Results for Sample Patient

Date of birth: MM/DD/YYYY
Sex at birth: Female
Medical record #: MR-000000
Client accession #: CA-000000
Diagnosis: Breast Cancer

Blood collection date: MM/DD/YYYY
Tumor collection date: MM/DD/YYYY
Specimen type: UNSTAINED SLIDE
Specimen site: Breast
Tumor content: 20%

Ordering provider: Sample Physician
Order ID: AS5345355
Received date: MM/DD/YYYY
Report date: MM/DD/YYYY

Tumor genomic alterations

Analytes tested: DNA, RNA, Protein (IHC)
Clinical trials available: Yes, see page 4 for details

Tumor mutation burden (TMB): Low (3 mut/Mb)
Microsatellite status (MSI): Stable

Biomarker details	Associated therapies	Level of evidence[1]	Analyte
BRCA1 (M1775R) VAF: 67% `Likely germline`	**Possible benefit:** olaparib, talazoparib	A	DNA
	niraparib, rucaparib	C	
HER2 (Low) Intensity: 1+ %Cells: 20	**Possible benefit:** fam-trastuzumab deruxtecan-nxki	A	Protein (IHC)
PIK3CA (H1047R) VAF: 82%	**Possible benefit:** alpelisib + fulvestrant, capivasertib + fulvestrant, inavolisib + palbociclib + fulvestrant	A	DNA
	everolimus, everolimus + exemestane, temsirolimus	C	

[1] Level of evidence classification: Level A - FDA-approved or included in guidelines for patient's tumor type; Level B - strong evidence from clinical studies; Level C - FDA-approved for another tumor type or moderate evidence from clinical studies; Level D - case reports.

Incidental pathogenic or likely pathogenic germline findings with hereditary cancer risk

BRCA1 (M1775R). See page 4 for more information

Likely germline indicates a variant that is suspected to be of germline origin. These are incidental non-validated findings that may have clinical implications. **Confirmatory germline testing is recommended.** Natera offers germline testing through Empower.

Tumor-specific biomarkers with no actionable alterations

AKT1, BRAF, BRCA2, ERBB2, ESR1, NTRK1, NTRK2, NTRK3, PTEN, RET

IHC result

IHC biomarker (clone)	IHC results	Approved by
PD-L1 (22C3)	%CPS: 0 Negative	Dr. Sample Pathologist MM/DD/YYYY
HER2 (4B5)	Intensity: 1+ %Cells: 20 Low	Dr. Sample Pathologist MM/DD/YYYY

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IHC interpretation thresholds

IHC biomarker (clone)	Negative	Equivocal or not significant	Positive
HER2 (4B5)	0 or 1+/2+ and >0% and ≤10%	2+ and >10% or 3+ and >0% and ≤10%	1+ and >10% (Low) or 3+ and >10%
PD-L1 (22C3) CPS	0 and <9	Not Applicable	≥10

Tumor genomic alteration details

Genomic alteration: BRCA1 (M1775R) VAF: 67%

HGVS: c.5324T>G p.Met1775Arg	**Coordinate:** chr17:41203088
Alteration type: Missense	**Transcript ID:** ENST00000357654
Read depth: 1696	**Location:** 20/23

The BRCA1 gene encodes a nuclear phosphoprotein that plays a role in maintaining genomic stability by DNA repair via homologous recombination (HR) (PMID: 22193408). This alteration, which does not lie within any known functional domain, is predicted to result in the loss of Brca1 protein function (PMID: 23239986, 31143303). The FDA has approved olaparib and talazoparib in HER2-negative breast cancer patients harboring germline BRCA1 alterations.

Drug Evidence for olaparib:

- In a Phase II study, 62 patients with breast cancer harboring BRCA loss of function mutations were treated with olaparib. An overall survival of 11 months and a median progression free survival of 3.7 months were reported (PMID: 25366685).
- In a Phase III study, 205 Her2-negative breast cancer patients harboring germline BRCA mutations were treated with olaparib. A total of 59.9% of patients responded with a median progression-free survival of 7 months (PMID: 28578601).

Drug Evidence for niraparib:

- In a Phase III study, 138 patients with ovarian, fallopian tube, or primary peritoneal cancer harboring a germline BRCA mutation were treated with niraparib. A median progression-free survival of 21 months was reported (PMID: 27717299).
- In a Phase I study, 15 patients with breast cancer harboring BRCA1 mutations were treated with niraparib. A total of 93.3% of patients responded (PMID: 35788722).

Drug Evidence for rucaparib:

- In a Phase II study, 16 patients with ovarian cancer harboring germline BRCA1 mutations were treated with rucaparib. A total of 19% of patients responded to treatment (PMID: 27002934).
- In a Phase I/II study, four patients with breast cancer harboring germline BRCA1/2 mutations were treated with rucaparib. Complete response was achieved by 1 patient with partial response in 3 patients (PMID: 28264872).

Drug Evidence for talazoparib:

- In a Phase III study, 287 breast cancer (triple negative breast cancer or hormone receptor-positve) patients harboring germline BRCA1 (n=133) and BRCA2 (n=154) mutations were treated with talazoparib. The median progression-free survival and overall survival were 8.6 and 22.3 months, respectively. The overall response and clinical benefit rates were 62.6% and 68.6%, respectively (PMID: 30110579).
- In a feasibility study, thirteen patients with HER2 negative breast cancer harboring germline BRCA1/2 mutations were treated with talazoparib in a neoadjuvant setting. All patients showed response with a median tumor reduction of 88% and ranging from 30-98% (PMID: 29238749).

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Protein biomarker: HER2 (Low)

Drug Evidence for fam-trastuzumab deruxtecan-nxki:

- In a Phase III study, 557 patients with HER2-low (IHC 1+ or IHC 2+/ISH -) metastatic breast cancer were treated with fam-trastuzumab deruxtecan. A median overall survival of 23.4 months, and a median progression-free survival of 9.9 months were reported (PMID: 35665782).
- In a Phase II trial, heavily pretreated HER2-low (IHC 2+/ISH- or IHC 1+) gastric/gastroesophageal junction adenocarcinoma patients were treated with fam-trastuzumab deruxtecan. The treatment resulted in an objective response rate (ORR) of 26.3%, median progression-free survival (mPFS) of 4.4 months, and median overall survival (mOS) of 7.8 months in patients with IHC 2+/ISH-, and an ORR of 9.5%, mPFS of 2.8 months, and mOS of 8.5 months in patients with IHC 1+ (PMID: 36379002).

Genomic alteration: PIK3CA (H1047R) VAF: 82%

HGVS: c.3140A>G p.His1047Arg	**Coordinate:** chr3:178952085
Alteration type: Missense	**Transcript ID:** ENST00000263967
Read depth: 2100	**Location:** 21/21

The PIK3CA gene encodes a member of the phosphoinositide 3-kinase (PI3K) family, an important node in the PI3K–PKB/AKT signaling pathway, integrating biological signals from the RAS/RAF–MEK–ERK signaling pathway, stimulating cell proliferation, angiogenesis, and cell survival by evading apoptosis (PMID: 21986133, 37509254). This alteration, which lies within the PIK3 kinase, catalytic domain, is predicted to result in the gain of Pik3ca protein function (PMID: 15647370, 16322248, 16432179, 26270481, 26863299, 28808038, 30371878). The FDA has approved alpelisib in combination with fulvestrant, capivasertib in combination with fulvestrant, inavolisib in combination with palbociclib in combination with fulvestrant in HR-positive HER2-negative breast cancer patients harboring PIK3CA H1047R.

Drug Evidence for inavolisib + palbociclib + fulvestrant:

- In a Phase I/Ib trial, hormone receptor-positive, human epidermal growth factor receptor 2-negative, locally advanced or metastatic breast cancer patients with PIK3CA mutations were treated with inavolisib in combination with palbociclib and endocrine therapy (either letrozole or fulvestrant). Among 25 patients with measurable disease receiving treatment with inavolisib, palbociclib and fulvestrant, overall response rate was 52%, including 1 complete response and 14 partial responses. Progression free survival was 23.3 months (PMID: 39236276).
- In the Phase III INAVO120 trial, hormone receptor-positive, human epidermal growth factor receptor 2-negative, locally advanced or metastatic breast cancer patients with PIK3CA mutations were randomized to receive treatment with either inavolisib (n=161) or placebo (n=164) in combination with palbociclib and fulvestrant. The median progression-free survival was 15.0 months among those treated with inavolisib compared with 7.3 months in the placebo group (hazard ratio (HR)= 0.43; P<0.001). Overall response rate was 58% vs. 25% in the inavolisib and placebo groups, respectively (PMID: 39476340).

Drug Evidence for alpelisib + fulvestrant:

- In a Phase III study, 169 patients with HR-positive, HER2-negative advanced breast cancer harboring PIK3CA mutations were treated with alpelisib + fulvestrant. An overall responce rate of 26.6% and a median progression free survival of 11 months was reported (PMID: 31091374).

Drug Evidence for everolimus:

- In a retrospective study, two patients with HR positive, HER2 negative breast cancer harboring PIK3CA mutations in the helicase or kinase domains were treated with everolimus plus anastrozole. Partial response was achieved by one patient and stable disease was reported in the other patient (PMID: 24912489).
- In a retrospective study, six patients with hormone receptor positive breast cancer harboring PIK3CA H1047R mutations were treated with everolimus. A median progression free survival of 8.8 months was reported (PMID: 31088410).

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Drug Evidence for capivasertib + fulvestrant:

- In a Phase III study, 155 patients with hormone receptor positive, HER2 negative breast cancer harboring AKT pathway activating mutations (PIK3CA or AKT1 gain of function or PTEN loss of function) were treated with capivasertib + fulvestrant. A total of 28.8% of patients had objective responses, and a median progression free survival of 7.3 months was reported in this cohort (PMID: 37256976).

Drug Evidence for temsirolimus:

- In a combined study of several Phase I studies, 23 patients with solid tumors harboring various PIK3CA mutations, including H1047R/L, were treated with temsirolimus or other PI3K/AKT/mTOR pathway inhibitors. A total of 39% of patients responded (7 with a partial response and 2 with stable disease) (PMID: 22271473).
- In combined Phase I studies, 17 patients with solid tumors harboring various PIK3CA mutations including H1047# were treated with temsirolimus or other PI3K/AKT/mTOR inhibitor. A total of 35% of patients responded (PMID: 21216929).

Drug Evidence for everolimus + exemestane:

- A retrospective Bolero-2 study (n= 88 with PIK3CA mutations in exon 9 or 20) exploring the genetic landscape of tumors of HR+/HER2- advanced breast cancer patients treated with either placebo + exemestane or everolimus + exemestane. Prolonged progression free survival (PFS) by twofold in was observed in patients with treated with nonsteroidal aromatase inhibitors and reported greater PFS benefit from everolimus in those with PIK3CA helical domain (exon 9) mutations (5.2 months) than in those with PIK3CA kinase domain (exon 20) mutations (6.8 months). PFS benefit with everolimus was maintained regardless of the alteration status of PIK3CA, FGFR1, and CCND1 or the pathways of which they are components (PMID: 26503204).

Incidental germline findings details

Genomic alteration: BRCA1 (M1775R) VAF: 67%	
HGVS: c.5324T>G p.Met1775Arg	**Coordinate:** chr17:41203088
Alteration type: Missense	**Transcript ID:** ENST00000357654
Read depth: N/A	**Location:** 20/23

This variant has been reported in multiple individuals with breast and/or ovarian cancer (Miki et al. 1994. PubMed ID: 7545954; Hall et al. 2009. PubMed ID: 19241424; Fackenthal et al. 2012. PubMed ID: 22034289; Zheng et al. 2018. PubMed ID: 30130155; Carter et al. 2018. PubMed ID: 30322717; George et al. 2021. PubMed ID: 33646313). In vitro studies demonstrate that this variant disrupts BRCA1 protein function (Clapperton et al. 2004. PubMed ID: 15133502; Phelan et al. 2005. PubMed ID: 15689452; Varma et al. 2005. PubMed ID: 16101277; Nikolopoulos et al. 2007. PubMed ID: 17493881; Findlay et al. 2018. PubMed ID: 30209399; Fernandes et al. 2019. PubMed ID: 30765603). This variant is located within the C-terminal BRCT domain of the BRCA1 gene, which is predicted to be a hot spot for missense variation (Dines et al. 2020. PubMed ID: 31911673). Another missense variant at the same amino acid residue (p.Met1775Lys) has also been reported in patients with hereditary cancer and was shown to be functionally damaging (Tischkowitz et al. 2008. PubMed ID: 18285836; Drikos et al. 2009. PubMed ID: 19452558; Findlay et al. 2018. PubMed ID: 30209399; Southey et al. 2021. PubMed ID: 34887416). This variant is reported in 0.016% of alleles in individuals of African descent in gnomAD and has been interpreted as pathogenic by multiple clinical laboratories in ClinVar (Variation ID: 17694). This variant is interpreted as pathogenic.

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Clinical trials

Potential trials based on genomic targets identified.

Biomarker	NCT ID	Study phase
BRCA1 (M1775R)	NCT01042379	Phase 2
PIK3CA (H1047R)	NCT04762979	Phase 2
PIK3CA (H1047R)	NCT05768139	Phase 1/Phase 2
BRCA1 (M1775R)	NCT04890613	Phase 1
PIK3CA (H1047R)	NCT04802759	Phase 1/Phase 2
PIK3CA (H1047R)	NCT06993844	Phase 1/Phase 2
BRCA1 (M1775R)	NCT06488378	Phase 1
BRCA1 (M1775R)	NCT03742245	Phase 1

Variants of unknown significance

Alteration	Alteration Type	VAF %
ABCC12 (I1035T)	Missense	45
ABI1 (L378fs)	Frameshift	18
ABI3BP (L191I)	Missense	28
AC002310.13 (P7R)	Missense	39
ACHE (E274Q)	Missense	35
ADAMTS2	Amplification	-
ADD3 (R509W)	Missense	41
AIM1	Amplification	-
AKAP10 (c.1185+2_1185+4delTAAinsAT)	Splice Donor Variant	59
ANK1 (S1727T)	Missense	42
ANK3 (c.3846-6_3859delGCTCAGATTTTGGCTTGCAG)	Splice Acceptor Variant	29
ANO1	Amplification	-
ANXA1 (Y21D)	Missense	38
AP3B2 (E985*)	Stop Gain	32
AR (ARv7)	Alternative Transcript	-

Alteration	Alteration Type	VAF %
MICAL3 (P1816A)	Missense	61
MROH8 (R787L)	Missense	45
MSRB2	Amplification	-
MSX2	Amplification	-
MYH7B (V1617_K1624del)	Inframe Deletion	22
MYH9 (E465Q)	Missense	43
MYOCD (I904F)	Missense	64
NEURL1B	Amplification	-
NKX2-5	Amplification	-
NLRC5 (R661S)	Missense	37
NOX4 (Y497fs)	Frameshift	15
NPAP1 (D1016Y)	Missense	40
NPAS4 (A222T)	Missense	25
NRAP (R288K)	Missense	5
NRP2 (R507H)	Missense	37

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Alteration	Alteration Type	VAF %
ARMC3	Amplification	-
ATG5	Amplification	-
ATP6V1B1 (A508T)	Missense	9
BBOX1 (S374T)	Missense	26
BEGAIN (T353_E363delinsK)	Inframe Deletion	20
BEND3	Amplification	-
BMP2K (Q466_Q468del)	Inframe Deletion	29
BRF1 (E362Q)	Missense	37
BTNL9	Amplification	-
BVES	Amplification	-
C10orf67	Amplification	-
C11orf80 (A31_A34dup)	Inframe Insertion	20
C6orf203	Amplification	-
C6orf226 (R56G)	Missense	41
C9orf66	Deletion	-
CACNG7 (T255M)	Missense	28
CADPS (R372C)	Missense	38
CAGE1 (S636F)	Missense	38
CALD1 (V519M)	Missense	39
CBS (P6T)	Missense	54
CBWD1	Deletion	-
CD101 (W448R)	Missense	40
CENPJ (R1169C)	Missense	64
CENPU (V195F)	Missense	65
CFHR3	Amplification	-
CHD8 (K520I)	Missense	35

Alteration	Alteration Type	VAF %
NSG2	Amplification	-
OR2D2 (T137A)	Missense	28
OR4C11	Amplification	-
OR4N4 (V194E)	Missense	21
OR4P4	Amplification	-
OR4S2	Amplification	-
OTUD1	Amplification	-
OTUD7A (A833T)	Missense	47
PARG (G391E)	Missense	54
PCSK9 (R525S)	Missense	41
PDSS2	Amplification	-
PIK3CA (Y432H)	Missense	43
PLA2G16	Amplification	-
POPDC3	Amplification	-
POU2F2 (P160_Q167del)	Inframe Deletion	9
PRDM1	Amplification	-
PREP	Amplification	-
PRG3 (R111H)	Missense	12
PRR7 (S148P)	Missense	44
PRRC2A (P1163R)	Missense	40
PSME4 (c.5257_5263+11delTCTGCAGGTAACATTGCT)	Splice Donor Variant	26
PTF1A	Amplification	-
QRSL1	Amplification	-
RARRES3	Amplification	-
RASGEF1C	Amplification	-
RFX3	Deletion	-

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Alteration	Alteration Type	VAF %
DACH2 (N50K)	Missense	63
DAO (P103T)	Missense	45
DDAH1 (G94E)	Missense	41
DDX5 (A280V)	Missense	23
DMRT1	Deletion	-
DMRT2	Deletion	-
DMRT3	Deletion	-
DOCK8	Deletion	-
DRG2 (L195_S199del)	Inframe Deletion	51
EGFL6 (T87S)	Missense	40
ELFN1 (R119W)	Missense	39
ELOVL2 (V224L)	Missense	37
EMR1 (N150T)	Missense	7
FABP7 (D111E)	Missense	39
FAM115C (A819V)	Missense	42
FAM115C (H766R)	Missense	37
FAM153A	Amplification	-
FAM227B (H129L)	Missense	25
FANCM (K1510M)	Missense	40
FCRL5 (Q233K)	Missense	28
FNDC3B (V100M)	Missense	38
GDAP1L1 (D155H)	Missense	41
GEMIN4 (T90A)	Missense	65
GLI4 (A115T)	Missense	39
GLIS3	Deletion	-
GNL2 (c.1303-2A>T)	Splice Acceptor Variant	51
GRM5 (T175N)	Missense	9
GRM6	Amplification	-

Alteration	Alteration Type	VAF %
RIC8A (M222fs)	Frameshift	55
RNF141 (T49M)	Missense	42
RNF44	Amplification	-
RP11-683L23.1	Deletion	-
RPTOR (V1144M)	Missense	21
RTN4IP1	Amplification	-
RYR2 (A2542D)	Missense	26
SAFB2 (M682_R686del)	Inframe Deletion	42
SAV1 (K24N)	Missense	39
SDK2 (I199T)	Missense	26
SH3BP2 (P246S)	Missense	35
SIGLEC14	Amplification	-
SLC17A3 (D86E)	Missense	37
SLC9A8 (C186S)	Missense	35
SMARCA2	Deletion	-
SMC1A (Y1085fs)	Frameshift	17
SOX7 (Q225*)	Stop Gain	37
SPG11 (I185V)	Missense	43
SYNE2 (N5536H)	Missense	37
SYNE2 (Q5863L)	Missense	42
SYNPO2 (I260T)	Missense	41
TACC2 (D2770E)	Missense	26
TBC1D3B	Deletion	-
TDRD6 (E2087*)	Stop Gain	38
TDRD7 (S309T)	Missense	34
TLX3	Amplification	-
TMEM87B (T296R)	Missense	35
TNIK (c.3448+1_3448+8del GTAAGTCT)	Splice Donor Variant	26

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Alteration	Alteration Type	VAF %
HERC1 (V225L)	Missense	40
HLA-DQB2 (F39I)	Missense	7
HNRNPUL2 (R397P)	Missense	68
HNRNPUL2-BSCL2 (R397P)	Missense	68
ILK (L38*)	Stop Gain	27
ITGA10 (V631G)	Missense	38
KANK1	Deletion	-
KCNH1 (D830N)	Missense	56
KCNV2	Deletion	-
KIAA0020	Deletion	-
KIAA1109 (Q3274P)	Missense	78
KLHL20 (P251H)	Missense	58
KRTAP2-4	Deletion	-
LAMC1 (A1239G)	Missense	23
LDB2 (G313V)	Missense	38
LHX9	Amplification	-
LIN28B	Amplification	-
LRBA (D2109N)	Missense	39
LRIT1 (R429Q)	Missense	5
MACF1 (E1184K)	Missense	7
MAML2 (C54Y)	Missense	23
MARCO (A514V)	Missense	17
MICAL3 (E1822G)	Missense	51

Alteration	Alteration Type	VAF %
TPBGL	Amplification	-
TPM4 (R238G)	Missense	36
TRRAP (L81F)	Missense	40
TTBK2 (H925Y)	Missense	39
TTN (T26210I)	Missense	39
UBR4 (F2123L)	Missense	36
UGT2B28	Deletion	-
UIMC1	Amplification	-
USP24 (V2474L)	Missense	40
VLDLR	Deletion	-
WDFY4 (V3156M)	Missense	51
WWP1 (H820R)	Missense	10
ZBTB4 (M451T)	Missense	69
ZC3HAV1 (c.444+1G>T)	Splice Donor Variant	24
ZFP2	Amplification	-
ZNF354A	Amplification	-
ZNF354B	Amplification	-
ZNF354C	Amplification	-
ZNF454	Amplification	-
ZNF76 (L11F)	Missense	7
ZNF764 (P7R)	Missense	39
ZNF879	Amplification	-

Methodology

Altera™ test is a Next Generation Sequencing tumor/normal exome and tumor RNA Seq assay that detects substitutions, insertions, deletions, copy number events, and fusions in tumor tissue. MET exon 14 skipping, AR-v7, EGFRvIII, and EGFRvIV variants are also detected in RNA. Genomic DNA is extracted from the patient's normal (peripheral white blood cells) 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.

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Sequence data are analyzed using various validated bioinformatics tools and custom Next Generation Sequencing Pipeline NG2-LDT 1.17.0. 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. Level of evidence provided for each gene drug association is based on the AMP/ASCO/CAP standards and guidelines for the interpretation of somatic sequence variants (PMID: 27993330).

Copy number events (amplifications/deletions) reported are focal in nature (<25mb).

Allele fraction is dependent on tumor content. Tumor content is not taken into account when reporting allele fractions.

Tumor Mutation Burden (TMB) is determined by measuring the number of somatic mutations occurring in sequenced genes, counting all mutations expected to change the aminoacid 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) and ≥180x for the normal sample. 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 in the IHC Thresholds table appearing in the report.

The following Food and Drug Administration (FDA) approved antibody clones are used: PATHWAY anti-HER2 (4B5)/Roche and PD-L1 22C3 IHC pharmDx/ Agilent.

These assays have not been validated on decalcified specimens. Results should be interpreted with caution given the possibility of false negative results 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 Natera/GHI upon request.

Digital images of immunohistochemical (IHC) stained tissues for manual scoring and/or image review are visualized using the Philips Image Management System Version 3.3. Image analysis software is not utilized.

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 fraction in hotspots, 5% in other regions), or in a region not included 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

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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

The pre-analytic phase of this test was performed by Natera, Inc. 201 Industrial Rd. Suite 410, San Carlos, CA 94070 (CLIA ID 05D1082992) and Genomic Health, Inc., 445 N 5th St, 1st Floor, Phoenix, AZ, 85004 (CLIA ID 03D2048606). The analytic phase of this test was performed by Genomic Health, Inc., 445 N 5th St, 1st Floor, Phoenix, AZ, 85004 (CLIA ID 03D2048606). The post-analytic phase of this test was performed by NSTX, Inc. 13011 McCallen Pass, Building A Suite 110, Austin, TX 78753 (CLIA ID 45D2093704) and Genomic Health, Inc., 445 N 5th St, 1st Floor, Phoenix, AZ, 85004 (CLIA ID 03D2048606). The performance characteristics of this test were developed by Genomic Health Inc. This test has not been approved by the U.S. FDA. The assay has been validated as a Laboratory Developed Test per institutional and applicable CLIA regulation. The test is not FDA cleared or approved as a companion diagnostic. These laboratories are regulated under CLIA as qualified to perform high-complexity testing.

This test is used for screening purposes and is not intended to be a stand-alone diagnostic tool. It should also be noted that the data interpretations are based on our current understanding of genes and variants and are current 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 germline evaluation testing in light of such information is at the discretion of the ordering physician. Variants of Unknown Significance (VUS) may associate with potential therapies in the future. Genomic Health Inc. does not update reports or send notification regarding reclassification of these alterations. 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.

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