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TO: UCH Medical Staff, Housestaff, Patient Care Centers, and Outpatient Clinics, University Chicago Comprehensive Cancer Center

FROM: Lauren L. Ritterhouse, MD, PhD, Jeremy P. Segal, MD, PhD, Megan McNerney, MD, PhD, Y. Lynn Wang, MD, PhD, FCAP, and Carrie Fitzpatrick, PhD

RE: Update to UChicago OncoPlus Universal Cancer Mutation Analysis Panel: MSI Calling

Announcement
The Clinical Genomics Laboratory in the Division of Genomic and Molecular Pathology is pleased to announce a significant update to our universal cancer mutation analysis panel, OncoPlus v3.0. The OncoPlus panel is a targeted next generation sequencing (NGS) assay that is designed to interrogate 1,213 cancer-related genes for assessments of both solid tumors and hematological malignancies, with a subset clinically reported for personalized care.

Via this update, we are implementing 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)”.

This updated calling algorithm will allow for screening of patients with Lynch syndrome as well as to identify patients who may be eligible for immunotherapy. The remainder of the OncoPlus panel will remain unchanged and includes the genes detailed below.

Test information
Genes reported for mutations, insertions and deletions (147): ABL1, AKT1, ALK, APC, ARID1A, ARID2, ASXL1, ATM, ATR, ATRX, AXL, B2M, BAP1, BCOR, BCO1, BIRC3, BLM, BRAF, BRCA1, BRCA2, BTK, CALR, CBL, CBLB, CCND1, CCND2, CCND3, CDH1, 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, MET, MLH1, MLH3, MPL, MRE11A, MSH2, MSH6, MTOR, MYD88, NBN, NF1, NF2, NFE2L2, NOTCH1,
NOTCH2, NPM1, NRAS, PALB2, PBRM1, PDGFRA, PDGFRB, PHF6, PIK3CA, PIK3CB, PIK3R1, PLCG2, POLE, POT1, PPP2R1A, PTC1, PTEN, PTPN11, RAD21, RAD51, RB1, RET, RUNX1, SDHB, SDHC, SDHD, SETBP1, SF3B1, SMAD4, SMARCB1, SMC1A, SMC3, SMO, SRSF2, STAG2, STK11, TERT, TET2, TP53, TSC1, TSC2, U2AF1, VHL, WT1, and ZRSR2.

Genes reported for copy number gains and losses (136): ABL1, AKT1, ALK, APC, ARID1A, ARID2, ASXL1, ATM, ATR, AXL, B2M, BAP1, BIRC3, BLM, BRAF, BRCA1, BRCA2, CALR, CBL, CBLB, CCND1, CCND2, CCND3, CDH1, CDKN2A, CEBPA, CHEK1, CHEK2, CSF1R, CSF3R, CTCF, CTNNB1, CUX1, CXCR4, DAXX, DDR2, DDX41, DICER1, DNMT3A, EGFR, EP300, EPHA3, EPHA5, ERBB2, ERBB3, ERBB4, ERCC3, ESRI, 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, MET, MLH1, MLH3, MPL, MRE11A, MSH2, MSH6, MTOR, MYD88, NBN, NF1, NF2, NFE2L2, NOTCH1, NOTCH2, NPM1, NRAS, PALB2, PBRM1, PDGFRA, PDGFRB, PIK3CA, PIK3CB, PIK3R1, PLCG2, POLE, POT1, PPP2R1A, PTC1, PTEN, PTPN11, RAD21, RAD51, RB1, RET, RUNX1, SDHB, SDHC, SDHD, SETBP1, SF3B1, SMAD4, SMARCB1, SMC3, SMO, SRSF2, STK11, TERT, TET2, TP53, TSC1, TSC2, U2AF1, VHL, and WT1.

Genes reported for fusions/translocations: ALK, RET, and ROS1.

The test procedure involves DNA extraction and quantity/quality assessment, fragmentation and library preparation, followed by pooled capture targeting the desired genomic loci. Next generation sequencing (NGS) is performed on the HiSeq 2500 system (Illumina) and downstream analysis for quality control and detection of mutations is performed via custom-design bioinformatics pipelines on a HIPAA-compliant high performance computing system within the Center for Research Informatics (CRI). 147 genes will be reported for mutations and indels, 136 genes will be reported for copy number changes, and 3 genes will be reported for fusion events.

Specimen Requirements
Acceptable specimens include formalin-fixed, paraffin-embedded (FFPE) tissue or cytology (DiffQuick) aspirate smears for solid tumors, or blood/bone marrow aliquots for hematological malignancies (purple-top tubes). Specimens should contain >20% tumor cells and enough total cells to produce adequate DNA yield (typically >50,000 total cells). Specimens with less than 20% tumor cells may be tested at the discretion of the attending molecular pathologist. For accurate MSI calling, >40% tumor cells is required.
Test ordering
The test can be ordered through Epic using the same codes as before, as demonstrated below:

a. LABAPNGPLSM for Bone Marrow

b. LABAPNGPLB for Peripheral Blood
c. LABAPNGPLF for FFPE:

Reporting and Test limitations
The basic report format will remain similar to our current OncoPlus format, with mutations, copy numbers, and fusions listed in addition to the MSI status. While the assay itself is intended for performance on specimens with >20% tumor cells, accurate detection of MSI has been assessed for specimens with >40% tumor cells. False-negative results of all types may occur when there is a lower than adequate tumor cell burden.

Testing Frequency and Turnaround Time
Testing will be performed at least once weekly, Monday through Friday during day shifts only. Expected turnaround time is 12-18 business days following receipt of adequate specimens.

Additional Questions
Additional questions may be directed to the Division of Genomic and Molecular Pathology at 773-702-4946 or Dr. Lauren Ritterhouse at 773-702-8491 or Dr. Jeremy Segal at 773-702-3674.