Diagnosis: See pathology report for HBWI-21-2470

Primary Tumor Site: Colorectal **Tumor Content (%):** 70 **Block #:** C1

AuraSeq Solid Tumor Test Results

POSITIVE Genes for Detected Clinical Variants: ***KRAS, PIK3CA; MSI-H and MLH1 Promoter Hypermethylated by PCR (see below)***

NEGATIVE Genes (Undetectable, disease-specific): ***NRAS, HRAS, BRAF***

Approved Therapies Associated with Response: ***8***

Summary of Clinically Significant Variants

Gene Variant	FDA Approved Therapies (for patient's tumor type)	FDA Approved Therapies (for other tumor types)	Therapies with Resistance	Potential Clinical Trials (by Gene)
KRAS c.35G>T p.G12V	Pembrolizumab (KEYTRUDA) Nivolumab (OPDIVO)	Binimetinib (MEKTOVI) Cobimetinib (COTELLIC) Trametinib (MEKINIST) Selumetinib (KOSELUGO)	Cetuximab (ERBITUX) Panitumumab (VECTIBIX)	NCT02465060 NCT02693535 NCT02857270 NCT02079740 NCT03087071 NCT03981614
PIK3CA c.3140A>G p.H1047R	None	Alpelicib (PIQRAY) Idelalisib (ZYDELIG)	None	NCT03994796 NCT04589845 NCT02861300

Full MSI and MLH1 Promoter Hypermethylation reports attached

Tier I and II Variant Details

Gene Symbol	Exon	Nucleotide Change	Amino Acid Change	VAF (%)	Effect on Protein	Category
KRAS	2	c.35G>T	p.G12V	19.2	Gain-of-function	Tier I - Level A

The KRAS (homologous to the oncogene from the Kirsten rat sarcoma virus) protein and other members of the RAS family are central mediators downstream of growth factor receptor signaling and therefore are critical for cell proliferation, survival, and differentiation. Somatic missense mutations in the KRAS gene lead to single amino acid substitutions and are generally independent of EGFR mutations. The most frequent alterations are detected in codons 12 (~82% of all reported KRAS mutations) and 13 (~17%) in exon 2 of the KRAS gene. Mutations in other positions, such as codons 61 and 146, have also been reported; however, these alterations account for a minor proportion (1–4%) of KRAS mutations. KRAS mutations in codons 12 and 13 appear to play a major role in the progression of colorectal cancer (CRC). KRAS mutations are found in approximately 30-40% of patients with CRC, and it has been shown that tumors harboring KRAS gene mutations in exons 2, 3, or 4 are associated with poor response to EGFR targeted therapies such as panitumumab or cetuximab (1,2). A subsequent study, however, has shown that the use of cetuximab was associated with longer overall survival (OS) in CRC patients with tumors harboring the KRAS NM_ 004985.3: c.38G>A (p.Gly13Asp) mutation, commonly known as p.G13D, than other KRAS-mutated tumors (3).
References: 1- J Clin Oncol. 2008;26(3):374-9; 2- N Engl J Med. 2013;369(11):1023-34; 3- JAMA. 2010;304(16):1812-20.

Gene Symbol	Exon	Nucleotide Change	Amino Acid Change	VAF (%)	Effect on Protein	Category
PIK3CA	21	c.3140A>G	p.H1047R	24.8	Gain-of-function	Tier II - Level C

Phosphatidylinositol-4,5-bisphosphate 3-kinases (PI3K) are a family of lipid kinases involved in cell growth, proliferation, and survival, among other processes, by recruiting downstream signaling molecules such as AKT1. The PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha) gene encodes for the p110a catalytic subunit of the PI3K heterodimer. Mutant PIK3CA has been found in several tumors, including colorectal cancer (CRC), gliomas, gastric cancer, breast cancer, endometrial cancer, and lung cancer. Somatic mutations in PIK3CA have been found in 10-30% of CRC tumors (1). Multiple PI3K inhibitors, including buparlisib (2), taselisib (3), and copanlisib (4), are under investigation in patients with PIK3CA-mutated or PTEN-mutated solid tumors. Retrospective studies have suggested improved survival with postoperative aspirin use in patients whose CRC harbors a PIK3CA mutation (5).
References: 1- Science. 2004;304(5670):554.; 2- Gynecol Oncol. 2014;133(2):346-52; 3- Gynecol Oncol. 2014;135(2):312-7; 4- Ann Oncol. 2016;27(10):1928-40; 5- J Clin Oncol. 2013;31(34):4297-305.

Details on FDA approved Therapies (with Response or Resistance) and Clinical Trials

Pembrolizumab (KEYTRUDA)

Pembrolizumab (KEYTRUDA) is a monoclonal antibody that binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2, releasing PD-1 pathway-mediated inhibition of the immune response, including the anti-tumor immune response. Keytruda is specifically indicated for the treatment of patients with unresectable or metastatic melanoma, NSCLC with no EGFR or ALK alterations and PD-L1 expression = 1%, SCLC, HNSCC, and for the treatment of adult and pediatric patients with unresectable or metastatic, microsatellite instability-high (MSI-H) or mismatch repair deficient solid tumors, and for tumor mutational burden-high (TMB-H) cancers. For the complete drug label text, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/125514s084lbl.pdf

Nivolumab (OPDIVO)

Nivolumab (OPDIVO) is a human monoclonal antibody that blocks the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Binding of the PD-1 ligands, PD-L1 and PD-L2, to the PD-1 receptor found on T cells, inhibits T-cell proliferation and cytokine production. Opdivo is specifically indicated for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor. For the complete drug label, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/125554s022lbl.pdf.

Binimetinib (MEKTOVI)

Binimetinib (MEKTOVI) is a kinase inhibitor indicated, in combination with encorafenib, for the treatment of patients with unresectable or metastatic melanoma with a BRAF V600E or V600K mutation. For the complete drug label, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/210498lbl.pdf

Cobimetinib (COTELLIC)

Cobimetinib (COTELLIC), is a MEK inhibitor, FDA-approved for the treatment of patients with unresectable or metastatic melanoma with a BRAF V600E or V600K mutation, in combination with vemurafenib. For the complete drug label, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/206192s000lbl.pdf

Trametinib (MEKINIST)

Trametinib (MEKINIST) is a MEK inhibitor, FDA-approved for the treatment of patients with unresectable or metastatic melanoma with BRAF V600E or V600K mutations, as detected by an FDA-approved test. For the complete drug label, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/204114s001lbl.pdf

Selumetinib (KOSELUGO)

Selumetinib (KOSELUGO) is a MEK1/2 inhibitor, approved by the FDA for neurofibromatosis type 1 with symptomatic, inoperable plexiform neurofibromas, and previously given investigational use only as adjuvant treatment for patients with stage III or IV differentiated thyroid cancer. For the complete drug label, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/213756s000lbl.pdf

Alpelicib (PIQRAY)

Alpelicib (PIQRAY) is an FDA approved kinase inhibitor indicated in combination with fulvestrant for the treatment of postmenopausal women, and men, with hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative, PIK3CA-mutated, advanced or metastatic breast cancer as detected by an FDA-approved test following progression on or after an endocrine-based regimen. For the complete drug label, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/212526s000lbl.pdf.

Idelalisib (ZYDELIG)

Idelalisib (ZYDELIG), a PI3K inhibitor, is an FDA-approved drug for the treatment of patients with relapsed chronic lymphocytic leukemia (CLL), follicular lymphoma and small lymphocytic lymphoma (SLL). For the complete drug label, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/206545lbl.pdf

Cetuximab (ERBITUX)

Cetuximab (ERBITUX), is a chimeric monoclonal antibody directed against EGFR. FDA approved alone or in combination to treat advanced squamous cell carcinoma of the head and neck and to treat K-Ras mutation negative, EGFR expressing metastatic colorectal cancer. For the complete drug label, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/125084s0228lbl.pdf

Panitumumab (VECTIBIX)

Panitumumab (VECTIBIX) is a recombinant, human monoclonal antibody that binds specifically to EGFR. FDA approved for the treatment of metastatic colorectal carcinoma (mCRC) patients with disease progression or following certain chemotherapy regimens. Vectibix is not recommended for the treatment of mCRC patients with KRAS mutations (codons 12 or 13), detected by an FDA-approved test. For the complete drug label, go to: https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/125147s080lbl.pdf

NCT02465060

Title: NCI-MATCH: Targeted Therapy Directed by Genetic Testing in Treating Patients With Advanced Refractory Solid Tumors, Lymphomas, or Multiple Myeloma. This phase II trial studies how well treatment that is directed by genetic testing works in patients with solid tumors or lymphomas that have progressed following at least one line of standard treatment or for which no agreed upon treatment approach exists. Genetic tests look at the unique genetic material (genes) of patients' tumor cells. Patients with genetic abnormalities (such as mutations, amplifications, or translocations) may benefit more from treatment which targets their tumor's particular genetic abnormality. Identifying these genetic abnormalities first may help doctors plan better treatment for patients with solid tumors, lymphomas, or multiple myeloma. For more information contact University of Alabama at Birmingham Cancer Center Recruiting, Birmingham, Alabama, United States, 35233 (or other recruiting centers throughout the USA). Contact: Carla I. Falkson (Principal Investigator), Ph: 205-934-0309.

NCT02693535

Title: TAPUR: Testing the Use of Food and Drug Administration (FDA) Approved Drugs That Target a Specific Abnormality in a Tumor Gene in People With Advanced Stage Cancer (TAPUR). The purpose of the study is to learn from the real world practice of prescribing targeted therapies to patients with advanced cancer whose tumor harbors a genomic variant known to be a drug target or to predict sensitivity to a drug. For more information contact: Emory University Winship Cancer Institute, Atlanta, GA, 30322; Contact: Somini John; phone: 404-778-7664; e-mail: somini.m.john@emory.edu. Principal Investigator: Olatunji Alese, MD

NCT02857270

Title: A Phase 1 Study of an ERK1/2 Inhibitor (LY3214996) Administered Alone or in Combination With Other Agents in Advanced Cancer. Massachusetts General Hospital, Boston, Massachusetts, United States, 02114. Contact Ph: 617-643-3614 ; Principal Investigator: Ryan Sullivan

NCT02079740

Title: An Open Label, Two-Part, Phase Ib/II Study to Investigate the Safety, Pharmacokinetics, Pharmacodynamics, and Clinical Activity of the MEK Inhibitor Trametinib and the BCL2-Family Inhibitor Navitoclax (ABT-263) in Combination in Subjects With KRAS or NRAS Mutation-Positive Advanced Solid Tumors. Trametinib and Navitoclax in Treating Patients With Advanced or Metastatic Solid Tumors. Dana-Farber Cancer Institute, Boston, Massachusetts, United States, 02215. Contact: Ryan B. Corcoran Ph: 877-726-5130. Principal Investigator: Ryan B. Corcoran

NCT03087071

Title: A Phase II Enrichment Study of Panitumumab as a Single Agent or in Combination With Trametinib in Cetuximab-Refractory Stage IV Colorectal Cancer Patients. The goal of this clinical research study is to learn if panitumumab alone or in combination with trametinib can help to control advanced colorectal cancer. The safety of these drugs will also be studied. For more information contact University of Texas MD Anderson Cancer Center, Houston, TX, 77030. Contact Clinical Research Operations; e-mail: CR_Study_Registration@mdanderson.org

NCT03981614

Title: Binimetinib and Palbociclib or TAS-102 in Treating Patients With KRAS and NRAS Mutant Metastatic or Unresectable Colorectal Cancer. This phase II trial studies how well binimetinib and palbociclib work compared to TAS-102 in treating patients with KRAS and NRAS mutation positive colorectal cancer that has spread to other places in the body or cannot be removed by surgery. Binimetinib and palbociclib may stop the growth of tumor cells by blocking some of the enzymes needed for cell growth. Drugs used in chemotherapy, such as TAS-102, work in different ways to stop the growth of tumor cells, either by killing the cells, by stopping them from dividing, or by stopping them from spreading. Giving binimetinib and palbociclib may work better compared to TAS-102 alone in treating patients with colorectal cancer. This study is sponsored by NCI. For more information contact: Emory University Hospital/Winship Cancer Institute, Atlanta, GA 30322; contact person: KJ Lee phone: 404 -778-3173 email: kyungjong.lee@emory.edu; Principal Investigator: Mehmet Akce

NCT03994796

Title: Genetic Testing in Guiding Treatment for Patients With Brain Metastases.This phase II trial studies how well genetic testing works in guiding treatment for patients with solid tumors that have spread to the brain. Several genes have been found to be altered or mutated in brain metastases such as NTRK, ROS1, CDK or PI3K. Medications that target these genes such as abemaciclib, GDC-0084, and entrectinib may stop the growth of tumor cells by blocking some of the enzymes needed for cell growth. This study is sponsored by NCI and Genentech, Inc. For further informations contact: Emory University Hospital Midtown, Atlanta, GA, 30308; phone: 888-946-7447 , 404-778-1868, 404-851-7115; Principal Investigator: Jim Zhong

NCT04589845

Title: Tumor-Agnostic Precision Immuno-Oncology and Somatic Targeting Rational for You (TAPISTRY) Platform Study.TAPISTRY is a Phase II, global, multicenter, open-label, multi-cohort study designed to evaluate the safety and efficacy of targeted therapies or immunotherapy as single agents or in rational, specified combinations in participants with unresectable, locally advanced or metastatic solid tumors determined to harbor specific oncogenic genomic alterations or who are tumor mutational burden (TMB)-high as identified by a validated next-generation sequencing (NGS) assay. Participants with solid tumors will be treated with a drug or drug regimen tailored to their NGS assay results at screening. Participants will be assigned to the appropriate cohort based on their genetic alteration(s). Treatment will be assigned on the basis of relevant oncogenotype, will have cohort-specific inclusion/exclusion criteria, and, unless otherwise specified, will continue until disease progression, loss of clinical benefit, unacceptable toxicity, participant or physician decision to discontinue, or death, whichever occurs first. This study is sponsored by Hoffmann-La Roche. For further informations contact: University Cancer & Blood Center, LLC; Research, Athens, GA, 30607; Reference Study ID Number: BO41932 www.roche.com/about_roche/roche_worldwide.htm 888-662-6728 (U.S. and Canada) Global-Roche-Genentech-Trials@gene.com.

NCT02861300

Title: CB-839 + Capecitabine in Solid Tumors and Fluoropyrimidine Resistant PIK3CA Mutant Colorectal Cancer. For more information contact: University Hospitals, Seidman Cancer Center, Case Comprehensive Cancer Center, Cleveland, OH, 44106. Principal Investigator: David Bajor, MD; phone: 216-286-4414; e-mail: david.bajor@uhhospitals.org

Tier III: Variants of Unknown Significance (VUS)

None Detected

Methods and Limitations

Genomic DNA extracted from this patient's sample was used for multiplex PCR amplification using Ion AmpliSeqTM primers and reagents to detect somatic variants located in over 2,800 different hotspots (covered by 207 amplicons) on the 50 genes described in the Genes Tested section below. These 50 genes are involved in pathways that have been associated with cancer and response to certain targeted therapies. Amplicons were sequenced using next generation sequencing (NGS) technology on the Ion Torrent S5 XL sequencer and analyzed with Torrent Suite Software (version 5.8.0). The Feb. 2009 assembly of the human genome (hg19, GRCh37) is used as a reference. The DNA sequences for this panel of genes can be found at http://www.ncbi.nlm.nih.gov/refseq/rsg/ using the accession numbers listed in the Genes Tested section below. Variant nomenclature is based on the convention recommended by the Human Genome Variation Society (http://varnomen.hgvs.org/). The 50 genes included are not sequenced in their entirety. Variants outside the 207 amplicons included in this assay, as well as structural variants, will not be detected. This assay performs with 100% (95%CI 95.1%-100.0%) sensitivity and 100% (95%CI 67.6%-100.0%) specificity. The specimen is judged undetectable for any of the tested genes if variants are present at a frequency below 3% of total DNA, at a mean coverage of 2000X and a minimum coverage of 200X, or below 8% at a minimum coverage of 100X for single nucleotide variants (SNVs). Small insertions and deletions (Indels) are detected when present at a minimum frequency of 3% of total DNA, at a mean coverage of 2000X and a minimum coverage of 500X. This is a qualitative assay so the variant allele frequency (VAF) values are estimated. This report includes variants, and their estimated VAF, classified based on a tiered evidence-based system according to AMP, ASCO, and CAP guidelines (PMID: 27993330), as follows:

- Tier I: Variants of Strong Clinical Significance (therapeutic, prognostic & diagnostic) with Level A (FDA approved therapies, and included in professional guidelines) or Level B (well-powered studies with consensus from experts in the field, including potential germline pathogenic variants associated with cancer predisposition) evidence.
- Tier II: Variants of Potential Clinical Significance (therapeutic, prognostic & diagnostic) with Level C (FDA approved therapies for different tumor types or investigational therapies, and/or included in multiple small published studies with some consensus) or Level D (preclinical trials or a few case reports without consensus) evidence.
- Tier III: Variants of Uncertain Significance (VUS).
- Benign and likely-benign variants (Tier IV), or UNDETECTABLE variants are NOT reported.

This test is performed on DNA isolated from the tumor sample only, therefore the somatic, or germline, nature of reported variants is inferred. FDA approved drugs and clinical trials listed in this report are referenced for information purposes only and are not guaranteed to offer clinical benefit to the patient. Treatment decision responsibilities reside entirely with the treating physician.

Genes Tested

ABL1 (NM_005157.4), AKT1 (NM_005163.2), ALK (NM_004304.4), APC (NM_000038.5), ATM (NM_000051.3), BRAF (NM_004333.4), CDH1 (NM_004360.3), CDKN2A (NM_000077.4), CSF1R (NM_005211.3), CTNNB1 (NM_001904.3), EGFR (NM_005228.3), ERBB2 (NM_004448.2), ERBB4 (NM_005235.2), EZH2 (NM_004456.4), FBXW7 (NM_033632.3), FGFR1 (NM_001174067.1), FGFR2 (NM_000141.4), FGFR3 (NM_000142.4), FLT3 (NM_004119.2), GNA11 (NM_002067.2), GNAQ (NM_002072.3), GNAS (NM_000516.4), HNF1A (NM_000545.5), HRAS (NM_005343.2), IDH1 (NM_005896.2), IDH2 (NM_002168.3), JAK2 (NM_004972.3), JAK3 (NM_000215.3), KDR (NM_002253.2), KIT (NM_000222.2), KRAS (NM_033360.3), MET (NM_000245.2), MLH1 (NM_000249.3), MPL (NM_005373.2), NOTCH1 (NM_017617.3), NPM1 (NM_002520.6), NRAS (NM_002524.4), PDGFRA (NM_006206.4), PIK3CA (NM_006218.2), PTEN (NM_000314.6), PTPN11 (NM_002834.3), RB1 (NM_000321.2), RET (NM_020975.4), SMAD4 (NM_005359.5), SMARCB1 (NM_003073.4), SMO (NM_005631.4), SRC (NM_198291.2), STK11 (NM_000455.4), TP53 (NM_000546.5), and VHL (NM_000551.3).

Electronically Signed By:

Rathore M.D., Sumra

Signed Date: 05-14-2021

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Catherine I. Dumur, Ph.D., HCLD(ABB)

Signed Date: 05-13-2021

Disclaimer

This test is a Laboratory Developed Test that was developed and its performance characteristics were determined by the Bernhardt Laboratories at Aurora Diagnostics. This test has not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA does not require this test to go through pre-market review. This test is used for clinical purposes and should not be regarded as investigational or for research. The Molecular Diagnostics Laboratory is certified under Clinical Laboratory Improvement Amendment of 1988 as qualified to perform high

complexity clinical laboratory testing.

Testing Performed : Aurora Diagnostics , 5008 Mustang Road, Jacksonville, FL 32216. CLIA # 10D0645099.

Specimen: . Colon Right; HBWI-21-2470 Block #: C1 Tumor Content (%): 70

Microsatellite Instability (MSI)

Result: MSI - HIGH

COMMENT: The observed MSI-High pattern may indicate a DNA mismatch repair-deficient (dMMR) phenotype, which may be secondary to a somatic or germline genetic alteration. MLH1 promoter hypermethylation testing and/or BRAF mutation testing, is indicated (1). In addition, the monoclonal antibody Pembrolizumab (KEYTRUDA) is specifically indicated by the FDA for the treatment of patients with unresectable or metastatic, MSI-H or mismatch repair deficient solid tumors.
Reference: 1- Sepulveda et al., J Mol Diagn 2017;19(2):187-22.

REFLEX MLH1 PROMOTER HYPERMETHYLATION STUDIES PENDING; ADDENDUM REPORT TO FOLLOW.

Note(s)

Tumor content was estimated from an H&E slide.

Methodology:
Microsatellite instability (MSI) by PCR is performed using the patient's paired normal and tumor DNA from paraffin embedded tissue. The DNA is isolated using manual microdissection. A multiplex PCR reaction is performed using primers specific to five loci with mononucleotide repeats, BAT-25, BAT-26, NR-21, NR-24 and MONO-27. Instability is reported as MSI-High (2 or more of the 5 markers shows instability), MSI-Low (1 of the markers shows instability) or MSI-Stable (no instability detected in any of the 5 markers). This assay also uses 2 pentanucleotide markers to verify that normal and tumor DNA tested is from the same patient sample. The sensitivity of the assay is 18% of tumor noted from review of the H&E or 9% tumor DNA in the context of normal DNA. This assay performs with 100% sensitivity (95% CI 73.5% to 100.0%) and 100% specificity (95% CI 85.2% to 100.0%). Factors that may affect the performance of the assay include quality and quantity of DNA material, age of sample, and sample preparation. MSI by PCR is frequently done in conjunction with IHC for MMR. The performance characteristics of this test have been determined by Aurora Diagnostics/Bernhardt Laboratories. This test has not been approved by the FDA. The FDA has determined that such clearance or approval is not necessary. This laboratory is CLIA certified to perform high complexity clinical testing.

Clinical Significance:
Concomitant DNA mismatch repair (MMR) testing by IHC and microsatellite instability (MSI) testing by PCR effectively identifies patients with colorectal and endometrial carcinomas who are at increased risk for Lynch Syndrome (LS). The syndrome is characterized by the development of approximately 2-6% of colorectal cancers and 3-5% endometrial cancers, frequently diagnosed at an early age. Screening for LS in patients with colorectal and/or endometrial cancers has clinical implications for prevention of a second cancer in the affected patient and incident cancer in family members through risk-reducing strategies and heightened surveillance. Microsatellite instability is the molecular fingerprint of a deficient MMR system, and it can be frequently observed in sporadic colorectal cancers (about 15%) due to somatic abnormalities, usually hypermethylation of the MLH1 gene promoter, and a mutated BRAF gene (about 60% of the cases). Instability is a change in length of the microsatellite allele due to insertion or deletion of repeat units during DNA replication and failure of the DNA MMR system to correct these errors. On the other hand, microsatellite instability in the absence of MLH1 promoter hypermethylation and in the absence of a BRAF mutation, is likely to be associated with LS, thus initiating definitive genetic testing and genetic counseling is strongly recommended for MSI-High (MSI-H) tumors, and for MSI-Low (MSI-L) tumors if high clinical suspicion of LS exists. In addition, the monoclonal antibody Pembrolizumab (KEYTRUDA) is specifically indicated by the FDA for the treatment of patients with unresectable or metastatic, MSI-H or mismatch repair deficient solid tumors.

Testing Performed : Aurora Diagnostics , 5008 Mustang Road, Jacksonville, FL 32216. CLIA # 10D0645099.

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Dinesh Pradhan M.D.
Signed Date: 05-06-2021

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Signed Date: 05-06-2021

MOLECULAR RESULTS:

Specimen: Colon Right; HBWI-21-2470 Block #: C1 Tumor Content (%): 70

MLH1 Hypermethylation Assay

Result: **MLH1 Hypermethylation Present.**

Interpretation

The loss of MLH1 protein expression, as detected by immunohistochemistry, is most likely due to a somatic epigenetic modification (hypermethylation) of the MLH1 gene promoter region. Even though there have been a few reported cases of germline hypermethylation of CpG islands on the MLH1 promoter region (1), the presence of such hypermethylation has been documented in approximately 20% of sporadic colorectal cancers (2), which correlated with loss of MLH1 expression and high microsatellite instability (MSI-H). Thus, tumors exhibiting immunohistochemical loss of MLH1 in the presence of MLH1 promoter methylation are presumed more likely to be a sporadic cancer.

REFERENCES: 1- World J Gastroenterol. 2008;14(48):7329-34. 2- PLoS One. 2013;8(3):e59064.

Note(s)

Tumor content was estimated from an H&E slide.

Comment

METHOD: This assay detects the level of methylation of five CpG sites located in the region spanning from -209 to -181 from the transcription start site of the MLH1 gene (Ensembl gene: ENSG00000076242; HGNC:7127) after bisulfite conversion and PCR amplification followed by pyrosequencing. This approach allows to differentiate 5-methylcytosine (mC) from unmethylated cytosine (C) in a single pyrosequencing reaction for each patient sample. Samples showing a %Methylation average across the 5 CpG sites less than or equal to 10.5% are considered to be Unmethylated, whereas those showing >10.5% are considered to be Hypermethylated. This assay has a limit of detection of 5% methylated DNA in a background of unmethylated DNA. However, samples with less than 40% tumor content are subjected to microdissection to enrich for tumor cells. This assay performs with 100% (95%CI 83.2%-100.0%) sensitivity, 100% (95%CI 75.8%-100.0%) specificity and 100% (95%CI 89.0%-100.0%) accuracy.

Testing Performed : Aurora Diagnostics , 5008 Mustang Road, Jacksonville, FL 32216. CLIA # 10D0645099.

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Rathore M.D., Sumra

Signed Date: 05-13-2021

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Signed Date: 05-13-2021