REVIEW

cIMPACT-NOW update 7: advancing the molecular classification of ependymal tumors

David W. Ellison[1]; Kenneth D. Aldape[2]; David Capper[3]; Maryam Fouladi[4]; Mark R. Gilbert[5]; Richard J. Gilbertson[6]; Cynthia Hawkins[7]; Thomas E. Merchant[8]; Kristian Pajtler[9]; Sriram Venneti[10]; David N. Louis[11]

[1] Department of Pathology, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105, USA.
[2] Center for Cancer Research, Laboratory of Pathology, National Cancer Institute, Bethesda, MD.
[3] Department of Neuropathology, Charité Universitätsmedizin, Berlin, Germany.
[4] Brain Tumor Center, Division of Oncology, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH.
[5] Neuro-Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD.
[6] Department of Oncology, Cambridge Cancer Centre, Robinson Way, Cambridge, CB2 0RE, UK.
[7] Division of Pathology, The Hospital for Sick Children, Toronto, Ontario Canada.
[8] Department of Radiation Oncology, St. Jude Children's Research Hospital, 262 Danny Thomas Place, MS 210, Memphis, TN 38105.
[9] Division of Pediatric Neurooncology, German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany.
[10] Department of Pathology, University of Michigan, Ann Arbor, MI 48109.
[11] Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114.

Keywords
ependymoma, cIMPACT, classification.

Corresponding author:
David W. Ellison, Department of Pathology, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105 (E-mail: *David.Ellison@stjude.org*)

Received 12 May 2020
Accepted 25 May 2020
Published Online Article Accepted 5 June 2020

doi:10.1111/bpa.12866

Abstract

Advances in our understanding of the biological basis and molecular characteristics of ependymal tumors since the latest iteration of the World Health Organization (WHO) classification of CNS tumors (2016) have prompted the cIMPACT-NOW group to recommend a new classification. Separation of ependymal tumors by anatomic site is an important principle of the new classification and was prompted by methylome profiling data to indicate that molecular groups of ependymal tumors in the posterior fossa and supratentorial and spinal compartments are distinct. Common recurrent genetic or epigenetic alterations found in tumors belonging to the main molecular groups have been used to define tumor types at intracranial sites; *C11orf95* and *YAP1* fusion genes for supratentorial tumors and two types of posterior fossa ependymoma defined by methylation group, PFA and PFB. A recently described type of aggressive spinal ependymoma with *MYCN* amplification has also been included. Myxopapillary ependymoma and subependymoma have been retained as histopathologically defined tumor types, but the classification has dropped the distinction between classic and anaplastic ependymoma. While the cIMPACT-NOW group considered that data to inform assignment of grade to molecularly defined ependymomas are insufficiently mature, it recommends assigning WHO grade 2 to myxopapillary ependymoma and allows grade 2 or grade 3 to be assigned to ependymomas not defined by molecular status.

Ependymal tumors make up a heterogeneous category of central nervous system (CNS) gliomas with variably expressed morphologic, immunophenotypic and ultrastructural ependymal features (10). The current WHO classification (2016) lists: subependymoma (WHO grade 1), myxopapillary ependymoma (WHO grade 1), the classic ependymoma with its three histological subtypes—papillary, clear cell and tanycytic (WHO grade 2), anaplastic ependymoma (WHO grade 3) and one genetically defined type—ependymoma, *RELA* fusion-positive (WHO grade 2/ 3). However, aspects of this scheme are not ideal; for example, in some clinical settings, there is a poor association between tumor grading and outcome (4). In addition, recent studies using either DNA methylation profiling to demonstrate molecular groups of ependymoma or genome-wide sequencing to determine the genomic landscape of the disease support the proposition that ependymomas with similar morphologic features from across the neuraxis have distinct origins and oncogenic events of clinicopathologic significance and potential therapeutic utility (12,14,15,17). Seeking to improve the current classification, cIMPACT working committee 2 (WC2) considered a scheme based around molecularly defined types of ependymoma.

BACKGROUND TO THE MOLECULAR CLASSIFICATION OF EPENDYMOMA

Methylation and gene expression profiling studies have provided evidence for at least nine molecular groups of ependymoma across the three principal anatomic compartments of the CNS: supratentorial (ST), posterior fossa (PF) and spinal cord (SC) (3,14,15,21–23). These molecular groups have distinct molecular alterations and clinicopathologic characteristics, and their identification has clinical utility.

One molecular group at each anatomic site consists almost entirely of tumors with the morphologic features of subependymoma (15). Of the two remaining ST molecular groups, one is dominated by ependymomas with *C11orf95-RELA* fusion genes and the other contains tumors with a high frequency of *YAP1-MAMLD1* fusions (1,17). Occasionally, other fusion genes are present in ST ependymomas; for example, *C11orf95* has been reported to partner with *MAML2* and *YAP1*, and *YAP1* with *FAM118B*. In addition, some ST ependymomas have no detectable fusion gene and rare ependymomas with *C11orf95-RELA* fusions arise in the PF (DWE & KDA—personal observations). Across reported datasets, *C11orf95* and *YAP1* contribute most often to pathogenic fusions in the ST-RELA and ST-YAP1 molecular groups, respectively. These two groups of ST ependymoma are distinguished by their clinical characteristics. However, while a difference in patient outcome was reported for the ST-RELA and ST-YAP1 groups in one retrospective study (15), *RELA* fusion status was not found to be prognostic in another trial-based study (13). The clinical significance of rare genetic fusion events in ST ependymomas remains unclear.

Unlike ST ependymomas, PF ependymomas lack recurrent mutations (12,17). However, methylation profiling divides them into two main groups, PFA and PFB, which are also distinguished by global levels of histone H3 K27-trimethylation (16). This epigenetic mark is high in PFB ependymomas, but low in PFA tumors. PFA ependymomas occur mainly in infants, while PFB tumors arise mainly in older children and adults. Patient outcome might also be different; most, but not all, studies show that PFA ependymomas have a significantly worse prognosis (13,15,16,21,23).

Among SC ependymomas are the myxopapillary tumors that predominate in adult patients. These form one methylation group; classic ependymomas form a second and a rare third group consists of spinal subependymomas. Recently, an aggressive SC ependymoma characterized by early dissemination throughout the neuraxis, an anaplastic morphology and *MYCN* amplification has been reported (5,19). Other data indicate that, in adults, classic ependymomas and myxopapillary ependymomas have a similar outcome, suggesting that the latter might be more appropriately assigned to WHO grade 2 (20).

For subependymomas and myxopapillary ependymomas, the relationship between morphology and methylome group is imprecise. Some classic ependymomas without anaplastic features fall into the subependymoma or myxopapillary ependymoma molecular group (22). In addition, the clinical relevance of such findings is unclear; as yet, there is no indication that classification by molecular group provides more clinical utility than the current morphologic classification of these two tumor types.

The above findings have provided impetus to update the classification of ependymomas and, alongside other datasets, have prompted a series of recommendations from cIMPACT WC2:

- Ependymomas should be classified by anatomic site and by molecular group or an associated genetic alteration, so that classification of the disease reflects its underlying biology.
- ST ependymomas should be classified according to the genes, *C11orf95* and *YAP1*, that contribute to most pathogenic gene fusions in each of the two major molecular groups.
- PF ependymomas should be classified according to the two most prevalent molecular groups, PFA and PFB.
- SC ependymomas with *MYCN* amplification should be recognized as a distinctive type of ependymoma with a poor outcome.
- Meaningful data related to the outcome of patients on clinical trials are not yet available for a WHO grade to be assigned to types of ependymoma defined by molecular alterations.
- The rare subependymoma should continue to be identified by morphologic criteria; no clear clinical utility is yet attached to the identification of a subependymoma molecular group at each anatomic site.
- SC myxopapillary ependymomas should continue to be identified by morphologic criteria but designated WHO grade 2, because clinical trial datasets do not support a WHO grade 1 clinical behavior.
- Morphologic subtypes of the classic ependymoma (papillary, clear cell, tanycytic) should be recognized as distinctive patterns in the histopathological description of ependymomas; but, affording no specific clinical utility, they should no longer be included in the classification of the disease.

These recommendations were used to produce a novel classification of ependymal tumors (Table 1). In this classification, a diagnosis of subependymoma or myxopapillary ependymoma is made on morphologic criteria. Other ependymomas would be classified according to anatomic site and the results of molecular testing, if available. If molecular testing has been undertaken, yet no result generated to place an ependymoma among the molecularly defined tumor types in the classification, then the histologically defined diagnosis "ependymoma" is used with the suffix "NEC" (not elsewhere classified). When molecular testing is unavailable, then "ependymoma" is used with the suffix "NOS" (not otherwise specified) (11).

PRACTICAL ASPECTS OF CLASSIFYING EPENDYMOMAS

Longstanding controversy surrounds the clinicopathological utility of grading ependymal tumors (4); though use of WHO grade in the therapeutic stratification of adult patients with ST ependymoma remains established practice (20). Our

Table 1. Recommended ependymal tumor types

Tumor type	WHO grade
Supratentorial ependymoma, *C11orf95* fusion-positive	
Supratentorial ependymoma, *YAP1* fusion-positive	
Supratentorial ependymoma	Grade 2/ 3
Posterior fossa ependymoma, Group PFA	
Posterior fossa ependymoma, Group PFB	
Posterior fossa ependymoma	Grade 2/ 3
Spinal ependymoma, *MYCN*-amplified	
Spinal ependymoma	Grade 2/ 3
Myxopapillary ependymoma	Grade 2
Subependymoma	Grade 1

Sufficient data are currently unavailable for a WHO grade to be assigned to molecularly defined ependymomas.

proposed classification allows only a histologically defined diagnosis of "ependymoma" to be made at any of the three anatomic sites. However, in the upcoming edition of the WHO classification, several tumor types can be assigned to more than one grade, including ependymal tumors. As currently for the ependymoma, *RELA* fusion-positive, a pathologist will be able to assign either grade 2 or grade 3 to an ependymoma defined by morphologic criteria (Table 1), and in a change from previous editions of the classification anaplastic ependymoma will not be listed.

A range of diagnostic tests can be used to discover the molecular alterations that define the new types of ependymoma. Sequencing might demonstrate the fusion genes of the two types of ST ependymoma and interphase fluorescence *in situ* hybridization (iFISH) to demonstrate rearrangement of *C11orf95*, *RELA* or *YAP1* can support the presence of a fusion gene (17). iFISH can also show the defining amplification of the spinal ependymoma, *MYCN*-amplified. Immunohistochemistry to assess the expression of H3 K27-trimethylation can be used to distinguish PFA and PFB ependymomas (16).

DNA methylation profiling is proving to be a powerful tool for the classification of CNS tumors; it works with formalin-fixed paraffin-embedded derivatives and can provide a diagnosis from small tissue samples (2). It is also a powerful adjunct when histopathological features converge on more than one possible diagnosis. For example, the histopathology of ST ependymomas overlaps with several tumor types that were originally identified by methylation profiling, especially the CNS high-grade neuroepithelial tumor with *MN1* alteration (18). Because of its ability to determine whether a ST high-grade neuroepithelial tumor with some ependymal features should not be classified as an ependymoma or whether a PF ependymoma falls into the PFA or PFB molecular group, WC2 proposes consideration of methylation profiling as a front-line diagnostic test when ependymoma is part of the histopathological differential diagnosis.

WC2 considered chromosome 1q gain as a molecular marker to be used in the classification of PF ependymomas. Gain of 1q is present in 15-20% of PF ependymomas and has been reliably and reproducibly associated with a poor outcome and pattern of progression among patients with these tumors (6–8,13). However, among nine subtypes of PFA ependymoma discovered by methylation profiling, outcome was highly variable and subtypes with a poor prognosis could be enriched for 1q gain (subtype PFA-1c) or not (subtype PFA-1e) (14). Considering these data, WC2 proposes that reference to 1q status is placed in the integrated diagnosis among other molecular information (9), rather than used to define a tumor type in the classification.

In conclusion, WC2 proposes a classification of ependymal tumors that extends the principle of defining CNS tumors by characteristic molecular alterations. An integrated and tiered approach to reporting the diagnosis is recommended for capturing information on molecular characteristics alongside histopathological features.

ACKNOWLEDGMENTS

This paper has been reviewed by the Steering Committee and Clinical Advisory Panel of cIMPACT-NOW and by the International Society of Neuropathology Executive.

David Capper has a patent pending entitled DNA methylation-based method for classifying tumor species of the brain (EP3067432A1). Other authors have no conflict of interest to declare.

REFERENCES

1. Andreiuolo F, Varlet P, Tauziede-Espariat A, Junger ST, Dorner E, Dreschmann V *et al* (2019) Childhood supratentorial ependymomas with YAP1-MAMLD1 fusion: an entity with characteristic clinical, radiological, cytogenetic and histopathological features. *Brain Pathol* **29**:205–216.
2. Capper D, Jones DTW, Sill M, Hovestadt V, Schrimpf D, Sturm D *et al* (2018) DNA methylation-based classification of central nervous system tumours. *Nature* **555**:469–474.
3. Cavalli FMG, Hubner JM, Sharma T, Luu B, Sill M, Zapotocky M *et al* (2018) Heterogeneity within the PF-EPN-B ependymoma subgroup. *Acta Neuropathol* **136**:227–237.
4. Ellison DW, Kocak M, Figarella-Branger D, Felice G, Catherine G, Pietsch T *et al* (2011) Histopathological grading of pediatric ependymoma: reproducibility and clinical relevance in European trial cohorts. *J Negat Results Biomed.* **10**:7.
5. Ghasemi DR, Sill M, Okonechnikov K, Korshunov A, Yip S, Schutz PW *et al* (2019) MYCN amplification drives an aggressive form of spinal ependymoma. *Acta Neuropathol* **138**:1075–1089.
6. Godfraind C, Kaczmarska JM, Kocak M, Dalton J, Wright KD, Sanford RA *et al* (2012) Distinct disease-risk groups in pediatric supratentorial and posterior fossa ependymomas. *Acta Neuropathol* **124**:247–257.
7. Kilday JP, Mitra B, Domerg C, Ward J, Andreiuolo F, Osteso-Ibanez T *et al* (2012) Copy number gain of 1q25 predicts poor progression-free survival for pediatric intracranial ependymomas and enables patient risk stratification: a prospective European clinical trial cohort analysis on behalf of the Children's Cancer Leukaemia Group (CCLG), Societe Francaise d'Oncologie Pediatrique

(SFOP), and International Society for Pediatric Oncology (SIOP). *Clinical Cancer Research* **18**:2001–2011.
8. Korshunov A, Witt H, Hielscher T, Benner A, Remke M, Ryzhova M *et al* (2010) Molecular staging of intracranial ependymoma in children and adults. *J Clin Oncol* **28**:3182–3190.
9. Louis DN, Perry A, Burger P, Ellison DW, Reifenberger G, von Deimling A *et al* (2014) International Society of Neuropathology-Haarlem consensus guidelines for nervous system tumor classification and grading. *Brain Pathol* **24**:429–435.
10. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Ellison DW, Figarella Branger D *et al* (2016) WHO Classification of Tumours of the Central Nervous System. Lyon: IARC.
11. Louis DN, Wesseling P, Paulus W, Giannini C, Batchelor TT, Cairncross JG *et al* (2018) cIMPACT-NOW update 1: not otherwise specified (NOS) and not elsewhere classified (NEC). *Acta Neuropathol* **135**:481–484.
12. Mack SC, Witt H, Piro RM, Gu L, Zuyderduyn S, Stutz AM *et al* (2014) Epigenomic alterations define lethal CIMP-positive ependymomas of infancy. *Nature* **506**:445–450.
13. Merchant TE, Bendel AE, Sabin ND, Burger PC, Shaw DW, Chang E *et al* (2019) Conformal radiation therapy for pediatric ependymoma, chemotherapy for incompletely resected ependymoma, and observation for completely resected, supratentorial ependymoma. *J Clin Oncol* **37**:974–983.
14. Pajtler KW, Wen J, Sill M, Lin T, Orisme W, Tang B *et al* (2018) Molecular heterogeneity and CXorf67 alterations in posterior fossa group A (PFA) ependymomas. *Acta Neuropathol* **136**:211–226.
15. Pajtler KW, Witt H, Sill M, Jones DT, Hovestadt V, Kratochwil F *et al* (2015) Molecular classification of ependymal tumors across all CNS compartments, histopathological grades, and age groups. *Cancer Cell* **27**:728–743.
16. Panwalkar P, Clark J, Ramaswamy V, Hawes D, Yang F, Dunham C *et al* (2017) Immunohistochemical analysis of H3K27me3 demonstrates global reduction in group-A childhood posterior fossa ependymoma and is a powerful predictor of outcome. *Acta Neuropathol* **134**:705–714.
17. Parker M, Mohankumar KM, Punchihewa C, Weinlich R, Dalton JD, Li Y *et al* (2014) C11orf95-RELA fusions drive oncogenic NF-kappaB signalling in ependymoma. *Nature* **506**:451–455.
18. Sturm D, Orr BA, Toprak UH, Hovestadt V, Jones DTW, Capper D *et al* (2016) New brain tumor entities emerge from molecular classification of CNS-PNETs. *Cell* **164**:1060–1072.
19. Swanson AA, Raghunathan A, Jenkins RB, Messing-Junger M, Pietsch T, Clarke MJ *et al* (2019) Spinal cord ependymomas with MYCN amplification show aggressive clinical behavior. *J Neuropathol Exp Neurol.* **78**:791–797.
20. Vera-Bolanos E, Aldape K, Yuan Y, Wu J, Wani K, Necesito-Reyes MJ *et al* (2015) Clinical course and progression-free survival of adult intracranial and spinal ependymoma patients. *Neuro Oncol* **17**:440–447.
21. Wani K, Armstrong TS, Vera-Bolanos E, Raghunathan A, Ellison D, Gilbertson R *et al* (2012) A prognostic gene expression signature in infratentorial ependymoma. *Acta Neuropathol* **123**:727–738.
22. Witt H, Gramatzki D, Hentschel B, Pajtler KW, Felsberg J, Schackert G *et al* (2018) DNA methylation-based classification of ependymomas in adulthood: implications for diagnosis and treatment. *Neuro Oncol* **20**:1616–1624.
23. Witt H, Mack SC, Ryzhova M, Bender S, Sill M, Isserlin R *et al* (2011) Delineation of two clinically and molecularly distinct subgroups of posterior fossa ependymoma. *Cancer Cell* **20**:143–157.