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:	Colon
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: **Metastatic malignant neoplasm of sigmoid colon**

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
CTNNB1 (S23_D32delinsN)				Yes
KRAS (A146T)			cetuximab, panitumumab	Yes
TUMOR MUTATION BURDEN (TMB)				
LOW (3 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, ERBB2, KRAS, NRAS****. 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).*

[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:	CTNNB1 (S23_D32delinsN)	**Drug**	**Status**
Alteration Type:	Inframe Deletion	See Clinical Trials Section	
Coordinate:	chr3:41266070		
Allele Frequency:	31%		
Transcript ID:	ENST00000349496		
Origin:	DNA		
Read Depth:	651		
Location:	3/15		

Biomarker Summary

The catenin beta 1 (CTNNB1) gene encodes the protein beta-catenin, a key mediator of WNT signaling, as WNT activation induces stabilization of beta-catenin (Schneikert J and Behrens J, 2007; PMID: 16840506). The beta-catenin protein plays a critical role in formation of adherens junctions (AJs), which is required for the regulation of cell-cell adhesion (Tanabe S et al., 2016; PMID: 27574551). In colorectal cancer (CRC), it has been reported that membranous beta-catenin expression was statistically reduced in the invasive front (IF). Also, loss of membranous beta-catenin in the tumor center (TC) was more common among N2 tumors. Further, expression of beta-catenin in TC and IF, and their mean, was associated with longer disease-free survival (DFS) among CRC patients. Additionally, multivariate analysis suggested that tumor stage and mean beta-catenin expression were prognostic for longer DFS (hazard ratio=0.33; p=0.01) (Kamposioras K et al., 2013; PMID: 24123033). Mutations at the phosphorylation sites or loss of exon 3 in CTNNB1 are associated with increased beta-catenin protein stability and activation of the Wnt pathway (Polakis P, 1999; PMID: 10072352, Abraham SC et al., 2002; PMID: 11943721, Tanaka Y et al., 2001; PMID: 11731417, Takahashi Y et al., 2006; PMID: 16523258, Notani D et al., 2010; PMID: 20126258, Lévy L et al., 2004; PMID: 15060161). There are no approved therapies targeting CTNNB1 mutation, but WNT pathway inhibitors are in clinical trials for solid tumors (Tanwar PS et al., 2011; PMID: 21695255, Kogan Y et al., 2012; PMID: 22356261, Lachenmayer A et al., 2012; PMID: 22811581, Zhu Jet al., 2012; PMID: 22964660). Studies suggest that tumors with CTNNB1 mutations may also respond to sorafenib by inhibiting signaling through the WNT/beta-catenin pathway. In these tumors, sorafenib decreased the beta-catenin protein levels, and reduced liver-related Wnt target genes in experimental models (Lachenmayer A et al., 2012; PMID: 22811581). In a different study, a combination of sorafenib and beta-catenin inhibitor was suggested to be effective in suppressing Wnt-driven tumor cell proliferation (Muche S et al., 2014; PMID: 25202044). Pre-clinical studies have also shown that CTNNB1-mutant cancers have an increased sensitivity for tyrosine kinase inhibitors and the BCL2 inhibitor navitoclax (Zaman GJR et al., 2017; PMID: 28751540, Delgado E et al., 2015; PMID: 25457204, Basu A et al., 2013; PMID: 23993102).

Molecular Function

CTNNB1 (S23_D32delinsN) is an inframe deletion in exon 3, which lies within the ubiquitination recognition motif of the Ctnnb1 protein from amino acids 23 to 32 (Al-Fageeh M et al., 2004; PMID: 15064718). This inframe deletion has not been functionally characterized and hence, its impact on Ctnnb1 protein remains unclear. However, mutations in exon 3 (at specific codons 32, 33, 37, 41 and 47) leading to loss of phosphorylation sites, or in-frame deletions leading to loss of exon 3, can eliminate the phosphorylation sites, resulting in failure of the β-catenin protein to be targeted for proteasomal degradation (Gao C et al., 2017; PMID: 29435196). The S23 is one of the phosphorylation sites for GSK-3β, and alterations at S23, S23R, have been reported to result in enhanced Wnt transcription activity by 2.36 ± 1.01 fold compared to the wild type CTNNB1 control (Manring HR and Antinozzi PA., Cancer Res 2012;72(8 Suppl):Abstract# 2249). Also, other in vitro studies have shown that the related W25_D32del is predicted to confer a gain of function to the Ctnnb1 protein, as demonstrated by nuclear accumulation of Ctnnb1 (Takayasu H et al., 2001; PMID: 11309340, Wei Y et al., 2000; PMID: 10698519).

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Genomic Alteration	
Alteration:	KRAS (A146T)
Alteration Type:	Missense
Coordinate:	chr12:25378562
Allele Frequency:	64%
Transcript ID:	ENST00000256078
Origin:	DNA
Read Depth:	2052
Location:	4/6

Therapeutic Implication	
Drug	**Status**
cetuximab (Erbitux)	PREDICTED NON-BENEFICIAL/ REDUCED BENEFIT
panitumumab (Vectibix)	PREDICTED NON-BENEFICIAL/ REDUCED BENEFIT

Biomarker Summary

The KRAS proto-oncogene, GTPase (KRAS) gene encodes a member of the small GTPase superfamily and a key regulator of the MAPK, PI3K/AKT/mTOR pathways (di Magliano MP and Logsdon CD., 2013; PMID: 23622131). KRAS protein transduces signals by activating the RAF/MEK/ERK and PI3K/mTOR signaling cascade, thereby regulating cell proliferation, survival and differentiation (Pylayeva-Gupta Y et al., 2011; PMID: 21993244, Colicelli J et al., 2004; PMID: 15367757). KRAS is one of the most frequently mutated oncogenes in human cancers (Kortüm KM et al., 2015; PMID: 25743686, Lohr JG et al., 2014; PMID: 24434212). Oncogenic KRAS mutations are known to block EGFR signaling and mediate resistance to EGFR monoclonal antibodies therapy in colorectal cancers (CRC) (Amado RG et al., 2008; PMID: 18316791, Bokemeyer C et al., 2011; PMID: 21228335, Peeters M et al., 2010; PMID: 20921462), and EGFR tyrosine kinase therapy in non-small cell lung cancer (NSCLC) (Riely GJ et al., 2008; PMID: 18832458). Based on clinical guidelines for colon and rectal cancers, patients with any known KRAS mutation (exon 2, 3, 4) or NRAS mutation (exon 2, 3, 4) should not be treated with anti-EGFR monoclonal antibodies (cetuximab and panitumumab) (Van Cutsem E et al., 2011; PMID: 21502544, Douillard JY et al., 2013; PMID: 24024839, Zill OA et al., 2018; PMID: 29776953). The use of MEK inhibitors is predicted to be beneficial in tumors with KRAS mutations (Gilmartin AG et al., 2011; PMID: 21245089). However, clinical studies have suggested limited efficacy of MEK inhibitors in KRAS-mutated tumors. Hence, combinations of MEK inhibitors with other targeted therapies have been suggested to address the limited efficacy observed in these tumors (Adjei A et al., 2008; PMID: 18390968, Infante J et al., 2014; PMID: 24915778, Lito P et al., 2014; PMID: 24746704, Hochster HS et al., 2015; PMID: 25322874, Blumenschein GR et al., 2015; PMID: 25722381, Zhu Z et al., 2014; PMID: 24444711). A Phase 2 trial of selumetinib (a MEK inhibitor) in patients with KRAS-mutant, progressing CRC on first-line therapy reported PR and SD for four weeks in 3/31 and 16/31 of patients, respectively; notably, 3 patients were reported to have SD lasting for >1 year (Hochster HS et al., 2015; PMID: 25322874). A Phase 2 trial of temsirolimus (monotherapy or in combination with irinotecan) in patients with KRAS-mutant CRC reported SD in 24/64 and 40/63 patients, respectively; all responders had low levels of mutant KRAS in plasma samples (Spindler KL et al., 2013; PMID: 23514584). A Phase 2 study comparing selumetinib vs capecitabine in CRC patients reported SD in 10/34 of patients in the selumetinib group compared to 15/35 in the capecitabine group (Bennouna J et al., 2011; PMID: 20127139). Pre-clinical studies have reported that treatment of KRAS-mutant CRC models with MEK inhibitor (binimetinib or trametinib) combined with a Cdk4/6 inhibitor (palbociclib) resulted in greater tumor inhibition in vitro and in vivo in CRC cell lines and PDX models, than either treatment alone (Ziemke EK et al., 2016; PMID: 26369631, Lee MS et al., 2016; PMID: 27167191).

Molecular Function

KRAS (A146T) is missense mutation in exon 4, which lies within a GTP binding region of the Kras protein (Cai X et al., 2014; PMID: 24760004). Mutations of this residue presumably alter the local structure such that the GTP-bound state is much favored over GDP-bound form. In vitro and in vivo models have demonstrated the transforming capability of the KRAS A146T allele, although at lower efficiency compared with Kras codon 12 and 13 mutations (Edkins S et al., 2006; PMID: 16969076). Additionally, A146T results in impaired GTPase activity, increased intrinsic and GEF-mediated nucleotide exchange rate which results in the accumulation of GTP-bound Kras, increased phosphorylation of Mek, Erk, and Akt and therefore is transforming in cell culture and promoting tumor formation in mouse models (Poulin EJ et al., 2019; PMID: 30952657, Tyner JW et al., 2009; PMID: 19075190, Janakiraman M et al., 2010; PMID: 20570890, Weißbach S et al., 2020; PMID: 32079091). Pre-clinical evidence suggests that KRAS exon 4 mutations may predict sensitivity to MEK inhibition and resistance to EGFR-targeted inhibitors. Sensitivity to MEK inhibition leading to growth suppression is thought to occurs via downregulation of cyclin D1, increase in p27 expression, and hypo-phosphorylation of RB1 (Janakiraman M, 2010; PMID: 20570890). In a phase III trial, low-grade serous ovarian carcinoma patients harboring a KRAS mutation had 3.4 times the odds of responding to treatment with binimetinib, as compared to patients without KRAS mutation (Grisham RN et al., JCO.2021.39.15_suppl.5519).

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Drug Evidence Detail

Literature Supporting Therapeutic Implication

Drug	Biomarker	Therapeutic Implication
cetuximab (Erbitux)	KRAS (A146T)	PREDICTED NON-BENEFICIAL/ REDUCED BENEFIT

A meta-analysis of 22 studies that include 2395 CRC patients treated with cetuximab and panitumumab suggests that mutations in KRAS exons 3 and 4, NRAS, BRAF and PIK3CA and non functional PTEN predict resistance to anti EGFR therapies. Mutations in KRAS exons 3 and 4, BRAF, PIK3CA and non-functional PTEN (mutations or loss of protein expression) significantly predicted poor ORR (OR = 0.26, OR = 0.29, OR = 0.39, and OR = 0.41, respectively). Significantly shorter PFS applied to mutations in KRAS exons 3 and 4 (HR = 2.19), NRAS (HR = 2.30) and BRAF (HR = 2.95) and non-functional PTEN (HR = 1.88). Significantly shorter OS applied to mutations in KRAS exons 3 and 4 (HR = 1.78), NRAS (HR = 1.85), BRAF (HR = 2.52), PIK3CA (HR = 1.43) and alterations in PTEN (HR = 2.09).

https://www.ncbi.nlm.nih.gov/pubmed/24666267 *(Therkildsen C et al., Acta Oncol. 2014 Jul;53(7):852-64)*

A retrospective analyses of a large cell-free DNA (cfDNA) deep-sequencing data set of 70 cancer genes from 21,807 patients with treated, late-stage cancers across >50 cancer types revealed subclonal alterations and emerging resistance in 948 CRC samples that harbored the KRAS (AMP, A146P, A146S, A146T, A146V, A18D, A59T, D33E, F156V, G12A, G12C, G12D, G12R, G12S, G12V, G13C, G13D, K117N, L19F, Q22K, Q61H, Q61K, Q61L, Q61R, and V14I) and were associated with resistance to panitumumab and cetuximab.

https://pubmed.ncbi.nlm.nih.gov/29776953/ *(Zill OA et al., Clin Cancer Res. 2018 Aug 1;24(15):3528-3538)*

Drug	Biomarker	Therapeutic Implication
panitumumab (Vectibix)	KRAS (A146T)	PREDICTED NON-BENEFICIAL/ REDUCED BENEFIT

A meta-analysis of 22 studies that include 2395 CRC patients treated with cetuximab and panitumumab suggests that mutations in KRAS exons 3 and 4, NRAS, BRAF and PIK3CA and non functional PTEN predict resistance to anti EGFR therapies. Mutations in KRAS exons 3 and 4, BRAF, PIK3CA and non-functional PTEN (mutations or loss of protein expression) significantly predicted poor ORR (OR = 0.26, OR = 0.29, OR = 0.39, and OR = 0.41, respectively). Significantly shorter PFS applied to mutations in KRAS exons 3 and 4 (HR = 2.19), NRAS (HR = 2.30) and BRAF (HR = 2.95) and non-functional PTEN (HR = 1.88). Significantly shorter OS applied to mutations in KRAS exons 3 and 4 (HR = 1.78), NRAS (HR = 1.85), BRAF (HR = 2.52), PIK3CA (HR = 1.43) and alterations in PTEN (HR = 2.09).

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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
CTNNB1 (S23_D32delinsN)	BCL2 inhibitors: (Venetoclax, Navitoclax [ABT-263], APG-1252), pan-CLK inhibitor: (SM08502), CTNNB1 inhibitors: (Tegatrabetan [Tegavivint, BC2059]), PORCN inhibitors: (LGK974, CGX1321, ETC-1922159 [ETC-159]), RAF inhibitors: (Sorafenib)	NCT02675946 NCT02521844
KRAS (A146T)	KRAS anti-sense: (AZD4785), KRAS inhibitor: (BI 170196), MEK inhibitors: (Binimetinib, Cobimetinib, Selumetinib, Trametinib, PD0325901, E6201, Refametinib [BAY 869766, BAY86-9766, RDEA119], Pimasertib [AS703026, MSC1936369B], HL-085), ERK inhibitors: (LY3214996, KO-947, LTT462, Ulixertinib [BVD-523, VRT752271], ASN007, MK-8353), RAF-MEK dual inhibitors: (RO5126766), Farnesyltransferase inhibitors: (Tipifarnib [Zarnestra, R115777], FTI-277), Focal adhesion kinase (FAK) inhibitors: (Defactinib)	NCT03340506 NCT03377361 NCT03520075 NCT02613650

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
ACRBP (Q105L)	Missense	13
ACTL9 (R35W)	Missense	27
AKR1D1 (R261H)	Missense	24
ANKRD13D (V123I)	Missense	26
ARMC5 (E784D)	Missense	5
ASTN2 (V571I)	Missense	31
ATP6V0A2 (A292T)	Missense	19
BAHD1 (R613*)	Stop Gain	34
BRINP3 (F108V)	Missense	8
C11orf63 (P241L)	Missense	31
CACNA1E (R2041*)	Stop Gain	42
CAMK1 (T28M)	Missense	65
CAPZA3	Amplification	.
CCDC135 (P759L)	Missense	25
CCDC149 (E149A)	Missense	5
CCIN (A200S)	Missense	5
CDH8 (R4W)	Missense	26
CHST11 (E257K)	Missense	24
CNNM1 (E455*)	Stop Gain	33
CNPY1 (L63R)	Missense	20
CNTNAP4 (I997T)	Missense	26
COL6A3 (A2536V)	Missense	37
CORO1B (F40fs)	Frameshift	29
CPS1 (R888H)	Missense	28
DGAT2L6 (A309T)	Missense	7
DIP2B (V963G)	Missense	10
DLG4 (A257V)	Missense	21
DMD (E1044Q)	Missense	6
DMRTB1 (A27V)	Missense	6
DSG4 (R256K)	Missense	26
EDA2R (Q135K)	Missense	24
ELL3 (c.846_866+2delAGATATACCAGACTACCTCCTGT)	Splice Donor Variant	36
EPS8 (E296fs)	Frameshift	28
EPS8 (K301N)	Missense	28
EPS8	Breakpoint: Inversion	.
ESR2 (A357V)	Missense	24
EZR (R526C)	Missense	25
FAM132A (A145V)	Missense	28
FAM135B (V885I)	Missense	19
FAM21B	Deletion	.
FBN1 (T1299M)	Missense	38
FERD3L (A120V)	Missense	20
FLRT2 (A591E)	Missense	38
GAS7 (R16W)	Missense	23
GYS2_BCAT1	Breakpoint: Deletion	.
HCN4 (R378H)	Missense	35
HNRNPA2B1 (E74G)	Missense	17
IL17RB (I332fs)	Frameshift	27
INVS (R597Q)	Missense	26
IQSEC3 (R429Q)	Missense	16
ITGA2B (C87*)	Stop Gain	20
ITGB1BP2 (E67A)	Missense	6
KHDC3L (R30Q)	Missense	18
KIF17 (V827M)	Missense	31
KLHDC1 (S363Y)	Missense	19
LRP1 (R1999H)	Missense	30
MAP4K4 (R1184P)	Missense	28
MAPK6 (R304Q)	Missense	37
MARS2 (R556*)	Stop Gain	32
MDFIC (E201K)	Missense	20
MLH3 (Y1179H)	Missense	13
MLXIPL (Q539H)	Missense	5
MSR1 (V383I)	Missense	23
MST1 (C183R)	Missense	57
NCAM1 (R841Q)	Missense	30
NIN (V1775M)	Missense	21
NLRP5 (A593T)	Missense	10
NME7 (R165I)	Missense	17
NPBWR2 (K257T)	Missense	13
NPHS1 (L1240M)	Missense	15
NRG3 (A34V)	Missense	25

Alteration	Alteration Type	Allele Freq
NRXN1 (G1171A)	Missense	27
OR51I1 (L224R)	Missense	27
OR5B12 (R227C)	Missense	9
OR6B1 (F209L)	Missense	20
PCDHB15 (E203K)	Missense	27
PCLO (P456L)	Missense	21
PDE3A	Amplification	.
PHRF1 (A569S)	Missense	7
PIGA (R77*)	Stop Gain	58
PIK3C2G	Amplification	.
PJA1 (C613Y)	Missense	27
PKM (D238N)	Missense	37
PLCZ1	Amplification	.
PPP1R3G (A352V)	Missense	19
RAI1 (E935K)	Missense	30
RERGL	Amplification	.
RHOBTB1 (P389T)	Missense	27
RICTOR (S1571L)	Missense	51
RP11-296A16.1 (c.*1153_*1173+2delAGATATACCAGACTACCTCCTGT)	Splice Donor Variant	36
RYR2 (A2439T)	Missense	21
SCO1 (L135fs)	Frameshift	20
SIK2 (H134R)	Missense	32
SLC12A3 (R943Q)	Missense	44
SLC17A3 (G449R)	Missense	36
SLC25A33 (G235E)	Missense	30
SLC6A13 (R577Q)	Missense	15
SLCO1C1 (S362R)	Missense	8
SNX29 (G336V)	Missense	21
SPATA6L (E114A)	Missense	7
SPESP1 (I230fs)	Frameshift	36
SSTR3 (R334H)	Missense	30
STK11IP (S752F)	Missense	31
SYPL2 (R9H)	Missense	33
SYT6 (I384T)	Missense	25
TCF20 (K1710R)	Missense	6
TEKT4 (T141A)	Missense	6
TRAF7 (T636M)	Missense	24
TRPV4 (V562I)	Missense	24
TTI1 (A263V)	Missense	25
TYK2 (C1140F)	Missense	26
VCX3B	Deletion	.
ZC3H7B (E555A)	Missense	8
ZEB2 (E1105K)	Missense	27
ZFHX2 (A558T)	Missense	22
ZNF160 (R521H)	Missense	17
ZNF502 (F527I)	Missense	62
ZNF546 (R545I)	Missense	16
ZNF804B (N351T)	Missense	20
ZNF853 (R632H)	Missense	22
ZNF880 (S519P)	Missense	15

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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 1.4.0. 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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