AT THE FOREFRONT
UChicago Medicine

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Molecular Pathology OncoPlus Panel Report

Patient Name:	[redacted]			Accession #:	**PO19-6306**
Med. Rec. #:	3895634	Encounter #:	77471879	Collected:	9/6/2019
DOB (Age):	4/10/1949 (Age: 70)	Client:	1 - University of Chicago Medical Center	Received:	9/6/2019
Gender:	F	Location:	NPDL(UCH)	Completed:	9/30/2019
Physician:	Raphael Borok, MD				

Molecular Diagnostics Report

Clinical Indication
Metastatic carcinoma, favor high grade serous carcinoma

Specimen Information
Specimen Source: Omentum
Specimen Type: Formalin-fixed, paraffin-embedded tissue
Accession Number: PO19-6306, A2 (1867, B, Precision Diagnostics) (CGL4549.S1)

Pathology Assessment: The approximate tumor cell percentage in the tested area of the specimen is 40%.

Pathogenic Findings
FGFR1 Amplification
TP53 c.817C>T, p.R273C (NM_000546.5) (VAF: 83%)

Ancillary Findings
Tumor Mutational Burden Result: 9 mutations per megabase
Microsatellite Instability Result: Microsatellite Stable (MSS)

Findings of Uncertain Clinical Significance
APC Loss
BAP1 Loss - Equivocal
CALR c.89G>C, p.G30A (NM_004343.3) (VAF: 38%)
CXCR4 c.343G>A, p.A115T (NM_001008540.2) (VAF: 32%)
EGFR Loss
FANCA c.3293A>G, p.E1098G (NM_000135.3) (VAF: 51%)
FLT3 c.2754-5del, p.? (NM_004119.2) (VAF: 95%)
IKZF1 Loss
NOTCH2 c.325T>C, p.C109R (NM_024408.4) (VAF: 50%)
NOTCH2 c.3479A>G, p.H1160R (NM_024408.4) (VAF: 52%)
RAD21 c.841G>T, p.V281L (NM_006265.2) (VAF: 48%)
TSC2 c.3610+6G>A, p.? (NM_000548.4) (VAF: 47%)

Interpretation
FGFR1 Amplification

Amplification of FGFR1 is detected in this specimen. FGFR1 (Fibroblast Growth Factor Receptor 1) gene is located on chromosome 8p11.23. FGFR1 amplificaton is seen in approximately 3-4% of ovarian serous cancers (https://www.cbioportal.org, Mol Cancer Res. 2015 Mar;13(3):502-9).

TP53 c.817C>T, p.R273C (NM_000546.5)
This mutation in exon 8 of the TP53 tumor suppressor gene produces an arginine to cysteine substitution at amino acid 273 within the DNA binding domain of the p53 protein. R273, a DNA-contact amino acid, is one of the most frequently altered residues in human cancer (cancer.sanger.ac.uk/cosmic), with mutations to histidine and to cysteine being most common (Hum Mutat 2007;28(6):622629, Hum Mutat 2002;19:607-614). R273 mutations have been described to produce gain-of-function effects, impacting multiple intracellular pathways related to apoptosis, migration, and invasion (Cell 2009, 139(7): 1327-41, Reviewed in Cancer Cell 2014, 25(3): 304-17; Hum Mutat 2014, 35(5): 575-84). TP53 mutations have been reported in up to 96% of high-grade serous ovarian cancer cases (cbioportal.org; Nature. 2011, 474(7353):609-15; Nature. 2015, 521(7553):489-94). In this specimen, the p.R273C mutation is observed at a high variant allelic frequency (~83%), indicating a likely loss of heterozygosity (LOH) event at this locus.

All controls performed within acceptable limits.

Regions with Low Depth of Coverage
Note: This result is complicated by many areas with low depth of coverage, which is due to a combination of multiple copy number abnormalities and the generally poor quality of the DNA isolated from the specimen. For more information about low depth regions please contact the laboratory.

Test Information: OncoPlus (v5.0) - Somatic mutation testing by next-generation sequencing (NGS)

Genes analyzed for mutations and insertions/deletions (154): ABL1, AKT1, ALK, APC, ARID1A, ARID2, ASXL1, ATM, ATR, ATRX, AXL, B2M, BAP1, BCOR, BCORL1, BIRC3, BLM, BRAF, BRCA1, BRCA2, BTK, CALR, CBL, CBLB, CCND1, CCND2, CCND3, CDH1, CDK4, CDK6, CDKN2A, CEBPA, CHEK1, CHEK2, CSF1R, CSF3R, CTCF, CTNNA1, CTNNB1, CUX1, CXCR4, DAXX, DDR2, DDX3X, DDX41, DICER1, DNMT3A, EGFR, EP300, EPHA3, EPHA5, ERBB2, ERBB3, ERBB4, ERCC3, ESR1, ETV6, EZH2, FANCA, FAT3, FBXW7, FGFR1, FGFR2, FGFR3, FH, FLT3, FOXL2, GATA1, GATA2, GNA11, GNAQ, GNAS, GRIN2A, H3F3A, HIST1H3B, HIST1H3C, HNF1A, HRAS, IDH1, IDH2, IKZF1, ITPKB, JAK2, KDM6A, KDR, KIT, KMT2A, KRAS, MAP2K1, MAPK1, MDM2, MET, MLH1, MLH3, MPL, MRE11A, MSH2, MSH6, MTOR, MYC, MYCN, MYD88, NBN, NF1, NF2, NFE2L2, NOTCH1, NOTCH2, NPM1, NRAS, PALB2, PBRM1, PDGFRA, PDGFRB, PHF6, PIK3CA, PIK3CB, PIK3R1, PLCG2, POLE, POT1, PPP2R1A, PTCH1, PTEN, PTPN11, RAD21, RAD51, RB1, RET, RUNX1, SDHB, SDHC, SDHD, SETBP1, SF3B1, SMAD4, SMARCB1, SMC1A, SMC3, SMO, SRSF2, STAG2, STAT3, STAT5B, STK11, TERT (promoter only), TET2, TP53, TSC1, TSC2, U2AF1, VHL, WT1, ZRSR2

Genes analyzed for copy number variations (143 gene subset): ABL1, AKT1, ALK, APC, ARID1A, ARID2, ASXL1, ATM, ATR, AXL, B2M, BAP1, BIRC3, BLM, BRAF, BRCA1, BRCA2, CALR, CBL, CBLB, CCND1, CCND2, CCND3, CDH1, CDK4, CDK6, CDKN2A, CEBPA, CHEK1, CHEK2, CSF1R, CSF3R, CTCF, CTNNA1, CTNNB1, CUX1, CXCR4, DAXX, DDR2, DDX41, DICER1, DNMT3A, EGFR, EP300, EPHA3, EPHA5, ERBB2, ERBB3, ERBB4, ERCC3, ESR1, ETV6, EZH2, FANCA, FAT3, FBXW7, FGFR1, FGFR2, FGFR3, FH, FLT3, FOXL2, GATA2, GNA11, GNAQ, GNAS, GRIN2A, H3F3A, HIST1H3B, HIST1H3C, HNF1A, HRAS, IDH1, IDH2, IKZF1, ITPKB, JAK2, KDR, KIT, KMT2A, KRAS, MAP2K1, MAPK1, MDM2, MET, MLH1, MLH3, MPL, MRE11A, MSH2, MSH6, MTOR, MYC, MYCN, MYD88, NBN, NF1, NF2, NFE2L2, NOTCH1, NOTCH2, NPM1, NRAS, PALB2, PBRM1, PDGFRA, PDGFRB, PIK3CA, PIK3CB, PIK3R1, PLCG2, POLE, POT1, PPP2R1A, PTCH1, PTEN, PTPN11, RAD21, RAD51,

RB1, RET, RUNX1, SDHB, SDHC, SDHD, SETBP1, SF3B1, SMAD4, SMARCB1, SMC3, SMO, SRSF2, STAT3, STAT5B, STK11, TERT, TET2, TP53, TSC1, TSC2, U2AF1, VHL, WT1

Genes analyzed for fusions/translocations (3): ALK, RET, and ROS1

A full list of covered genomic regions within these genes is provided online - http://ucmclabhandbook.uchospitals.edu/

Methodology: DNA is isolated from this specimen using the FFPE DNA Extraction Kit (Qiagen). Following extraction, DNA is quantified using the Qubit fluorometric assay (Thermo Fisher Scientific). DNA is subjected to ultrasonic fragmentation and subsequent library preparation using adapter molecules containing patient-specific index sequences (HTP Library Preparation Kit, Kapa Biosystems). After library amplification, quantification and pooling, fragments originating from targeted genomic regions are enriched using a panel of biotinylated oligonucleotides (SeqCap EZ, Roche Nimblegen) supplemented with additional oligonucleotides (xGen Lockdown Probes, IDT). After subsequent amplification and pooled library quantification, libraries are sequenced in rapid run mode on a HiSeq 2500 system (Illumina) to produce 2 x 101 bp paired end sequencing reads. Sequencing data is analyzed via custom-designed bioinformatics pipelines on a University of Chicago HIPAA compliant high performance computing system, using the hg19 (GRCh37) human genome reference sequence for alignment.

Ancillary findings: OncoPlus includes a calling algorithm for microsatellite instability (MSI) which assesses 336 homopolymer microsatellite loci across the 1,213 genes captured as part of OncoPlus. These data are compared to a historical normal control sample set to determine the percentage of loci deemed unstable. If a specimen reaches a satisfactory threshold, it will reported as "Microsatellite Unstable/Mismatch Repair Deficient (MSI+/MMRd)", and if within the normal range will be reported as "Microsatellite Stable (MSS)". MSI is caused by DNA mismatch repair (MMR) deficiency, resulting in failure to repair the errors that normally occur during replication of repetitive DNA sequences. Defective DNA mismatch repair can have both genetic and epigenetic causes, and can be from either somatic or germline inactivation of the MMR apparatus. In addition to identifying patients at risk for Lynch syndrome, MSI status is a predictive biomarker for patients receiving immune checkpoint inhibitors as recently approved by the FDA (Science. 2017 Jul 28;357(6349):409-413). The OncoPlus tumor mutational burden (TMB) result is expressed as mutations per megabase of coding territory, and includes synonymous, non-synonymous and insertion/deletion mutations. Approximately 2.8 megabase of coding territory across 1135 genes is used for data collection to provide robust sampling, with germline findings subtracted via multiple filters. Studies suggest that increased TMB may predict improved response to immune checkpoint therapy (Nat Genet. 2019 Feb;51(2):202-206). Among melanomas and lung cancers, increasing neo-antigens were associated with improved outcome following immune checkpoint inhibition (N Engl J Med. 2014 Dec 4; 371(23): 2189–2199.; Science. 2015 Apr 3; 348(6230): 124–128). Elevated TMB was also associated with improved progression-free survival in a recent trial of combination immunotherapy (nivolumab /ipilimumab) in non-small cell lung cancer (N Engl J Med. 2018 May 31;378(22):2093-2104).

Limit of detection: For mutations, insertions and deletions, limit of detection is 10 percent mutant alleles (roughly corresponding to 20% tumor cells). Limit of detection for fusions/translocations is 20% tumor cells. Gene fusions can not be detected in the rare occurrence of a fusion between ALK, RET or ROS1 and a partner gene less than 100,000 bp distant. Limit of detection for copy number changes is >4X or <0.5X normal copy number, with relevant equivocal changes reported at >2X or <0.6X. Please contact the lab for more information.

Limitations: This test will not detect mutations in areas outside the targeted genomic regions. This test is not intended to detect minimal residual disease. >20% specimen tumor cells are required for high confidence

mutation and fusion calling, thus false-negative results may occur when there is a lower tumor cell burden. >40% specimen tumor cells are required for high confidence microsatellite instability (MSI) calling, and false-negative results (microsatellite stability) may occur when there is a lower tumor cell burden. Reduced sensitivity for insertions and deletions may be seen above 60bp in size depending on sequence composition. For copy number calling, sensitivity depends on the overall copy number alteration in tumor cells as well as the proportion of malignant cells in the specimen. All copy number results are gene-based only, the assay is not validated to report cytogenetic (larger scale) gains and losses. As with mutations, reduced detection sensitivity may be seen in specimens with low tumor cell burden. Fusion genes may not be detected in the rare occurrence that the partner gene or locus is less than 100,000 bp from the gene of interest. To ensure accuracy, TMB results are not provided if the specimen tumor cell burden estimate is below 30% or if other evidence suggests low tumor cell burden. This test evaluates for mutations in tumor tissue only, and is not intended for detection of germline mutations. Therefore if a hereditary/familial cancer is of clinical concern, additional clinical evaluation and genetic counseling prior to additional testing may be considered. Additional variants that are considered benign by the laboratory may not be reported. For information about these variants, please contact the laboratory or attending pathologist directly.

JEREMY SEGAL
Interpretation performed by the Attending Pathologist.
Electronically Signed Out By JEREMY SEGAL

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Disclaimer: This test was developed and its performance characteristics determined by The University of Chicago Clinical Genomics Laboratory. It has 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. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 ('CLIA') as qualified to perform high complexity clinical laboratory testing.

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Addendum Report

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Gender:	F	Location:	NPDL(UCH)	Completed:	9/30/2019
Physician:	Raphael Borok, MD				

Specimen(s) Received

A: Next Generation Sequencing, FFPE: Received are 4 blocks (A,B,H,J) from Precision Diagnostics (Oak Brook, IL.) labeled 1867 with a procedure date of 7/22/2019. These are accompanied by a report with the patient's name and date of birth 4/10/1949.

Addendum Comment

Immunohistochemical staining for PD-L1 is performed at the request of the submitting outside institution. This stain shows weak to moderate staining in 2 to 3% of the tumor cells.

PETER PYTEL
Interpretation performed by the Attending Pathologist.
Electronically Signed Out By PETER PYTEL

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