Medulloblastoma immunohistochemistry (Non-core)

Reason/Evidentiary Support

In the 2016 CNS WHO classification, medulloblastomas can be placed into one of four diagnostic molecular groups: WNT-activated, SHH-activated and *TP53*-wildtype, SHH-activated and *TP53*-mutant, and non-WNT/non-SHH (the latter encompassing group 3 and group 4 medulloblastoma as provisional diagnostic entities). These molecular groups are characterised by distinct clinical, pathological, and genetic attributes, and their use in integrated diagnoses alongside the histopathological variants of medulloblastoma provides information of prognostic and predictive utility. The groups of medulloblastomas were established by consensus from data in studies that had delineated molecular groups by gene expression profiling.[1] This approach remains the gold standard by which a medulloblastoma is assigned to a molecular group, but DNA methylation profiling is a reliable alternative.[2]

Some approaches that can be effectively applied to FFPE tissue use a restricted list of biomarkers to approximate molecular groups.[3,4] Included among these are immunohistochemical methods targeting surrogate markers of molecular groups, including nuclear β-catenin expression (WNT-activated), GAB1 (SHH-activated), YAP1 (WNT-activated or SHH-activated), and p53 (SHH, *TP53*-mutant), discussed in greater detail below.[5,6] While these immunohistochemical methods are relatively straightforward to develop in clinical histopathology laboratories, they may be challenging to interpret when only small subsets of tumour cells are immunopositive. Additionally, sequencing techniques (including NGS) can be utilized to identify signature mutations associated with distinct molecular groups, some of which provide additional predictive information for targeted therapies (e.g., within the SHH family). Furthermore (see also **Monosomy 6** and **MYC gene family amplification**), detection of copy number alterations can further aid in molecular subtyping (e.g., monosomy 6 for WNT-activated tumours and isodicentric 17q for groups 3 or 4).

<u>β-catenin Nuclear Expression (Immunohistochemistry)</u>

Upon WNT activation, β-catenin, encoded by the *CTNNB1* gene, translocates to the nucleus, where it interacts with transcription factors. Thus, nuclear β-catenin immunopositivity reflects activation of the WNT signalling pathway.

In the clinically relevant WNT-activated group of medulloblastoma, immunohistochemistry for β-catenin reveals reactivity in tumour cell nuclei, although immunostaining is often patchy or focal. Scattered single β-catenin nucleopositive cells should not be interpreted as definitive evidence of WNT activation and requires further analysis to WNT status (see next section).

<u>Immunohistochemistry with antibodies to β-catenin, GAB1, and YAP1 in the determination of medulloblastoma molecular groups</u>

While medulloblastoma molecular groups have been defined on the basis of gene expression and DNA methylation profiling,[7] one immunohistochemical method uses antibodies to β-catenin, GAB1, and YAP1 to place a medulloblastoma into one of three groups: WNT, SHH, and 'non-WNT, non-SHH'.[5,8] This immunohistochemical approach is designed for medulloblastomas and should not be applied to other types of tumours. All three antibodies should be used in the determination of molecular group, providing increased confidence in the result when tissue is limited or processing is suboptimal. In addition, while the combination of β-catenin, GAB1, and YAP1 is a single, broadly implemented approach, different laboratories may use variations on this combination; for example, some centres substitute filamin-A for YAP1 and some use OXTC2 and ant-p75 NGR when GAB cannot be optimized.[9]

Nuclear immunoreactivity for β-catenin signifies WNT pathway activation (Table 1), and WNT-activated medulloblastomas often demonstrate this in most cells, although in some preparations nuclear immunoreactivity may be patchy. As mentioned above, scattered single β-catenin nucleopositive cells should not be interpreted as definitive evidence of WNT activation. In difficult cases with equivocal β-catenin immunoreactivity or a low proportion of nucleopositive cells, widespread immunoreactivity for YAP1 and an immunonegative GAB1 preparation (Table 1) help to classify a medulloblastoma as WNT-activated. In addition, confirmation of WNT status should be sought using molecular analysis to demonstrate monosomy 6 (see **Monosomy 6**) or a *CTNNB1* mutation. SHH and 'non-WNT, non-SHH' medulloblastomas demonstrate immunoreactivity for β-catenin in the cytoplasm, but not the nucleus, of tumour cells. Cytoplasmic GAB1 immunoreactivity is a surrogate marker for SHH medulloblastomas, but is often weak or absent in nodular regions of tumours classified as desmoplastic/nodular or medulloblastoma with extensive nodularity (MBEN). WNT and SHH medulloblastomas show nuclear and cytoplasmic immunoreactivity for YAP1, but YAP1 is immunonegative in 'non-WNT, non-SHH' tumours. YAP1 expression can also be attenuated in nodular regions of desmoplastic/nodular and MBEN variants.

Table 1

MB molecular groups – immunohistochemical markers (see text)			
Antibodies to:	**WNT**	**SHH**	**non-WNT/non-SHH**
β-catenin	cytoplasmic & nuclear	cytoplasmic	cytoplasmic
GAB1	negative	positive	negative
YAP1	positive	positive	negative

References

1 Taylor MD, Northcott PA, Korshunov A, Remke M, Cho YJ, Clifford SC, Eberhart CG, Parsons DW, Rutkowski S, Gajjar A, Ellison DW, Lichter P, Gilbertson RJ, Pomeroy SL, Kool M and Pfister SM (2012). Molecular subgroups of medulloblastoma: the current consensus. *Acta Neuropathol* 123(4):465-472.

2 Schwalbe EC, Williamson D, Lindsey JC, Hamilton D, Ryan SL, Megahed H, Garami M, Hauser P, Dembowska-Baginska B, Perek D, Northcott PA, Taylor MD, Taylor RE, Ellison DW, Bailey S and Clifford SC (2013). DNA methylation profiling of medulloblastoma allows robust subclassification and improved outcome prediction using formalin-fixed biopsies. *Acta Neuropathol* 125(3):359-371.

3 Northcott PA, Shih DJ, Remke M, Cho YJ, Kool M, Hawkins C, Eberhart CG, Dubuc A, Guettouche T, Cardentey Y, Bouffet E, Pomeroy SL, Marra M, Malkin D, Rutka JT, Korshunov A, Pfister S and Taylor MD (2012). Rapid, reliable, and reproducible molecular sub-grouping of clinical medulloblastoma samples. *Acta Neuropathol* 123(4):615-626.

4 Schwalbe EC, Lindsey JC, Straughton D, Hogg TL, Cole M, Megahed H, Ryan SL, Lusher ME, Taylor MD, Gilbertson RJ, Ellison DW, Bailey S and Clifford SC (2011). Rapid diagnosis of medulloblastoma molecular subgroups. *Clin Cancer Res* 17(7):1883-1894.

5 Ellison DW, Dalton J, Kocak M, Nicholson SL, Fraga C, Neale G, Kenney AM, Brat DJ, Perry A, Yong WH, Taylor RE, Bailey S, Clifford SC and Gilbertson RJ (2011). Medulloblastoma: clinicopathological correlates of SHH, WNT, and non-SHH/WNT molecular subgroups. *Acta Neuropathol* 121(3):381-396.

6 Northcott PA, Korshunov A, Witt H, Hielscher T, Eberhart CG, Mack S, Bouffet E, Clifford SC, Hawkins CE, French P, Rutka JT, Pfister S and Taylor MD (2011). Medulloblastoma comprises four distinct molecular variants. *J Clin Oncol* 29(11):1408-1414.

7 Schwalbe EC, Lindsey JC, Nakjang S, Crosier S, Smith AJ, Hicks D, Rafiee G, Hill RM, Iliasova A, Stone T, Pizer B, Michalski A, Joshi A, Wharton SB, Jacques TS, Bailey S, Williamson D and Clifford SC (2017). Novel molecular subgroups for clinical classification and outcome prediction in childhood medulloblastoma: a cohort study. *Lancet Oncol* 18(7):958-971.

8 Ellison DW, Kocak M, Dalton J, Megahed H, Lusher ME, Ryan SL, Zhao W, Nicholson SL, Taylor RE, Bailey S and Clifford SC (2011). Definition of disease-risk stratification groups in childhood medulloblastoma using combined clinical, pathologic, and molecular variables. *J Clin Oncol* 29(11):1400-1407.

9 Pietsch T and Haberler C (2016). Update on the integrated histopathological and genetic classification of medulloblastoma - a practical diagnostic guideline. *Clin Neuropathol* 35(6):344-352.