Brigham and Women's Hospital
Department of Pathology
Center for Advanced Molecular Diagnostics
75 Francis Street, Boston, MA 02115
Tel (857) 307-1540 Fax (857) 307-1544

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 – HP-2221982

Original specimen collection date – 12/15/2014

Original pathologic diagnosis - Melanoma

Estimated percentage of neoplastic cells in submitted specimen - 70%

RESULTS:

There are 4265394 aligned, high-quality reads for this specimen with a mean of 51 reads across all targeted exons and 91% of all exons having more than 30 reads.

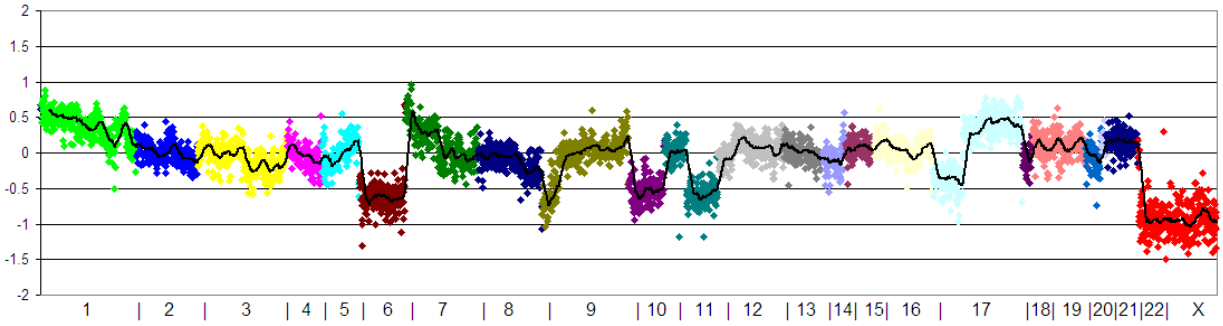

Figure legend: Plot of copy number variation by chromosomes which are color-coded. Sex chromosomes are excluded from the analysis. The vertical axis is the ratio of number of reads for this specimen and a panel of normals in log base 2 scale. A value of 0 denotes no difference from normal (diploid). 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:

NRAS c.181C>A (p.Q61K) , exon 2 - in 10% of 55 reads

Tier 2 variants: None identified.

CLIA certificate: 22D2040971

Laboratory Director
Dr. Neal Lindeman

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Tier 3 variants:

APC *c.4786C>T (p.Q1596*) , exon 14* - in 17% of 49 reads

TSC2 *c.2476C>A (p.L826M) , exon 22* - in 43% of 48 reads***

Tier 4 variants:

AKT3 *c.49G>A (p.E17K) , exon 12* - in 9% of 49 reads***
APC *c.6989C>T (p.S2330F) , exon 14* - in 11% of 55 reads***
BAP1 *c.1168_1169CC>TT (p.P390L) , exon 6* - in 13% of 45 reads***
EPHA3 *c.1666C>T (p.L556F) , exon 8* - in 12% of 42 reads***
ETV1 *c.546G>A (p.M182I) , exon 7* - in 12% of 56 reads***
FGFR4 *c.736_737CC>AT (p.P246M) , exon 7* - in 20% of 58 reads***
FLT1 *c.430G>A (p.E144K) , exon 27* - in 10% of 51 reads***
FLT3 *c.1442G>A (p.G481E) , exon 13* - in 17% of 35 reads***
H3F3A *c.95C>T (p.S32F)* - in 11% of 65 reads***
KDR *c.2406_2412GTCCATC>C (p.802_804LSI>F) , exon 14* - in 16% of 53 reads***
KDR *c.3299C>T (p.S1100F) , exon 7* - in 8% of 50 reads***
MECOM *c.2306C>T (p.S769L) , exon 5* - in 12% of 47 reads***
PIK3R1 *c.1153C>T (p.H385Y) , exon 10* - in 16% of 42 reads***
STAG1 *c.2932C>T (p.H978Y) , exon 8* - in 19% of 57 reads***
SYK *c.1045G>A (p.E349K) , exon 9* - in 10% of 51 reads***

NEGATIVE for mutations in the following genes with clinical relevance for this tumor type: BRAF, CDK4, CDKN2A

COPY NUMBER VARIATIONS:

1q21.3	MCL1	Low copy number gain
1q21.3	CRTC2	Low copy number gain
1q23.1	NTRK1	Low copy number gain
1q23.3	SDHC	Low copy number gain
1q23.3	DDR2	Low copy number gain
1q25.1	RFWD2	Low copy number gain
1q31.2	CDC73	Low copy number gain
1q32.1	PIK3C2B	Low copy number gain
1q32.1	MDM4	Low copy number gain
1q42.12	H3F3A	Low copy number gain
1q43	FH	Low copy number gain
1q43	AKT3	Low copy number gain
6p21.1	CCND3	Single copy deletion
6p21.2	CDKN1A	Single copy deletion
6p21.2	PIM1	Single copy deletion
6p21.31	FANCE	Single copy deletion
6q15	PNRC1	Single copy deletion
6q16.1	EPHA7	Single copy deletion
6q21	PRDM1	Single copy deletion

6q22.1	ROS1	Single copy deletion
6q23.3	MYB	Single copy deletion
6q23.3	TNFAIP3	Single copy deletion
6q25.1	ESR1	Single copy deletion
6q25.3	ARID1B	Single copy deletion
6q26	PARK2	Single copy deletion
6q26	QKI	Single copy deletion
15q22.31	MAP2K1	Low copy number gain
15q25.3	NTRK3	Low copy number gain
15q26.1	IDH2	Low copy number gain
15q26.1	BLM	Low copy number gain

Comments:

SOMATIC VARIANTS:

APC c.4786C>T (p.Q1596*) - Nonsense mutation of APC is likely to be loss-of-function mutation.

NRAS c.181C>A (p.Q61K) - NRAS-mutated melanoma can be targeted by MEK inhibitor***

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.

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.

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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.

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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