Medulloblastoma (MB) is an embryonal tumor of the cerebellum that arises for the vast majority from the cerebellar vermis or cerebellar hemishpeheres and accounts for around 20% of brain tumors in children. Multimodal treatment strategies involving surgery, radiation therapy and chemotherapy have markedly improved survival from this disease over the last 3 decades. The current management of MB is based on traditional prognostic factors used to risk-stratify patients: age, extent of resection, presence of metastatic disease and histology. Patients with standard-risk diseases have a 5-year event free survival (EFS) of 79%-85%, whereas high-risk patients have a 5-year EFS ranging between 55% to 70%.

A major limitation of the traditional risk-stratification of MB is that it fails to account for the molecular heterogeneity of this disease. Seminal work by Pomeroy et al., Cho et al., and Kool et al. has elucidated the genomic landscape of MB leading to a consenus recognition of 4 subgroups with distinct gene expression profiles, methylomes and clinicopathological features including demographics, prognosis and histology. Genetically-defined MB has recently been adopted by the World Health Organization in the 2016 updated classification of tumors of the central nervous system. A clinical test distinguishing these 4 subgroups will undeniably be the standard of care in the post-genomic personalized medicine era.

The Division of Pathology and the Cytogenetics Laboratory at CCHMC have partnered to develop the nation's first clinical methylation-derived medulloblastoma subrouping assay.

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Medulloblastoma Subgrouping

Methylation-derived Genetically-defined Medulloblastoma Subgrouping

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changing the outcome together

Medulloblastoma Subgroups

WNT: The WNT subgroup of MBs accounts for around 10% of tumors. These tumors occur in older children and adolescents with a peak incidence of 10-12 years and carry an excellent prognosis with survival rates of 90-95%. Histologically, the majority of WNT tumors are of the classic subtype; however they can rarely be of the large cell/anaplastic (LCA) variety and maintain a good prognosis. Genetically, the pathway is most frequently activated by a mutation in *CTNNB1* which encodes beta-catenin or, less commonly, mutations affecting other signaling intermediates in the pathway (*APC, AXIN1*). Up to 85% of WNT-activated tumors demonstrate monosomy 6 and/or a *CTNNB1* mutation.

Sonic hedgehog (SHH): The SHH subgroup of MBs accounts for around 30% of tumors. Tumors in this subgroup have a bimodal age distribution affecting infants (less than 3 years of age) and young adults (16 years and older) and have variable outcomes. Histologically, there is a clear predominance of nodular/desmoplastic histology and MB with extended nodularity phenotypes. SHH-activated MBs harbor mutations in *PTCH1* (~ 45%), *SMO* (~14% , more common in adults) and *SUFU* (~7.5% , more common in infants). *TP53*-mutant tumors are associated with amplifications of *GLI2*, *MYCN* or *SHH* and poorer prognosis.

Group 3: The group 3 subgroup of MBs accounts for approximately 20% of MBs, occurs more commonly in infants and children, and affects males about twice as often as females. This group carries the worst prognosis in MB irrespective of age; almost 50% of patients present with metastatic disease. Histologically, the group is mostly comprised of the classic or LCA phenotypes. *MYC* amplification and overexpression are a hallmark of this subgroup, and the most common cytogenetic aberration observed is isochromosome 17q.

Group 4: The group 4 subgroup of MBs accounts for the majority of MB cases (~40%), occurs more commonly in children at a peak age of 10 years, and affects males three times as often as females. Prognosis is variable and overall intermediate; almost one third of patients present with metastatic disease at diagnosis. Histologically, the group is mainly composed of tumors with the classic phenotype. Genetically, group 4 MB is characterized by *MYCN* amplification and minimal MYC expression; up to 80% of tumors harbor copy number alterations on chromosome 17 including 17p deletion, 17q gain or isochromosome 17q.

Methodology & Sensitivity

Methodology: Prior to performing methylation-derived subgrouping, a board-certified pathologist will provide diagnostic pathology analysis on H&E stained slides and diagnostic immunohistochemical staining (if needed) to confirm that the tumor tissue is a medulloblastoma. If confirmation is positive, the medulloblastoma methylation-derived subgrouping is performed in Cincinnati Children's CAP/CLIA certified cytogenetics laboratory using the Infinium Assay with the Illumina MethylationEPIC BeadChip platform. DNA is isolated according to standard protocols and bisulfite conversion is performed using the Zymo EZ DNA methylation kit. For formalin fixed paraffin embedded tissues (FFPE), the Qlamp DNA FFPE Tissue Kit is used to extract DNA,

and the Illumina Infinium HD Assay Kit FFPE Restore kit and the Zymo DNA Clean & Concentrator are performed. A support vector machine (SVM) was trained on a cohort of medulloblastoma samples to develop a methylation-derived sub-classification prediction algorithm. The MethylationEPIC BeadChip includes 46 of the 48 CpG dinucleotide signatures used in the classification scheme developed by Hovestadt et al. (2013). The 46 CpG dinucleotide signatures used in the classification scheme developed algorithm classifies and determines a probability for a medulloblastoma tumor into one of four subgroups: SHH, WNT, Group 3 or Group 4. Quality control parameters were accessed using Illumina Genome Studio V2011.1 (Methylation Module, version 1.9.1000)

Analytical Sensitivity: The sensitivity of the EPIC BeadChip to classify a true-positive Sonic hedgehog pathway-activated (SHH), Wnt-pathway activated (WNT) or a Group 4 MB is 100%. The current sensitivity to classify a true-positive Group 3 MB is at least 85.7%.

Turn-Around Time: 10-14 days **CPT Code**: 81406
If you are a researcher, please contact the Cytogenetics lab at 513-636-4474 prior to sending research samples.