receptors are composed of an extracellular ligand binding domain (LBD), a transmembrane (TM) region, and an intracellular kinase domain (KD). Upon ligand binding, NTRK proteins undergo oligomerization and phosphorylation of specific tyrosine residues in the KD, which results in activation of signal transduction pathways, leading to proliferation, differentiation, and survival in normal and neoplastic neuronal cells (Nakagawara A et al., 2001; PMID: 11431098). The NTRK3 fusion product results in constitutively activated or overexpressed kinase function conferring oncogenicity (Yeh I et al., 2016; PMID: 27477320). Patients with NTRK fusion-positive cancers respond well to first-generation TRK inhibitors, such as larotrectinib and entrectinib, which are approved by FDA for NTRK fusion-bearing tumors without a known acquired resistance mutation, regardless of histology. A case study reported durable response with entrectinib therapy in a patient with this fusion and a diagnosis of mammary analogue secretory carcinoma (MASC). However, the patient developed resistance to therapy due to acquired mutation in NTRK3 (Drilon A et al., 2016; PMID: 26884591). Tumors with certain acquired resistance mutations have been reported to respond to next-generation TRK inhibitors, including LOXO-195 and TPX-0005 (repotrectinib), which are being explored in clinical trials (Drilon A et al., 2017; PMID: 28578312, Cocco E et al., 2018; PMID: 30333516).

Molecular Function

ETV6/NTRK3 fusion results from the fusion of exon 4 of the transcription factor ETS variant gene 6 (ETV6) with exon 13 of the neurotrophin 3 receptor (NTRK3 or TrkC) gene. This fusion joins exons 1-4 of ETV6 with exons 13-20 of NTRK3, with NTRK3 contributing the binding domain and kinase domain at the 3'-end of the fusion gene (Church AJ et al., 2018; PMID: 29099503). NTRK fusions possess ligand-independent constitutive kinase activity, activate canonical downstream signaling pathways involved in growth and survival, transform primary cells in vitro and in vivo, and can occur in human cancers in a mutually exclusive pattern from other oncogenic drivers. The ETV6/NTRK3 fusion results in activation of multiple signaling cascades, including the ras protein (RAS) and phosphatidylinositol 3-kinase–protein kinase B (PI3K–AKT) pathways. NTRK3 activation has been reported to induce tumor growth and metastasis in certain cancers (Jin W et al., 2010; PMID: 20802235). In a case report of a patient with advanced triple negative breast cancer expressing ETV6/NTRK3 fusion, a rapid response to larotrectinib was reported (Landman Y et al., 2018; PMID: 29233640).

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Genomic Alteration

Alteration:	TP53 (H179R)
Alteration Type:	Missense
Coordinate:	chr17:7578394
Allele Frequency:	51%
Origin:	DNA
Read Depth:	1277
Location:	5/11

Therapeutic Implication

Drug	Status
See Clinical Trials Section	

Biomarker Summary

The tumor protein p53 (TP53) gene encodes a tumor suppressor protein 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 is more frequently mutated in basal-like triple-negative breast cancer (TNBC), with an enrichment of truncating mutations (Shi Y et al., 2018; PMID: 30007403). Loss of Tp53 confer chemotherapy resistance in cancer, which is partly responsible for the poor prognosis of TNBC (Gadhikar MA et al., 2013; PMID: 23839309). 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. WEE1 is a serine/threonine protein kinase that phosphorylates cyclin-dependent kinase 1 (Cdk1), and functions at the G2/M checkpoint of mitosis. Preclinical studies have demonstrated that cancer cell viability can be attenuated after Wee-1 inhibition. As such, cancer cells become sensitized to conventional therapy by Wee-1 inhibition, especially in cells with insufficient G1-arrest due to deficient p53 signaling (Wang Y et al., 2004; PMID: 14726685). Currently, clinical trials of WEE1 inhibitor are ongoing 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 study, TP53 mutations predict sensitivity to VEGF/VEGFR inhibitors in the clinic (Wheler JJ et al., 2016; PMID: 27466356).

Molecular Function

TP53 (H179R) is a missense mutation which lies within the DNA binding domain (DBD) of the Tp53 protein (Varna M et al., 2011; PMID: 21760703). In vitro studies have shown that H179R results in a loss of Tp53 protein function as demonstrated by failure to activate downstream gene transcription and increased survival (Ashur-Fabian O et al., 2007; PMID: 17361096, Mullany LK et al., 2015; PMID: 26585234), and also induces cancer gene signature through activation of Ras signaling (Solomon H et al., 2012; PMID: 22427690). TP53 mutations in the DBD domain are known to result in loss of function of p53 (Kato S et al., 2003; PMID: 12826609).

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Additional Significant Alterations Detail

Additional Significant Alteration		Therapeutic Implication
Alteration:	PEG3 (S640*)	**Status**
Alteration Type:	Stop Gain	No Drug or Clinical Trial
Coordinate:	chr19:57327891	
Allele Frequency:	20%	
Origin:	DNA	
Read Depth:	785	

Biomarker Summary

The tumor sample harbors a stop-gain mutation in the PEG3 gene, which is predicted to result in loss of functional Peg3 protein. Paternally expressed gene (PEG3) is a maternally imprinted tumor suppressor gene that is downregulated in gliomas, ovarian, breast cancers and other gynecologic cancer (Khoda T et al., 2001; PMID: 11260267, Dowdy SC et al., 2005; PMID: 16023706, Feng W et al., 2008; PMID: 18286529). PEG3 mutations have been documented in breasts cancer cases. In addition, PEG3 mutation was found to be associated with high tumor mutation burden and poor prognosis in breast cancer (Zhang M and Zhang J., 2020; PMID: 32729618).

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:	41%	
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 is associated with oncogenic events in diverse tumor types (Heidenreich B et al., 2014; PMID: 24657534, Pezzuto F et al., 2017; PMID: 28529542). TERT promoter hotspot mutations (C228T and C250T) have been documented in breast cancer, although at a very low (0.9%) frequency, one of which (C250T) was also found in a triple-negative breast cancer (TNBC) case (Shimoi T et al., 2018; PMID: 29222734). 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 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
crizotinib (Xalkori)	ETV6/NTRK3 (Fusion)	PREDICTED BENEFICIAL

In a case report of a 34-year-old female diagnosed with Mammary analogue secretory carcinoma (MASC), NGS testing revealed an ETV6-NTRK3 t(12;15)(p13.2;q25.3) rearrangement. The patient he patient was treated with crizotinib. With the initiation of therapy and the performance of intercostal neurolysis, she quickly experienced resolution of her pleuritic pain. Repeat CT imaging at 3 and 10 weeks revealed stable disease (2% and 19% reduction in disease burden, respectively), until 18 weeks when the disease progressed.

https://pubmed.ncbi.nlm.nih.gov/26884591/ *(Drilon A et al., Ann Oncol . 2016 May;27(5):920-6)*

Drug	Biomarker	Therapeutic Implication
entrectinib (Rozlytrek)	ETV6/NTRK3 (Fusion)	PREDICTED BENEFICIAL

In phase 1 or 2 clinical trials (ALKA-372-001, STARTRK-1, and STARTRK-2), which enrolled patients aged 18 years or older with metastatic or locally advanced NTRK fusion-positive solid tumors (10 different tumor types) who received entrectinib. All patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 0-2 and could have received previous anti-cancer therapy (except previous TRK inhibitors). The primary endpoints, the proportion of patients with an objective response and median duration of response, were evaluated by blinded independent central review in the efficacy-evaluable population (ie, patients with NTRK fusion-positive solid tumors who were TRK inhibitor-naive and had received at least one dose of entrectinib). At the data cutoff date for this analysis (May 31, 2018) the efficacy-evaluable population comprised 54 adults. Median follow-up was 12.9 months. Of the 54 patients, 31 (57%; 95% CI 43.2-70.8) had an objective response, of which four (7%) were complete responses and 27 (50%) partial responses. Median duration of response was 10 months (95% CI 7.1 to not estimable).

https://www.ncbi.nlm.nih.gov/pubmed/31838007 *(Doebele RC et al., Lancet Oncol. 2019 Dec 11. pii: S1470-2045(19)30691-6)*

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Drug	Biomarker	Therapeutic Implication
larotrectinib (Vitrakvi)	ETV6/NTRK3 (Fusion)	PREDICTED BENEFICIAL

In two global, multicenter, registrational clinical trials to assess the efficacy and safety of larotrectinib in patients with NTRK fusion-positive lung cancers, the objective response rate (ORR) by investigator assessment among 15 evaluable patients was 73% (95% CI, 45 to 92); one (7%) patient had a complete response, 10 (67%) had a partial response, three (20%) had stable disease, and one (7%) had progressive disease as best response. The median duration of response, progression-free survival, and overall survival were 33.9 months (95% CI, 5.6 to 33.9), 35.4 months (95% CI, 5.3 to 35.4), and 40.7 months (95% CI, 17.2 to not estimable), respectively. Among patients with baseline CNS metastases, the ORR was 63% (95% CI, 25 to 91).

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8830513/ *(Drilon A et al., JCO Precis Oncol. 2022 Jan;6:e2100418)*

Patients (pts) with non-primary CNS solid tumors with brain metastases, or primary CNS tumors harboring a TRK fusion treated with larotrectinib in 2 clinical trials (NCT02637687, NCT02576431) were identified. Larotrectinib was administered until disease progression (PD), withdrawal, or unacceptable toxicity. Disease status was investigator-assessed (RANO and RECIST). 14 pts were identified: 5 non-primary CNS solid tumors (3 lung cancer, 2 thyroid cancer; fusion type: 2 ETV6-NTRK3, 2 SQSTM1-NTRK3, 1 EPS15-NTRK1; age range 25–79 y) and 9 primary CNS tumors (3 glioma, 2 glioblastoma, 1 astrocytoma, 3 NOS; fusion type: 3 BCR-NTRK2, 2 KANK-NTRK2, 1 each of AFAP1-NTRK1, AGTPBP1-NTRK2, ETV6-NTRK3, SPECC1L-NTRK2; age range 2–79 y). In the 5 pts with non-primary CNS tumors, the best objective response to therapy was PR in 3 (60%, 1 pending confirmation), SD in 1 (20%), and not evaluable (NE) in 1 (20%). Duration of response ranged from 9+ to 13 mo. In the 9 pts with primary CNS tumors, disease control was achieved in all evaluable pts (primary PD not observed; 1 pt required dose increase). The best objective response to therapy was PR in 1 (11%; pending confirmation, −55% tumor shrinkage, ongoing at 3.7 mo), SD in 7 (78%; tumor shrinkage range −1% to −24% for pts with measurable disease, 5 had SD > 4 mo), and NE in 1 (11%). Duration of treatment ranged from 2.8–9.2+ mo.

https://ascopubs.org/doi/abs/10.1200/JCO.2019.37.15_suppl.2006 *(Drilon AE et al., Journal of Clinical Oncology 2019 37:15_suppl, 2006-2006)*

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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
ETV6/NTRK3 (Fusion)	NTRK inhibitors: (Larotrectinib, Utatrectinib [AZD745], Selitrectinib [LOXO-195, BAY2731954], PBI-200 [PBI 200, PBI200]), NTRK1-3/ROS1/ALK inhibitor: (Entrectinib), ALK/ROS1/NTRK/SRC multi-kinase inhibitor: (Repotrectinib [TPX-0005]), Pan ALK/MET/TRK inhibitor: (Crizotinib)	NCT02576431 NCT04589845 NCT03093116 NCT05300438
TERT (c.-124C>T)	TERT inhibitors: (Telomelysin [OBP-301], Imetelstat [GRN163L])	Not recruiting for tumor type
TP53 (H179R)	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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Patient:	Sample Patient	**Medical Record #:**	MR 000000
Sex at Birth:	Female	**Client Accession #:**	CA 000000
DOB:	MM/DD/YYYY	**Ordering Physician:**	Sample Physician

EXACT SCIENCES

Report Date: MM/DD/YYYY

Variants of Unknown Significance

Alteration	Alteration Type	Allele Freq
ACRC (S393L)	Missense	49
ACTR10 (E416D)	Missense	20
ADAMTS13 (R692C)	Missense	20
ADNP2 (E960K)	Missense	13
AGBL1 (S884Y)	Missense	12
AIM1 (A235T)	Missense	12
AK7 (H273Q)	Missense	27
ALKBH8 (E371K)	Missense	33
AMY2B (H346Y)	Missense	6
ANK2 (R1585T)	Missense	14
ANKMY2 (M1?)	Start Lost	24
ANKRD40 (S191L)	Missense	11
ANO7 (L16fs)	Frameshift	22
AP3B2 (I150M)	Missense	9
ARHGAP26 (H787N)	Missense	8
ARHGAP44 (E286K)	Missense	10
ARHGEF7 (I779V)	Missense	20
ATAD5 (Q501E)	Missense	18
ATP6V0D2 (R237Q)	Missense	11
ATP8B4 (R628*)	Stop Gain	8
BCL2L2 (K21M)	Missense	20
BCL2L2-PABPN1 (K21M)	Missense	20
BDKRB2 (H381Y)	Missense	7
BEND7 (E52*)	Stop Gain	9
BOD1L1 (L1843F)	Missense	10
BZRAP1 (E741Q)	Missense	11
BZRAP1 (R762S)	Missense	13
C2orf57 (S147T)	Missense	13
C9orf131 (S308F)	Missense	28
CACNA1H (P2306S)	Missense	16
CACNG7 (A183T)	Missense	19
CDH1 (F189L)	Missense	12
CDH13 (G308S)	Missense	10
CDH2 (I836T)	Missense	8
CDX1 (P94S)	Missense	18
CEP170B (V121L)	Missense	10
CEP89 (I363V)	Missense	12
CMTR2 (K732E)	Missense	13
CT45A5 (Q129H)	Missense	37
CTNNA2 (A191S)	Missense	11
CUL1 (Q505H)	Missense	12
CWC22 (S107F)	Missense	9
DCHS1 (P3171L)	Missense	17
DCST2 (P269S)	Missense	37
DIAPH1 (D381N)	Missense	5
DLC1 (E515K)	Missense	5
DOC2B (R61fs)	Frameshift	11
DPP9 (Q661*)	Stop Gain	14
DUSP15 (E207fs)	Frameshift	24
EFCAB1 (R3H)	Missense	7
ETV6_NTRK3	Breakpoint: Translocation	.
F2R (A311fs)	Frameshift	22
FBN1 (G538D)	Missense	7
FCRL2 (Y80C)	Missense	39
FTHL17 (H140Q)	Missense	9
FUS (A369D)	Missense	14
GGT7 (E47K)	Missense	25
GPR179 (K1216T)	Missense	23
GRIN1 (T926I)	Missense	22
H2AFJ (R4G)	Missense	12
HABP4 (R73K)	Missense	19
HCN1 (T375I)	Missense	11
HLTF (I1003M)	Missense	9
HMCN1 (E2340Q)	Missense	9
HOXD9 (Q46fs)	Frameshift	23
HSPG2 (R226G)	Missense	24
IGKV4-1/IGKC	Fused Genes (RNA)	.
IKZF1 (Y371C)	Missense	16
IP6K3 (A85V)	Missense	23
IQCE (G569S)	Missense	10
ITGA1 (S1040N)	Missense	16

Alteration	Alteration Type	Allele Freq
IVL (L492F)	Missense	30
KCNQ3 (I704T)	Missense	8
KIAA0100 (V26L)	Missense	15
KIAA0430 (P761A)	Missense	10
KIAA0754 (E1218K)	Missense	11
KIAA0754 (M193I)	Missense	9
KIAA1009 (E644K)	Missense	11
KIAA1199 (D330N)	Missense	8
KIF12 (G471R)	Missense	10
KIF26A (G540S)	Missense	18
KLHL29 (Q321*)	Stop Gain	19
KRT14 (R125C)	Missense	23
LCMT2 (R82C)	Missense	10
LINGO4 (G579E)	Missense	10
MAK (E503K)	Missense	23
MATK (R171C)	Missense	18
MINK1 (R768L)	Missense	8
MPEG1 (D548H)	Missense	8
MUC19 (E420fs)	Frameshift	12
MUC3A (T410I)	Missense	12
MUC4 (S983F)	Missense	29
MYH2 (R1059K)	Missense	43
NAALADL2 (Y165C)	Missense	11
NLN (A694T)	Missense	12
NLN (V502M)	Missense	10
NSD1 (R632Q)	Missense	16
OR10G9 (P181L)	Missense	6
PAK7 (T690A)	Missense	29
PANX2 (P594R)	Missense	27
PCDHB2 (V643A)	Missense	15
PDE11A (R237P)	Missense	15
PDX1 (H94N)	Missense	14
PPM1G (R463C)	Missense	20
PPP1R13B (R165H)	Missense	9
PPP2R2C (FQ438LE)	Missense	19
PRAMEF4 (S297*)	Stop Gain	39
PRPF40A (W160fs)	Frameshift	10
RALGDS (M902I)	Missense	8
REC8 (c.1060+1G>A)	Splice Donor Variant	17
RECQL4 (R8W)	Missense	37
RNF213 (D608_F609insK)	Inframe Insertion	23
ROBO1 (A777T)	Missense	8
RP11-318A15.7_MFSD11_SRSF2	Breakpoint: Deletion	.
SAMD9L (L1040P)	Missense	17
SEC23A (Q75*)	Stop Gain	10
SEC24B (P104A)	Missense	6
SETX (D1490N)	Missense	12
SLC25A44 (R247*)	Stop Gain	7
SLC27A3 (A100V)	Missense	37
SPATA32 (E204K)	Missense	14
SPEM1 (Q182*)	Stop Gain	35
SPHK2 (H52Y)	Missense	37
STAB1 (T730K)	Missense	8
STX2 (R124Q)	Missense	11
SYNGR4 (F33L)	Missense	50
TAF13 (R37Q)	Missense	9
TAF1L (L1518R)	Missense	5
THSD7B (M142T)	Missense	21
TMC2 (D102E)	Missense	14
TMEM8A (S687L)	Missense	14
TNIP3 (E273K)	Missense	18
TONSL (E26K)	Missense	10
TPT1 (E30Q)	Missense	31
TRA2B (R218L)	Missense	7
TRIM25 (V470M)	Missense	20
TRIM33 (S72L)	Missense	12
TTC17 (K109*)	Stop Gain	9
TTN (D24807N)	Missense	11
TTN (E25436*)	Stop Gain	12
UBQLN4 (D74N)	Missense	19
UNKL (S40L)	Missense	13

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Alteration	Alteration Type	Allele Freq
USP24 (E574K)	Missense	5
USP36 (R1078*)	Stop Gain	12
USP47 (L785V)	Missense	11
UTP15 (c.184-1G>C)	Splice Acceptor Variant	11
WDFY3 (Q2006*)	Stop Gain	7
WDR93 (Y223H)	Missense	8
ZBTB3 (D266H)	Missense	19
ZBTB3 (E377K)	Missense	16
ZNF831 (S930F)	Missense	11

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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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Electronically Signed By

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