Brigham and Women's Hospital
Department of Pathology
Center for Advanced Molecular Diagnostics
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Accession No.:
Unit Number(s):
Patient Name:
Birth Date:
Age & Sex at Diagnosis:

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Test Performed - MDOPANEL_B

Test Description - OncoPanel

Accession numbers on blocks/tissue submitted – PT-1121977

Original specimen collection date – 10/18/2014

Original pathologic diagnosis – Breast Cancer

Estimated percentage of neoplastic cells in submitted specimen - 30%

RESULTS:

There are 5982541 aligned, high-quality reads for this specimen with a mean of 155 reads across all targeted exons and 95% of all exons having more than 30 reads.

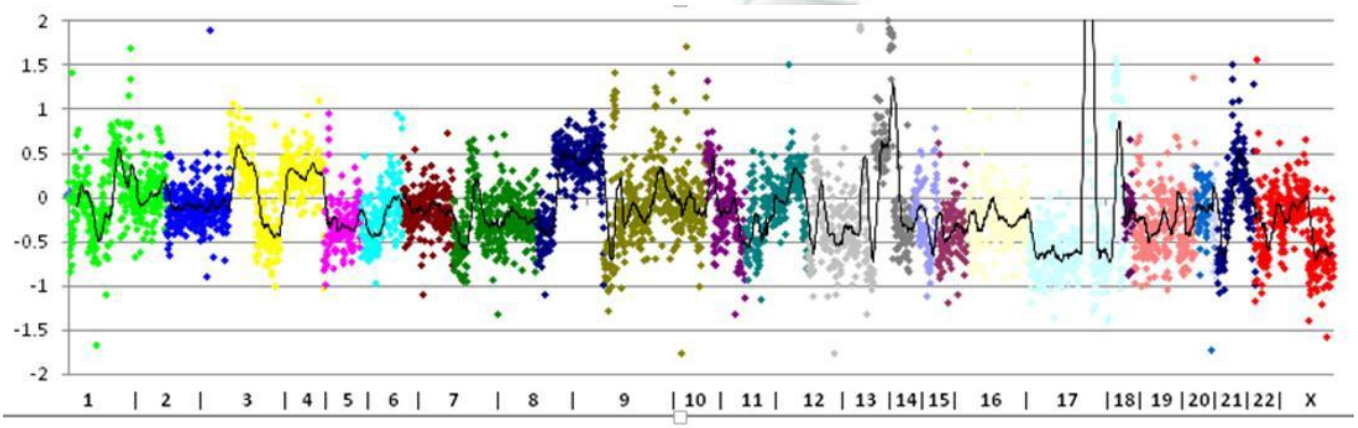

Figure legend: Plot of copy number variation by chromosomes which are color-coded. Sex chromosomes are excluded from the analysis. The vertical axis is the ratio of number of reads for this specimen and a panel of normals in log base 2 scale. A value of 0 denotes no difference from normal (diploid). When the sample contains 100% tumor cells, a value of -1 equals to 1 copy loss and 0.58 is 1 copy gain. The sensitivity and specificity of copy number variation evaluation by next-generation sequencing is affected by several factors, including the tumor percentage, ploidy, clonal heterogeneity, and the GC content of the gene of interest. For example, a sample with 20% tumor cells having a 5-copy amplification of a gene is indistinguishable from a sample with 100% tumor cells with 1 copy gain of the same gene. Confirmation of the copy number variation findings by Next-Gen Sequencing with a different testing platform is recommended.

DNA VARIANTS:

See Background section for tier definitions

Tier 1 variants: None identified.

Tier 2 variants:

TP53 c.613T_>C (p.Y205H), exon 2 - in 50% of 73 reads**

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Tier 3 variants:

BUB1B c.767G>A (p.R256K), exon 7 - in 51% of 99 reads**

Tier 4 variants:

MLL2 c.14546G>A (p.S4849N), exon 47 - 60% of 113 reads **

NEGATIVE for mutations in the following genes with clinical relevance for this tumor type: BRCA1, BRCA2, ERBB2, PALB2, PTEN

COPY NUMBER VARIATIONS:

1p12	NOTCH2	Low copy number gain
1q21.3	MCL1	Low copy number gain
1q23.3	SDHC	Low copy number gain
4p16.3	FGFR3	Single copy deletion
4p13	PHOX2B	Low copy number gain
8p23.1	GATA4	Single copy deletion
8p12	WRN	Single copy deletion
8p11.23	FGFR1	Single copy deletion
8q11.21	PRKDC	Low copy number gain
8q13.1	MYBL1	Low copy number gain
8q21.3	NBN	Low copy number gain
8q24.11	RAD21	Low copy number gain
8q24.11	EXT1	Low copy number gain
8q24.21	MYC	Low copy number gain
8q24.3	PTK2	Low copy number gain
9p24.1	JAK2	Single copy deletion
9p24.1	CD274	Low copy number gain
9p24.1	PDCD1LG2	Low copy number gain
12q14.1	CDK4	High copy number gain

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13q12.2	FLT3	Low copy number gain
13q12.2	FLT1	Low copy number gain
13q13.1	BRCA2	High copy number gain
17q12	ERBB2	High copy number gain
17q12	IKZF3	High copy number gain
17q23.2	BRIP1	Low copy number gain
22q11.21	MAPK1	Single copy deletion
22q11.22	PRAME	Single copy deletion
22q11.23	SMARCB1	Single copy deletion

Chromosomal Rearrangement:
Translocation review identifies:- an interchromosomal rearrangement involving SFXN2 at intron 1 and EWSR1 at intron 7 (breakpoints: 10:104479502,22:29680641). The significance of this fusion is unknown in this context.

INTERPRETATION:
SOMATIC VARIANTS:
BUB1B c.767G>A (p.R256K) - **BUB1B (BubR1) is a critical component of the mitotic checkpoint. Loss of BUB1B is associated with chromosomal instability in a variety of tumor types. This variant has not been previously reported.

TP53 c.613T>C (p.Y205H) - **Tumor protein p53 (TP53) gene is a tumour suppressor gene that is mutated in 12-80% of breast cancers, depending on subtype, and is associated with more aggressive tumor behavior (PMID: 16489069). This missense mutation has been reported in the COSMIC database in multiple tumor types, including bresat carcinoma.

*** These variants may have a role in cancer biology, or may have shown potential future clinical application in in vitro studies, but as yet no clinical role for this mutation has been established as standard-of-care in the published medical literature.

COPY NUMBER ALTERATIONS (CNA):
CNA analysis shows frequent segmental alterations and multiple foci of gene amplification:
High copy number gain of CDK4 at 12q14.1: CDK4 amplification promotes cell cycle progression and occurs in many cancer subtypes. Early phase clinical trials of CDK4/6 inhibitors (NCT01394016) have shown some promise in selected patients.

High copy number gain of ERBB2 at 17q12: High level copy number gains of ERBB2 and other genes on 17q is noted in this sample; amplification of ERBB2 is known to be clinically significant in breast cancer and a potential therapeutic target. Confirmatory testing for HER2 status by fluorescent in situ hybridization and/or immunohistochemistry is recommended (PMID: 23915743).

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TEST INFORMATION:

BACKGROUND:

Somatic genetic alterations in oncogenes and tumor-suppressor genes contribute to the pathogenesis and evolution of human cancers. These alterations can provide prognostic and predictive information and stratify cancers for targeted therapeutic information. We classify these alterations into five tiers using the following guidelines:

Tier 1: The alteration has well-established published evidence confirming clinical utility in this tumor type, in at least one of the following contexts: predicting response to treatment with an FDA-approved therapy; assessing prognosis; establishing a definitive diagnosis; or conferring an inherited increased risk of cancer to this patient and family.

Tier 2: The alteration may have clinical utility in at least one of the following contexts: selection of an investigational therapy in clinical trials for this cancer type; limited evidence of prognostic association; supportive of a specific diagnosis; proven association of response to treatment with an FDA-approved therapy in a different type of cancer; or similar to a different mutation with a proven association with response to treatment with an FDA-approved therapy in this type of cancer.

Tier 3: The alteration is of uncertain clinical utility, but may have a role as suggested by at least one of the following: demonstration of association with response to treatment in this cancer type in preclinical studies (e.g., in vitro studies or animal models); alteration in a biochemical pathway that has other known, therapeutically-targetable alterations; alteration in a highly conserved region of the protein predicted, in silico, to alter protein function; or selection of an investigational therapy for a different cancer type.

Tier 4: The alteration is novel or its significance has not been studied in cancer.

Tier 5: The alteration has been determined to have no clinical utility, either for selecting therapy, assessing prognosis, establishing a diagnosis, or determining hereditary disease risk.

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METHODOLOGY:

We have developed a cancer genomic assay to detect somatic mutations, copy number variations and structural variants in tumor DNA extracted from fresh, frozen or formalin-fixed paraffin-embedded samples. The OncoPanel assay surveys exonic DNA sequences of 299 cancer genes and 113 introns across 35 genes for rearrangement detection. DNA is isolated from tissue containing at least 20% tumor nuclei and analyzed by massively parallel sequencing using a solution-phase Agilent SureSelect hybrid capture kit and an Illumina HiSeq 2500 sequencer.

The 299 genes are: ABL1, AKT1, AKT2, AKT3, ALK, ALOX12B, APC, AR, ARAF, ARID1A, ARID1B, ARID2, ASXL1, ATM, ATRX, AURKA, AURKB, AXL, B2M, BAP1, BCL2, BCL2L1, BCL2L12, BCL6, BCOR, BCORL1, BLM, BMPR1A, BRAF, BRCA1, BRCA2, BRD4, BRIP1, BUB1B, CADM2, CARD11, CBL, CBLB, CCND1, CCND2, CCND3, CCNE1, CD274, CD58, CD79B, CDC73, CDH1, CDK1, CDK2, CDK4, CDK5, CDK6, CDK9, CDKN1A, CDKN1B, CDKN1C, CDKN2A, CDKN2B, CDKN2C, CEBPA, CHEK2, CIITA, CREBBP, CRKL, CRLF2, CRTC1, CRTC2, CSF1R, CSF3R, CTNNB1, CUX1, CYLD, DDB2, DDR2, DEPDC5, DICER1, DIS3, DMD, DNMT3A, EED, EGFR, EP300, EPHA3, EPHA5, EPHA7, ERBB2, ERBB3, ERBB4, ERCC2, ERCC3, ERCC4, ERCC5, ESR1, ETV1, ETV4, ETV5, ETV6, EWSR1, EXT1, EXT2, EZH2, FAM46C, FANCA, FANCC, FANCD2, FANCE, FANCF, FANCG, FAS, FBXW7, FGFR1, FGFR2, FGFR3, FGFR4, FH, FKBP9, FLCN, FLT1, FLT3, FLT4, GATA3, GATA4, GATA6, GLI1, GLI2, GLI3, GNA11, GNAQ, GNAS, GNB2L1, GPC3, GSTM5, H3F3A, HNF1A, HRAS, ID3, IDH1, IDH2, IGF1R, IKZF1, IKZF3, INSIG1, JAK2, JAK3, KCNIP1, KDM5C, KDM6A, KDM6B, KDR, KEAP1, KIT, KRAS, LINC00894, LMO1, LMO2, LMO3, MAP2K1, MAP2K4, MAP3K1, MAPK1, MCL1, MDM2, MDM4, MECOM, MEF2B, MEN1, MET, MITF, MLH1, MLL, MLL2, MPL, MSH2, MSH6, MTOR, MUTYH, MYB, MYBL1, MYC, MYCL1, MYCN, MYD88, NBN, NEGR1, NF1, NF2, NFE2L2, NFKBIA, NFKBIZ, NKX2-1, NOTCH1, NOTCH2, NPM1, NPRL2, NPRL3, NRAS, NTRK1, NTRK2, NTRK3, PALB2, PARK2, PAX5, PBRM1, PDCD1LG2, PDGFRA, PDGFRB, PHF6, PHOX2B, PIK3C2B, PIK3CA, PIK3R1, PIM1, PMS1, PMS2, PNRC1, PRAME, PRDM1, PRF1, PRKAR1A, PRKCI, PRKCZ, PRKDC, PRPF40B, PRPF8, PSMD13, PTCH1, PTEN, PTK2, PTPN11, PTPRD, QKI, RAD21, RAF1, RARA, RB1, RBL2, RECQL4, REL, RET, RFWD2, RHEB, RHPN2, ROS1, RPL26, RUNX1, SBDS, SDHA, SDHAF2, SDHB, SDHC, SDHD, SETBP1, SETD2, SF1, SF3B1, SH2B3,

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SLITRK6, SMAD2, SMAD4, SMARCA4, SMARCB1, SMC1A, SMC3, SMO, SOCS1, SOX2, SOX9, SQSTM1, SRC, SRSF2, STAG1, STAG2, STAT3, STAT6, STK11, SUFU, SUZ12, SYK, TCF3, TCF7L1, TCF7L2, TERC, TERT, TET2, TLR4, TNFAIP3, TP53, TSC1, TSC2, U2AF1, VHL, WRN, WT1, XPA, XPC, XPO1, ZNF217, ZNF708, ZRSR2.

Intronic regions are tiled on specific introns of ABL1, AKT3, ALK, BCL2, BCL6, BRAF, CIITA, EGFR, ERG, ETV1, EWSR1, FGFR1, FGFR2, FGFR3, FUS, IGH@, IGK@, IGL@, JAK2, MLL, MYC, NPM1, NTRK1, PAX5, PDGFRA, PDGFRB, PPARG, RAF1, RARA, RET, ROS1, SS18, TRA@, TRB@, TRG@.TMPRSS2.

For detailed methodology and protocol, please contact the Center for Advanced Molecular Diagnostics (857-307-1500).

These tests were developed and their performance characteristics determined by the Molecular Diagnostics Laboratory, Brigham and Women's Hospital. They have not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary.

References:

Wagle et al. High-throughput detection of actionable genomic alterations in clinical tumor samples by targeted, massively parallel sequencing. Cancer Discov. 2012 Jan;2(1):82-93.

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