Low-grade epilepsy-associated neuro-epithelial tumours (LEAT) in the 2016 WHO classification of tumours of the nervous system: what's old, new and blue?

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Abstract

Histological classification of brain tumours using the World Health Organization (WHO) system is the gold standard for treatment decision. Dizzying developments in molecular-genetic technology and research have rapidly advanced our current understanding of neuro-oncology. As a consequence, the WHO has invited their expert panels to revise the current classification system of tumours of the nervous system and to introduce for the first time, a molecular-genetic approach for selected tumour entities, thus setting a new gold standard in histopathology. In May 2016, the revised 4th Edition of the "blue book" for brain tumours was released and will have a major impact in stratifying diagnosis and treatment. However, as in previous editions, low grade neuro-epithelial tumours presenting with a mixed neuronal and glial phenotype and early-onset epilepsy as the major neurological symptom (herein designated as low-grade epilepsy-associated neuro-epithelial tumours, LEAT) have not been the subject of significant histological or molecular-genetic innovations, which will be critically reviewed by the Neuropathology Task Force of the International League against Epilepsy (ILAE). The Task Force also proposes a roadmap of how to develop a clinically meaningful and integrated clinico-pathological-genetic classification system for LEAT in the near future.

Introduction

The WHO series of organ-specific tumour classification systems is the gold standard for histological tumour typing, and compiled in the "blue book series". Hence, timely updates are required for each tumour entity to take account of improved knowledge of the underlying pathomechanisms and best patient management as new targets for precision medicine become available. In May 2016, the revised WHO classification of tumours of the central nervous system was released[1, 2] and has already revolutionized the arena of neuro-oncology. It is for the first time in the blue book series of brain tumours that molecular-genetic information is required for an integrated phenotypic-genotypic diagnosis[3], and this applies to diffusely infiltrating glial and embryonal tumours. Capacity building for molecular-genetic analysis is an ongoing challenge, but many centres around the world have already

implemented this new concept. Increasing availability of immunohistochemical surrogates for molecular genetic alterations[4] will also help in the practical application of the new WHO classification system. It was the consensus of the WHO panel to acknowledge the rapid developments in neuro-oncology since the breakthrough discoveries of 1p/19q co-deletions as diagnostic, prognostic and predictive biomarker in oligodendrogliomas [5-8], followed by discovery of MGMT (O6-methylguanine-DNA methyltransferase) hypermethylation in glioblastomas as a predictive biomarker for treatment response to temozolomide[9, 10], and the discovery of the R132H mutation in the IDH1 gene in diffuse gliomas [11, 12], which can also be detected immunohistochemically [13, 14].The growing number of randomized trials for best treatment of brain tumours, in which patients were stratified by molecular-genetic analysis of microscopically-reviewed surgical tissue, were most helpful to validate clinically meaningful patient cohorts[11, 15-18].

However, this successful multidisciplinary strategy has not been developed nor systematically applied for LEAT, and tumour entities lacking ample evidence for distinct molecular-genetic signatures have fallen behind this rapidly developing process. The ever-expanding gulf between Virchow's original concepts of cellular pathology (as a subjective diagnosis based on education and experience) and the evolving era of molecular pathology (as an objective diagnosis obtained from laboratory tests) will further divide the field into tumours with a targeted tumour therapy based on defined molecular alterations and those without. The latter applies in particular to rare low-grade tumour entities. These are difficult to recruit prospectively in sufficiently large series to generate meaningful survival data in reasonable time periods. Indeed, 'neurodevelopmental tumours' with a mixed glial and neuronal phenotype, a benign course, temporal lobe localization, and seizure onset at a young age as a major clinical symptom did not meet current WHO criteria for integrated histological and genetic classification. Such "low-grade epilepsy-associated brain tumours (LEAT)" cover a large spectrum of neuropathological entities (Table 1), and represent the second largest group of patients submitted to epilepsy surgery[19]. The International League against Epilepsy (ILAE) has charged its Commission on Diagnostic Methods and Neuropathology Task Force to address this controversial topic and we will discuss herein both, conceptual benefits of an integrated phenotypic-genotypic approach and also how to bridge the current knowledge gap.

The 2016 WHO classification system: what's new?

The 2016 WHO classification includes a number of novel tumour entities, such as the diffuse midline glioma, H3K27M mutated (WHO IV°) with predominantly astrocytic differentiation, occurring mostly in children[20]. The presence of a K27M mutation in either histone *H3F3A* or *HIST1H3B/C* is mandatory for the diagnosis of this tumour. With respect to epilepsy-associated brain tumours, a gangliocytic tumour was recently reported in a total of 12 adults presenting with temporal lobe epilepsy[21, 22]. This lesion is now included in the WHO blue book under the name of "multinodular and vacuolating neuronal tumour of the cerebrum". There is no grade assigned yet and it is unclear if it is neoplastic or hamartomatous. It is beyond the scope of this review, however, to systematically list all newly introduced tumour entities. The most important innovation in the new WHO 2016 classification is the panel of molecular genetic tests added to the neuropathologist's armamentarium for studying and classifying brain tumours[2], and which has also helped to discover clinically meaningful new entities[17]. The integrated histopathology report should describe, therefore, not only the cellular (microscopic) composition of tumour tissue but also their molecular (genetic) signature[3]. As a prominent example, a genetically driven tumour classification applies for all diffuse astrocytic and oligodendroglial tumours. The histological discrimination between astrocytomas, oligodendrogliomas and mixed oligo-astrocytomas has provoked longstanding controversy in past decades in trying to decipher strict histological criteria in both low or high grade subtypes [23]. It has been long recognized that the so called clear cell ("oligodendroglial") component was associated with a better response to chemotherapy[6], yet, interobserver agreement during histological work-up of surgical brain tumour specimens remained poor[24]. To solve this ongoing controversy, the WHO now refers to IDH and 1p/19q as molecular-genetic markers to objectively differentiate the spectrum of histopathologically diverse gliomas into clinically meaningful subgroups. Clinical trials confirmed that *IDH1* mutated gliomas have a better prognosis than *IDH1* wildtype[25] and that 1p/19q co-deletions are strongly associated with favourable outcome in patients with anaplastic gliomas[23, 26].

The 2016 WHO classification system: what's old?

Low-grade epilepsy associated neuro-epithelial tumours remain an ever challenging issue for histopathological classification throughout all editions of the WHO blue book, with a remarkable history of newly introduced tumour entities (Table 2). These tumours consist mostly of neuronal and mixed neuronal-glial variants as well as supratentorial low-grade gliomas (see Table 1). As a group, early-onset drug-resistant epilepsy (mean of < 15 years) is often the patient's major or only neurological symptom[19, 27]. Another differentiating feature from diffusely infiltrating gliomas are the tumour's frequent localization in the temporal lobe. This group of tumours has been previously termed, and is nowadays recognized as ***long-term*** epilepsy-associated tumours (LEAT)[28], with gangliogliomas (GG) and dysembryoplastic neuroepithelial tumours (DNT) as prominent examples. The term LEAT was originally introduced by the Bonn Epilepsy Centre because of a long history of drug-resistant epilepsy (> 2 years) and the epilepsy surgery approach (i.e. intent to treat epilepsy) in all of their patients [28]. Meanwhile, the clinical definition for "epilepsy" and "drug resistant epilepsy" has changed [29, 30], as has the concept of epilepsy surgery as last treatment resort[31, 32]. We suggest, therefore, to change it's definition to "low-grade epilepsy-associated neuro-epithelial tumours (LEAT)". However, this term does not meet the WHO concept for a nosological tumour classification. The same applies for terms such as "Epilepsoma" or "Epileptoma"[19, 33]. Compared to malignant gliomas, meningiomas or brain metastases, LEAT are rare, representing approximately 2-5% of the entire brain tumour cohort[34]. However, they are the second most common lesion in patients submitted to epilepsy surgery. The very broad histological spectrum of these neoplasms is another intriguing observation. This relates to their variable composition of astroglia, oligodendroglia, other clear cells or neurones, inflammatory cellular infiltrates, calcification or protein aggregation, and also to their variable patterns of papillary, rosetted, or nodular growth (Figure 1). Diffuse infiltration with clusters remote from the tumour mass is another frequent observation[19, 27]. Associated focal cortical dysplasia has been reported in vastly different frequencies, thereby representing another complex issue in need of clarification [19, 27]. Published evidence remains heterogeneous and controversial[19, 27, 35, 36], and the WHO panel could not develop or propose an integrated genotype-phenotype classification and grading system at present. Nevertheless, with recent studies advocating adjuvant chemo-radiation therapy in addition to surgery in low-grade gliomas[16] it is becoming increasingly important that LEAT are clearly distinguishable from IDH1/2-wildtype low-grade gliomas to avoid any hazards of over-

treatment[37]. Making the distinction is a challenge for the neuropathologist[37]. Improved imaging, leading to earlier surgery before recognition as an "long-term epilepsy-associated" tumour rather than a tumour presenting with seizures, sometimes piece-meal removal and finally the large variety of histological appearances, may result in an incorrect diagnosis as a diffuse low-grade glioma.

The 2016 WHO classification system: what's blue?

As histopathological classification systems will be continuously updated and revised, the WHO's current roadmap towards better understanding of carcinogenesis and personalized treatment needs to be adopted to LEAT. It is now generally accepted and also recognized in the 2016 WHO classification, that LEATs lack IDH1 or IDH2 mutations as well as 1p/19q co-deletions[11, 36, 38, 39]. Published literature on prevalent molecular alterations in LEAT point to the RAS/RAF/MAPK pathway and the PI3K/AKT/mTOR pathway (Figure 2). BRAF V600E mutations were most consistently reported as genetic driver in gangliogliomas (18-56%). It's variable detection also in DNT (0-50%)[40-48] may reflect, however, the aforementioned difficulties in separating both tumour entities at the microscopic level (see also[40, 49]). As BRAF V600E mutations were observed also in pleomorphic xanthoastrocytomas [42] and pilocytic astrocytomas[42, 50], their presence cannot be regarded yet as specific for a given tumour entity. In DNT, tyrosine kinase activating *FGFR1* gene mutations prevail (58-82%)[40, 49], whereas *MYB/MYBL1* alterations were encountered in 87% of angiocentric gliomas[40], another LEAT tumour entity associated with drug-resistant epilepsy and early seizure onset[51]. A methylation-based classification from visually selected tumour regions of formalin fixed and paraffin embedded tissue represents a promisingnew option[40, 52-54], but has not been systematically applied and validated for the entire LEAT spectrum. Use of mutation-specific antibodies, i.e. directed against the V600E BRAF mutation [55], may help to further explore the extent of cellular and genetic mosaicism [46, 48, 56]. It needs to be shown, however, if such features also contribute to the epileptogenic phenotype. These studies need a careful design based on a validated histopathological classification scheme.

In order to further develop this field, the Task Force recommends a specific scenario and environment for randomized controlled trials (RCT; Figure 3), which are largely missing in the

field of LEAT. Given that the majority of these tumours are rare and grow slowly, RCTs will need to include multiple centres to recruit sufficient patient numbers within a reasonable time period. An additional goal should be to develop reference pathology centres and bio-repositories of surgical brain specimens and matched blood samples across all different continents to allow for a systematic molecular testing, keeping pace with new technologies or biomarkers as they become available. Reliable assessment of the biological behaviour of a given LEAT and risk for malignant progression (reported as low in general with documented cases of malignant progression[57]) needs clarification, as the current WHO edition did not specify atypical LEAT variants (WHO II°). The tumour's epileptogenic potential to irritate remote cortical areas or recruit remote cortical networks is another challenging issue in need of clarification. Seizure semiology is determined by a tumour's localization within the brain, i.e. in the mesial or lateral temporal lobe [37]. However, satellite tumour cell infiltrates remote from the mass lesion, as described in CD34 immunoreactive gangliogliomas [19, 35, 58], may compromise a successful postsurgical outcome. These questions should be addressed in an RCT setting with systematic genotype-phenotype analysis which also help to clarify the best time period for postsurgical drug withdrawal after complete tumour resection. Although such RCTs do not aim for new interventional treatment targets, they will help to provide evidence for a comprehensive and clinically meaningful clinico-pathological and genetic tumour classification scheme in the near future.

ILAE's Task Force for Neuropathology was charged to address this issue and to launch an interdisciplinary agreement study for tumour grading and classification. This Task Force has recently been successful in the implementation of international consensus classification systems for Focal Cortical Dysplasia[59] and Hippocampal Sclerosis[60] in patients with drug-resistant epilepsy, and also introducing a collaborative virtual microscopy platform[61]. In a first agreement study of 30 tumours randomly selected from a multicentre epilepsy surgery series (German Neuropathology Reference Centre for Epilepsy Surgery in Erlangen), agreement amongst 25 invited colleagues from 12 countries experienced in epilepsy surgery programmes reached only 40% (unpublished data, R. Coras personal communication). Use of immunohistochemical markers to more reliably differentiate histopathological epilepsy-associated neuroepithelial tumours features, i.e. CD34 and MAP2[19, 58, 62], achieved slightly better agreement in the same series of tumour specimens (unpublished data, R. Coras

personal communication). The current situation is reminiscent, therefore, of that in oligodendrogliomas (as discussed above), and we should follow WHO's molecular-genetic vision to achieve a comprehensive and robust classification of LEAT.

Conclusion

By establishing an integrated phenotype-genotype diagnosis, the new 2016 WHO classification system has introduced a substantial change in our current neuropathological work-up. It represents a paradigm shift, that microscopic inspection alone is not sufficiently reliable to predict the clinical course and treatment response in a given brain tumour. In young patients with epilepsy, a distinct subgroup of brain tumours can be encountered (herein termed 'low-grade epilepsy-associated neuro-epithelial tumours'). They present with a large and often mixed phenotypic spectrum and are difficult to classify by existing schemes. Also, published series have reported a variable representation of genetic alterations. Randomized clinical trials have not yet been performed to approve clinically meaningful tumour entities. Therefore, the revised WHO classification 2016 contains no histopathological or molecular-genetic advances in relation to this group of tumours. Adapting WHO's vision is mandatory to establish a comprehensive clinico-pathological and genetic classification system for epilepsy-associated neuro-epithelial tumours in the near future.

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References

1. Louis, D.N. et al. WHO Classification of Tumours of the Central Nervous System (Revised 4th edition) (eds. Louis, D.N., Ohgaki, H., Wiestler, O.D. & Cavenee, W.K.) (IARC, Lyon, 2016).
2. Louis, D.N. et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol* **131**, 803-20 (2016).
3. Louis, D.N. et al. International Society Of Neuropathology--Haarlem consensus guidelines for nervous system tumor classification and grading. *Brain Pathol* **24**, 429-35 (2014).
4. Reuss, D.E. et al. ATRX and IDH1-R132H immunohistochemistry with subsequent copy number analysis and IDH sequencing as a basis for an "integrated" diagnostic approach for adult astrocytoma, oligodendroglioma and glioblastoma. *Acta Neuropathol* **129**, 133-46 (2015).
5. Jenkins, R.B. et al. A t(1;19)(q10;p10) mediates the combined deletions of 1p and 19q and predicts a better prognosis of patients with oligodendroglioma. *Cancer Res* **66**, 9852-61 (2006).
6. Cairncross, J.G. et al. Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. *J Natl Cancer Inst* **90**, 1473-1479 (1998).
7. Reifenberger, G. et al. in WHO classification of Tumours of the Central Nervous System (revised 4th edition) (eds. Louis, D.N., Ohgaki, H., Wiestler, O.D. & Cavanee, W.K.) 60-69 (International Agency for Research on Cancer, Lyon, 2016).
8. Reifenberger, J. et al. Molecular-Genetic Analysis of Oligodendroglial Tumors Shows Preferential Allelic Deletions on 19q and 1p. *American Journal of Pathology* **145**, 1175-1190 (1994).
9. Stupp, R. et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* **352**, 987-96 (2005).
10. Hegi, M.E. et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* **352**, 997-1003 (2005).
11. Yan, H. et al. IDH1 and IDH2 mutations in gliomas. *N Engl J Med* **360**, 765-73 (2009).
12. Parsons, D.W. et al. An integrated genomic analysis of human glioblastoma multiforme. *Science* **321**, 1807-12 (2008).
13. Capper, D. et al. Application of mutant IDH1 antibody to differentiate diffuse glioma from nonneoplastic central nervous system lesions and therapy-induced changes. *Am J Surg Pathol* **34**, 1199-204 (2010).
14. Capper, D. et al. Characterization of R132H mutation-specific IDH1 antibody binding in brain tumors. *Brain Pathology* **20**, 245-54 (2010).
15. Wick, W. et al. NOA-04 randomized phase III trial of sequential radiochemotherapy of anaplastic glioma with procarbazine, lomustine, and vincristine or temozolomide. *J Clin Oncol* **27**, 5874-80 (2009).
16. Buckner, J.C. et al. Radiation plus Procarbazine, CCNU, and Vincristine in Low-Grade Glioma. *N Engl J Med* **374**, 1344-55 (2016).
17. Sturm, D. et al. New Brain Tumor Entities Emerge from Molecular Classification of CNS-PNETs. *Cell* **164**, 1060-72 (2016).
18. Weller, M. et al. MGMT Promoter Methylation Is a Strong Prognostic Biomarker for Benefit from Dose-Intensified Temozolomide Rechallenge in Progressive Glioblastoma: The DIRECTOR Trial. *Clin Cancer Res* **21**, 2057-64 (2015).
19. Blumcke, I., Aronica, E., Urbach, H., Alexopoulos, A. & Gonzalez-Martinez, J.A. A neuropathology-based approach to epilepsy surgery in brain tumors and

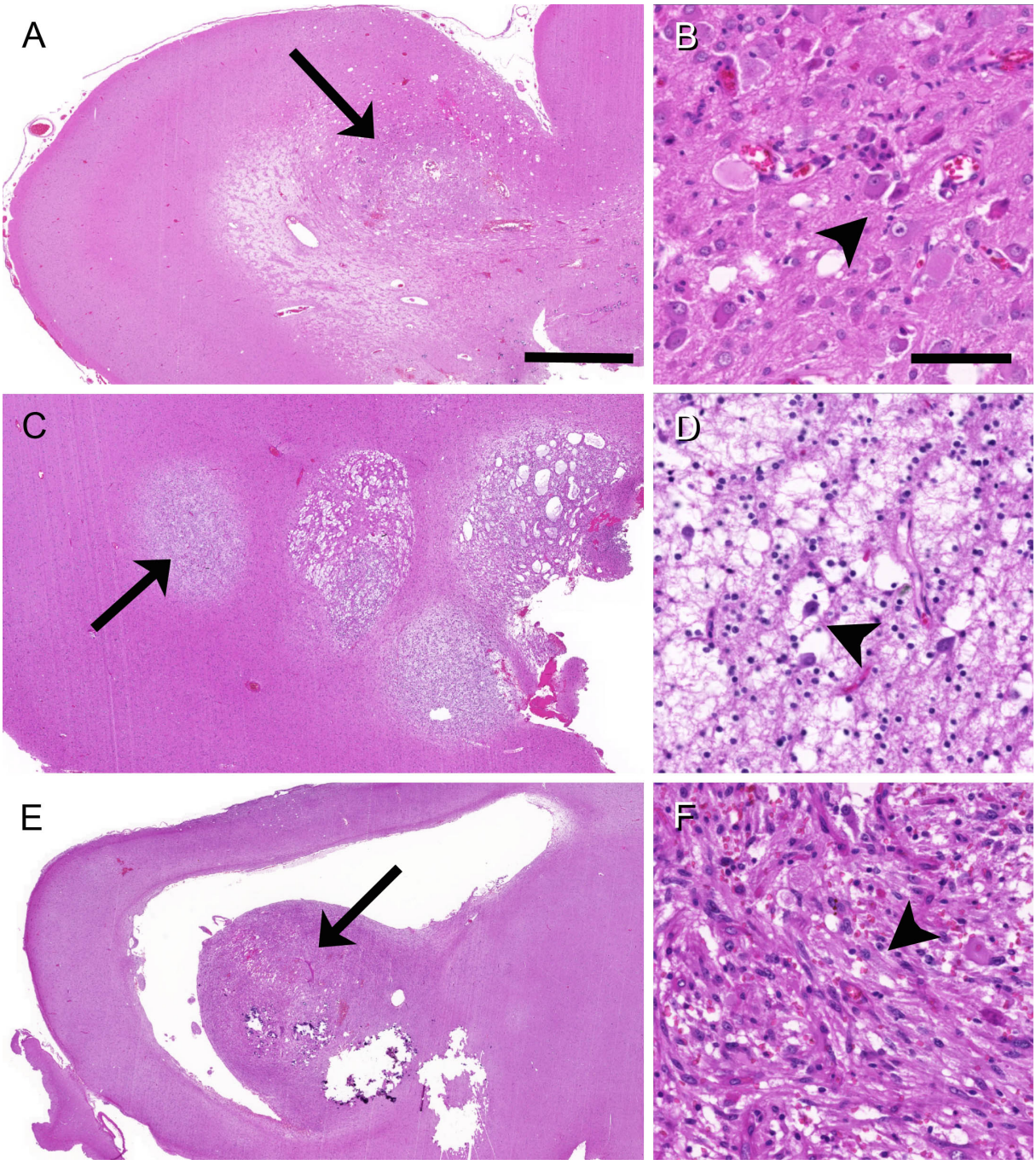
proposal for a new terminology use for long-term epilepsy-associated brain tumors. *Acta Neuropathol* **128**, 39-54 (2014).
20. Castel, D. et al. Histone H3F3A and HIST1H3B K27M mutations define two subgroups of diffuse intrinsic pontine gliomas with different prognosis and phenotypes. *Acta Neuropathol* **130**, 815-27 (2015).
21. Bodi, I. et al. Two cases of multinodular and vacuolating neuronal tumour. *Acta Neuropathol Commun* **2**, 7 (2014).
22. Huse, J.T. et al. Multinodular and vacuolating neuronal tumors of the cerebrum: 10 cases of a distinctive seizure-associated lesion. *Brain Pathol* **23**, 515-24 (2013).
23. Reifenberger, G. & Louis, D.N. Oligodendroglioma: toward molecular definitions in diagnostic neuro-oncology. *J Neuropathol Exp Neurol* **62**, 111-126 (2003).
24. Giannini, C. et al. Oligodendrogliomas: reproducibility and prognostic value of histologic diagnosis and grading. *J Neuropathol Exp Neurol* **60**, 248-62 (2001).
25. van den Bent, M.J. et al. IDH1 and IDH2 mutations are prognostic but not predictive for outcome in anaplastic oligodendroglial tumors: a report of the European Organization for Research and Treatment of Cancer Brain Tumor Group. *Clin Cancer Res* **16**, 1597-604 (2010).
26. Weller, M. et al. Combined 1p/19q loss in oligodendroglial tumors: predictive or prognostic biomarker? *Clin Cancer Res* **13**, 6933-7 (2007).
27. Thom, M., Blumcke, I. & Aronica, E. Long-term epilepsy-associated tumors. *Brain Pathol* **22**, 350-79 (2012).
28. Luyken, C. et al. The spectrum of long-term epilepsy-associated tumors: long-term seizure and tumor outcome and neurosurgical aspects. *Epilepsia* **44**, 822-30 (2003).
29. Fisher, R.S. et al. ILAE official report: a practical clinical definition of epilepsy. *Epilepsia* **55**, 475-82 (2014).
30. Kwan, P. et al. Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. *Epilepsia* **51**, 1069-77 (2010).
31. Blumcke, I. et al. International recommendation for a comprehensive neuropathologic workup of epilepsy surgery brain tissue: A consensus Task Force report from the ILAE Commission on Diagnostic Methods. *Epilepsia* **57**, 348-358 (2016).
32. Wiebe, S., Blume, W.T., Girvin, J.P. & Eliasziw, M. A randomized, controlled trial of surgery for temporal-lobe epilepsy. *N Engl J Med* **345**, 311-8 (2001).
33. Japp, A., Gielen, G.H. & Becker, A.J. Recent aspects of classification and epidemiology of epilepsy-associated tumors. *Epilepsia* **54 Suppl 9**, 5-11 (2013).
34. Jemal, A. et al. Cancer statistics, 2006. *CA Cancer J Clin* **56**, 106-30 (2006).
35. Blumcke, I. & Wiestler, O.D. Gangliogliomas: an intriguing tumor entity associated with focal epilepsies. *J Neuropathol Exp Neurol* **61**, 575-584 (2002).
36. Thom, M. et al. One hundred and one dysembryoplastic neuroepithelial tumors: an adult epilepsy series with immunohistochemical, molecular genetic, and clinical correlations and a review of the literature. *J Neuropathol Exp Neurol* **70**, 859-78 (2011).
37. Holthausen, H. & Blumcke, I. Epilepsy-associated tumours: what epileptologists should know about neuropathology, terminology, and classification systems. *Epileptic Disord* (2016).
38. Horbinski, C. et al. Isocitrate dehydrogenase 1 analysis differentiates gangliogliomas from infiltrative gliomas. *Brain Pathol* **21**, 564-74 (2011).

39. Prabowo, A.S. et al. Landscape of chromosomal copy number aberrations in gangliogliomas and dysembryoplastic neuroepithelial tumours. *Neuropathol Appl Neurobiol* (2015).
40. Qaddoumi, I. et al. Genetic alterations in uncommon low-grade neuroepithelial tumors: BRAF, FGFR1, and MYB mutations occur at high frequency and align with morphology. *Acta Neuropathol* (2016).
41. Dougherty, M.J. et al. Activating mutations in BRAF characterize a spectrum of pediatric low-grade gliomas. *Neuro Oncol* **12**, 621-30 (2010).
42. Schindler, G. et al. Analysis of BRAF V600E mutation in 1,320 nervous system tumors reveals high mutation frequencies in pleomorphic xanthoastrocytoma, ganglioglioma and extra-cerebellar pilocytic astrocytoma. *Acta Neuropathologica* **121**, 397-405 (2011).
43. Ramkissoon, L.A. et al. Genomic analysis of diffuse pediatric low-grade gliomas identifies recurrent oncogenic truncating rearrangements in the transcription factor MYBL1. *Proc Natl Acad Sci U S A* **110**, 8188-93 (2013).
44. Roth, J.J. et al. Diagnostic application of high resolution single nucleotide polymorphism array analysis for children with brain tumors. *Cancer Genet* **207**, 111-23 (2014).
45. Cruz, G.R. et al. Analysis of KIAA1549-BRAF fusion gene expression and IDH1/IDH2 mutations in low grade pediatric astrocytomas. *J Neurooncol* **117**, 235-42 (2014).
46. Prabowo, A.S. et al. BRAF V600E mutation is associated with mTOR signaling activation in glioneuronal tumors. *Brain Pathol* **24**, 52-66 (2014).
47. Zhang, J. et al. Whole-genome sequencing identifies genetic alterations in pediatric low-grade gliomas. *Nature genetics* **45**, 602-12 (2013).
48. Chappe, C. et al. Dysembryoplastic Neuroepithelial Tumors Share with Pleomorphic Xanthoastrocytomas and Gangliogliomas BRAF Mutation and Expression. *Brain Pathology* **23**, 574-83 (2013).
49. Rivera, B. et al. Germline and somatic FGFR1 abnormalities in dysembryoplastic neuroepithelial tumors. *Acta Neuropathol* (2016).
50. Korshunov, A. et al. Combined molecular analysis of BRAF and IDH1 distinguishes pilocytic astrocytoma from diffuse astrocytoma. *Acta Neuropathologica* **118**, 401-5 (2009).
51. Wang, M. et al. Monomorphous angiocentric glioma: a distinctive epileptogenic neoplasm with features of infiltrating astrocytoma and ependymoma. *J Neuropathol Exp Neurol* **64**, 875-81 (2005).
52. Holsken, A. et al. Adamantinomatous and papillary craniopharyngiomas are characterized by distinct epigenomic as well as mutational and transcriptomic profiles. *Acta Neuropathol Commun* **4**, 20 (2016).
53. Johann, P.D. et al. Atypical Teratoid/Rhabdoid Tumors Are Comprised of Three Epigenetic Subgroups with Distinct Enhancer Landscapes. *Cancer Cell* **29**, 379-93 (2016).
54. Pajtler, K.W. et al. Molecular Classification of Ependymal Tumors across All CNS Compartments, Histopathological Grades, and Age Groups. *Cancer Cell* **27**, 728-43 (2015).
55. Capper, D. et al. Assessment of BRAF V600E mutation status by immunohistochemistry with a mutation-specific monoclonal antibody. *Acta Neuropathol* **122**, 11-9 (2011).
56. Koelsche, C. et al. Mutant BRAF V600E protein in ganglioglioma is predominantly expressed by neuronal tumor cells. *Acta Neuropathol* **125**, 891-900 (2013).

57. Majores, M. et al. Tumor recurrence and malignant progression of gangliogliomas. *Cancer* **113**, 3355-63 (2008).
58. Blumcke, I. et al. The CD34 epitope is expressed in neoplastic and malformative lesions associated with chronic, focal epilepsies. *Acta Neuropathol* **97**, 481-490 (1999).
59. Blumcke, I. et al. The clinico-pathological spectrum of Focal Cortical Dysplasias: a consensus classification proposed by an ad hoc Task Force of the ILAE Diagnostic Methods Commission. *Epilepsia* **52**, 158-174 (2011).
60. Blumcke, I. et al. International consensus classification of hippocampal sclerosis in temporal lobe epilepsy: a Task Force report from the ILAE Commission on Diagnostic Methods. *Epilepsia* **54**, 1315-29 (2013).
61. Coras, R. et al. Good interobserver and intraobserver agreement in the evaluation of the new ILAE classification of focal cortical dysplasias. *Epilepsia* **53**, 1341-8 (2012).
62. Blumcke, I. et al. Distinct expression pattern of microtubule-associated protein-2 in human oligodendrogliomas and glial precursor cells. *J Neuropathol Exp Neurol* **60**, 984-993 (2001).
63. Schramm, J., Luyken, C., Urbach, H., Fimmers, R. & Blumcke, I. Evidence for a clinically distinct new subtype of grade II astrocytomas in patients with long-term epilepsy. *Neurosurgery* **55**, 340-7; discussion 347-8 (2004).
64. Blumcke, I., Luyken, C., Urbach, H., Schramm, J. & Wiestler, O.D. An isomorphic subtype of long-term epilepsy-associated astrocytomas associated with benign prognosis. *Acta Neuropathol* **107**, 381-388 (2004).
65. Zülch, K.J. Histological typing of tumours of the central nervous system (World Health Organization, Geneva, 1979).
66. Kleihues, P., Burger, P.C. & Scheithauer, B.W. (eds.) Histological typing of tumors of the central nervous system (Springer, Berlin, Heidelberg, New York, London, Paris, Tokyo, HongKong, Barcelona, Budapest, 1993).
67. Kleihues, P. & Cavenee, W.K. (eds.) Pathology and genetics of tumors of the nervous system (International Agency for Research on Cancer, Lyon, France, 2000).
68. Louis, D.N., Ohgaki, H., Wiestler, O.D. & Cavenee, W.K. (eds.) WHO classification of tumors of the central nervous system (International Agency for Research on Cancer, Lyon, France, 2007).
69. Agarwal, S. et al. Extraventricular neurocytomas: a morphological and histogenetic consideration. A study of six cases. *Pathology* **43**, 327-34 (2011).
70. Capper, D. et al. Mutation-specific IDH1 antibody differentiates oligodendrogliomas and oligoastrocytomas from other brain tumors with oligodendroglioma-like morphology. *Acta Neuropathol* **121**, 241-52 (2011).
71. Horstmann, S. et al. Genetic and expression profiles of cerebellar liponeurocytomas. *Brain Pathol* **14**, 281-9 (2004).
72. Bridge, J.A. et al. Identification of a novel, recurrent SLC44A1-PRKCA fusion in papillary glioneuronal tumor. *Brain Pathol* **23**, 121-8 (2013).
73. Solis, O.E. et al. Rosette-forming glioneuronal tumor: a pineal region case with IDH1 and IDH2 mutation analyses and literature review of 43 cases. *J Neurooncol* **102**, 477-84 (2011).
74. Gessi, M. et al. FGFR1 mutations in Rosette-forming glioneuronal tumors of the fourth ventricle. *J Neuropathol Exp Neurol* **73**, 580-4 (2014).
75. Gessi, M. et al. Absence of KIAA1549-BRAF fusion in rosette-forming glioneuronal tumors of the fourth ventricle (RGNT). *J Neurooncol* **110**, 21-5 (2012).

76. Rodriguez, F.J. et al. Disseminated oligodendroglial-like leptomeningeal tumor of childhood: a distinctive clinicopathologic entity. *Acta Neuropathol* **124**, 627-41 (2012).
77. Rodriguez, F.J. et al. High rate of concurrent BRAF-KIAA1549 gene fusion and 1p deletion in disseminated oligodendroglioma-like leptomeningeal neoplasms (DOLN). *Acta Neuropathol* **129**, 609-10 (2015).
78. Preuss, M. et al. Disseminated oligodendroglial-like leptomeningeal tumors: preliminary diagnostic and therapeutic results for a novel tumor entity [corrected]. *J Neurooncol* **124**, 65-74 (2015).

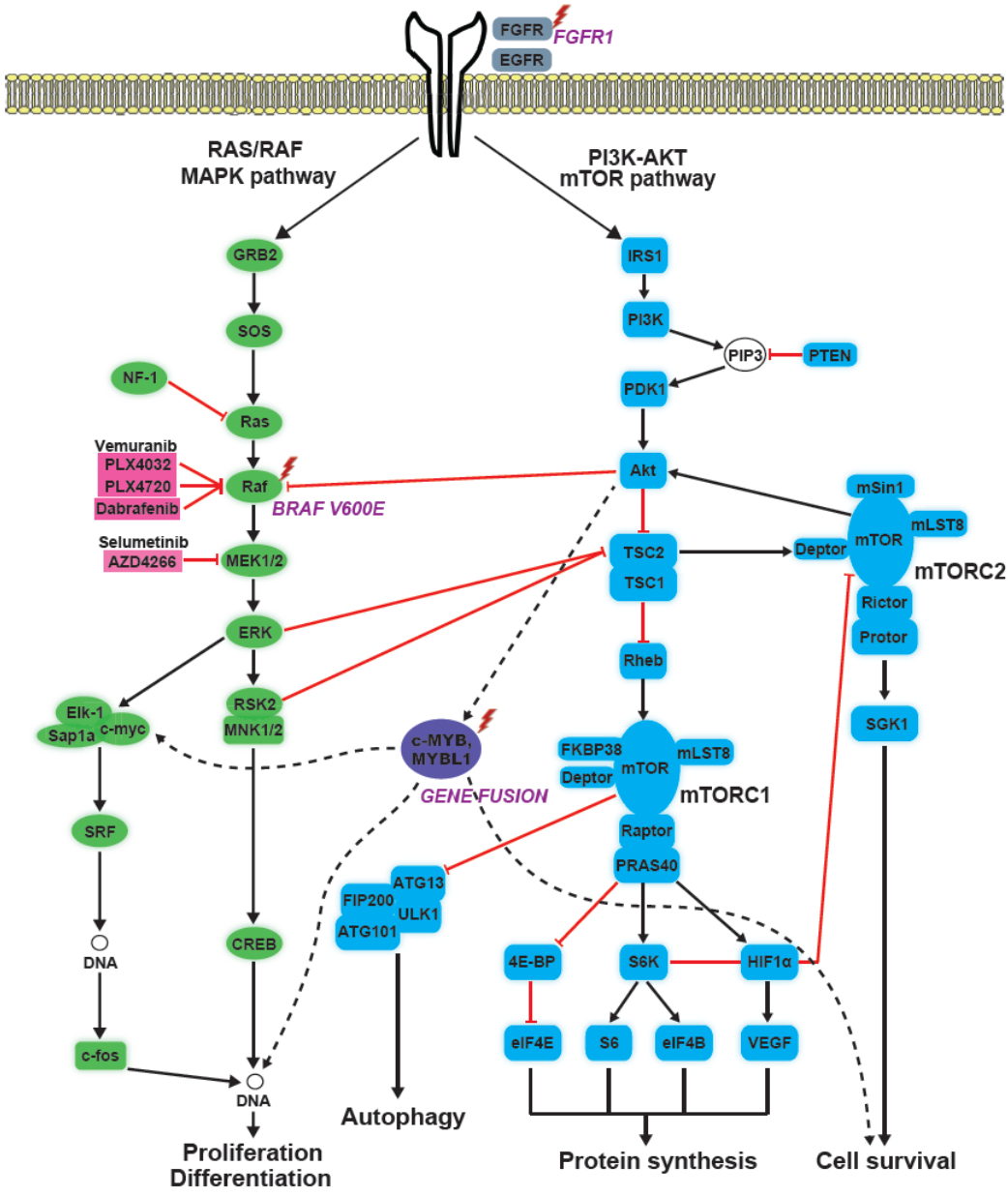
Figure 1: Is it all in one? The histopathological spectrum of nodular growth in low-grade epilepsy-associated neuro-epithelial tumours



Legend to Figure 1: Three examples of difficult-to-classify mixed neuronal-glial tumours in the temporal lobe, characterized by areas of nodular tumour growth (arrows in A, C, and E). All patients suffered from intractable epilepsy with seizure onset at young age. ***A-B:*** This nodule is composed of unequivocal clusters of ganglion cells (arrowhead in B), not otherwise explicable by anatomical location. ***C-D:*** Multinodular intracortical growth consisting of

oligodendroglia-like cells and floating neurones (so-called „specific glio-neuronal element“, arrowhead in D). ***E-F:*** Predominatly astroglial nodule with Rosenthal fibres (arrowhead in F) and protein droplets. All tumours showed other areas of diffuse cell infiltration, with or without aberrant CD34-immunoreactive cell clusters. Other tumours of this patient cohort presented with a different composition of same cytological features. It is an ever challenging question in disease classification whether to lump these variants together as one tumor entity, i.e. „epileptoma“ or specify n+1 new entities, i.e. ganglioglioma vs. DNT vs. composite glio-neuronal tumour ? Comprehensive molecular analysis (in a RCT setting, Figure 2) will help to solve this dilemma (see text for further discussion). Scale bar in A = 1mm (applies also to C, E), scale bar in B = 50µm (applies also to D, F).

Figure 2



Legend to Figure 2: Genetic alterations commonly found in LEAT connect 2 major pathways in cell metabolism, the RAS/RAF/MAPK pathway (green) and the PI3K/AKT/mTOR pathway (blue). Upstream in receptor signalling, mutations of FGFR1 have been described in DNT leading to activation of the RAS/RAF/MAPK pathway and the PI3K/AKT/mTOR pathway. BRAF is a component further downstream in the RAS/RAF/MAPK signalling cascade. Activation of this pathway by the V600E mutation leads to phosphrylation of MAPKs, which phosphorylate and regulate activities of partners such as transcriptions factors (CREB, c-fos) for cell proliferation and differentation. These functions made BRAF and MEK1/2 reasonable targets for therapeutic approaches (pink). On the other hand activation is tightly regulated

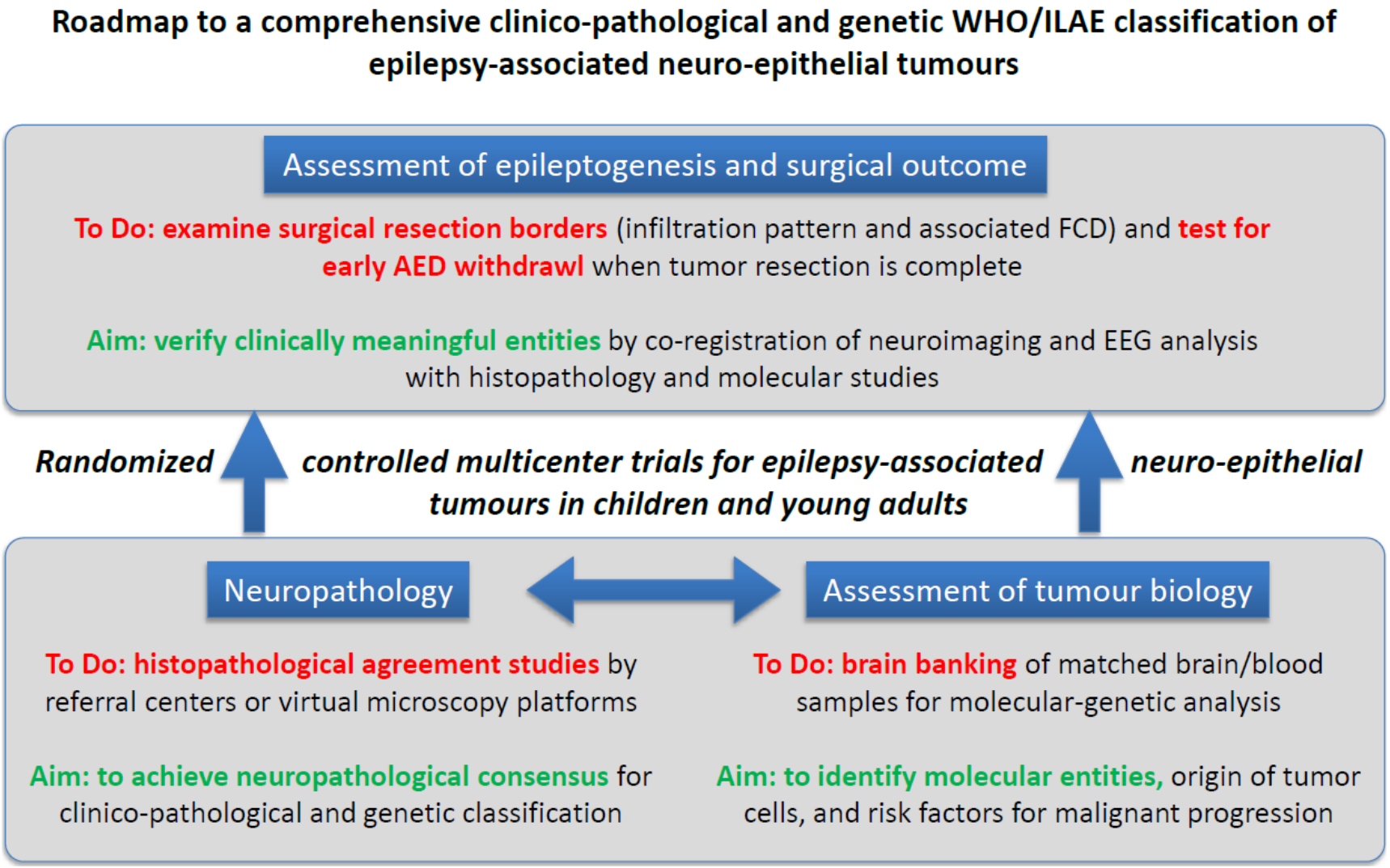
by substrates of the PI3K/AKT/mTOR signalling cascade. Especially, the inhibiting function of the 2 major complexes TSC and mTOR play a profound role in the regulation of autophagy, protein synthesis and cell survival. One of these transcription factors is c-myb/ mybl1 (purple) which is altered in angiocentric gliomas. All genetic alterations described are indicated with a lightening bolt.

Abbreviations:

- AKT (v-akt murine thymoma viral oncogene homologs 1, 2, 3) (alias: PKB): AKT1 (14q32); AKT2 (19q13); AKT3 (1q44)
- DEPTOR (8q24, DEP domain containing MTOR-interacting protein)
- FOS (v-fos FBJ murine osteosarcoma viral oncogene homolog) and FOS-like antigen): FOS (14q24); FOSB (19q13); FOSL1 (11q13); FOSL2 (2p23)
- GRB2 (17q25) (growth factor receptor-bound protein 2)
- INSR (19p13) (insulin receptor))
- MAPK (mitogen-activated protein kinase): MAPK1 (22q11) (alias: ERK2); MAPK3 (16p11) (alias: ERK1)
- MAP2K (mitogen-activated protein kinase kinase 1): MAP2K1 (15q22) (alias: MAPKK1, MEK1); MAP2K2 (19p13) (alias: MAPKK2, MEK2)
- MDM2 (12q15) (transformed mouse 3T3 cell double minute 2, p53 binding protein)
- MYC (8q24) (v-myc myelocytomatosis viral oncogene homolog (avian)
- MTOR (1p36) (mechanistic target of rapamycin)
- PI3K (phosphoinositide-3-kinase, catalytic, alpha, beta, delta, gamma polypeptide): PIK3CA (3q26); PIK3CB (3q22); PIK3CD (1p36); PIK3CG (7q22)
- PKD1 (14q11) (protein kinase D) (alias: PRKCM (mu)) (Wang et al., 2006)
- PTEN (10q23) (phosphatase and tensin homolog deleted on chromosome ten)
- RAF (v-raf murine sarcoma viral oncogene homolog): ARAF (Xp11); BRAF (7q34); RAF1 (3p25)
- RAS (RAS viral oncogene homolog): HRAS (11p15) (Harvey); KRAS (12p12) (Kirsten); NRAS (1p13) (neuroblastoma)
- RHEB (7q36) (ras homolog enriched in brain)
- SOS1 (2p21) (son of sevenless homolog 1)
- STAT (signal transducer and activator of transcription): STAT1 (2q32); STAT2 (12q13); STAT3 (17q21); STAT4 (2q32); STAT5a (17q11); STAT5b (17q11); STAT6 (12q13)

- TSC1 (9q34) (tuberous Sclerosis 1)
- TSC2 (16p13) (tuberous Sclerosis 2)

Figure 3


Roadmap to a comprehensive clinico-pathological and genetic WHO/ILAE classification of epilepsy-associated neuro-epithelial tumours
Assessment of epileptogenesis and surgical outcome
To Do: examine surgical resection borders (infiltration pattern and associated FCD) and test for early AED withdrawl when tumor resection is complete
Aim: verify clinically meaningful entities by co-registration of neuroimaging and EEG analysis with histopathology and molecular studies
Randomized controlled multicenter trials for epilepsy-associated neuro-epithelial tumours in children and young adults
Neuropathology
Assessment of tumour biology
To Do: histopathological agreement studies by referral centers or virtual microscopy platforms
To Do: brain banking of matched brain/blood samples for molecular-genetic analysis
Aim: to achieve neuropathological consensus for clinico-pathological and genetic classification
Aim: to identify molecular entities, origin of tumor cells, and risk factors for malignant progression

Legend to Figure 3: A useful and doable scenario for systematic investigations of meaningful clinico-pathological LEAT entities. We believe that the basis for a successfull WHO classification is a systematic genotype-phenotype assessment, validated by randomized and controlled clinical trials. These RCTs can be also used to clarify the benefit of early AED withdrawal after complete tumour resection, but do not primarily aim at novel interventional treatment options (as new drug targets are not validated yet in LEAT).

Table 1: The spectrum of epilepsy – associated neuro-epithelial tumours

Entity	Numbers (%)	Onset	Duration	Age OP
ANT I°	5 (0.4%)	**2.0**	13.0	19.7
GG I°	673 (48.7%)	**12.8**	12.7	24.9
GG II°/III°	77 (5.6%)	**14.2**	11.0	26.9
ISO I°	29 (2.1%)	**14.4**	17.7	27.9
DNT I°	256 (18.5%)	**14.7**	10.7	25.2
PA I°	81 (5.9%)	**14.8**	12.1	25.1
PXA II°	38 (2.7%)	*18.8*	12.2	29.3
OLIGO II°/III°	97 (7%)	*24.5*	12.5	38.6
ASTRO II°/III°	110 (8%)	*29.5*	6.7	36.2
Total	***1382***	***16.5***	***11.7***	***27.9***

Legend to Table 1: Summary of tumours collected in adults and children at the German Neuropathology Reference Center for Epilepsy Surgery in Erlangen. More than 80% of tumours present with seizure onset before age of 15 years (upper 6 rows), and localize in 77% to the temporal lobe[19], herein designated as low-grade epilepsy-associated neuro-epithelial tumours (LEAT). Early seizure onset and temporal localization separate LEAT from semi-benign and diffusely infiltrating gliomas also encountered in epilepsy surgery series (lower 3 rows). Age at epilepsy onset (mean in years); Epilepsy duration (mean in years); Age at operation (mean in years); ANT – angiocentric glioma WHO I°; GG – gangliogliomas WHO I° - III°; GG II° is not specified by the WHO classification, but used as clinical diagnosis ('analogue WHO II°') according to the proposal by Blumcke and Wiestler[35]; ISO - isomorphic astrocytoma variant (analogous to WHO I°[63, 64]); DNT – Dysembryoplastic neuroepithelial tumour WHO I°; PA – pilocytic astrocytoma WHO I°; PXA – pleomorphic xantoastrocytoma WHO II°; OLIGO – oligodendroglioma including mixed glioma WHO II° and III°; ASTRO – diffuse astrocytoma subtypes WHO II° and III°.

Table 2: Evolving WHO classification of neuronal and mixed neuronal-glial tumours over years 1979 – 2016					
	1979 *[65]	**1993**[66]**	**2000[67]**	**2007[68]**	**2016[1]**
Gangliocytoma	Composed predominantly of mature ganglion cells ***Grade I***	unchanged	Well differentiated, slowly growing neuroepithelial tumours composed of neoplastic, mature ganglion cells, either alone (GC ***Grade I***), or in combination with neoplastic glial cells (GG ***Grade I or II***). Tumours with anaplastic glial component "anaplastic GG" ***Grade III or IV***	Well differentiated, slowly growing neuroepithelial tumours composed of neoplastic, mature ganglion cells, either alone (GC Grade I), or in combination with neoplastic glial cells (GG ***Grade I***), observed in patients with long-term epilepsy. Anaplastic GG, ***Grade III*** Criteria for ***Grade II*** not established.	IDH1/2 wildtype[24, 26-28] BRAF V600E mutation in 18-56%[25, 29-36] ***Grade I*** Anaplastic GG, ***Grade III***
Ganglioglioma	Composed of mature ganglion cells and neoplastic glial cells ***Grades I or II***	unchanged			
Anaplastic (malignant) ganglioglioma	Gangliocytoma or ganglioglioma with anaplasia ***Grades III or IV***	Ganglioglioma with anaplasia in the glial component ***Grade III***			
Dysplastic gangliocytoma of cerebellum (Lhermitte-Duclos)	Described as a dysplastic variant of Gangliocytoma ***Grade I***	Described separately as a tumour-like lesion ***Grade I***	Discussed in association with Cowden Disease under Familial tumour syndromes Not clear if neoplastic or hamartomatous. If neoplastic, corresponds to ***Grade I***		PTEN (10q23) ***Grade I***
Paraganglioma		Identical to extra-adrenal paraganglioma orignating in filum terminale ***Grade I***	unchanged		

Desmoplastic infantile astrocytoma/ ganglioglioma		Mixed neuronal-glial neoplasm of infancy with desmoplasia Named as DIG ***Grade I***	Glial neoplasm of infancy with desmoplasia named as DIA Glial and neuronal neoplasm of infancy with desmoplasia named as DIG ***Grade I***		
Dysembryoplastic neuroepithelial tumour		Epilepsy-associated tumour with intracortical location, multinodular architecture and heterogeneous cellular composition ***Grade I***	Simple form with unique glioneuronal element; Complex form with glial nodules in combination with glioneuronal element Reference to non-specific histological forms (clinical features and imaging need to be considered) ***Grade I***	Simple form with unique glioneuronal element; Complex form with glial nodules in combination with glioneuronal element Non-specific histological form remains controversial ***Grade I***	IDH1/2 wildtype[24, 26-28] BRAF V600E mutation in (0-50%)[36, 39-48] FGFR1 mutation in 58-82%[40, 49] ***Grade I***
Central neurocytoma		Intraventricular tumour composed of uniform round cells with immunohistochemical (SYN) and ultrastructural features of neuronal differentiation ***Grade I***	Intraventricular tumour composed of uniform round cells with immunohistochemical (SYN) and ultrastructural features of neuronal differentiation Reference to extra-ventricular examples Upgraded: ***WHO II°***	Intraventricular tumour composed of uniform round cells with immunohistochemical (SYN) and ultrastructural features of neuronal differentiation ***Grade II***	
Extraventricular				Well-circumscribed	IDH1/2

neurocytoma				neoplasm composed of uniform round cells with neuronal differentiation, located in brain parenchyma ***Grade not assigned***	wildtype[69, 70] ***Grade II***
Cerebellar liponeurocytoma			Rare cerebellar neoplasm of adults with advanced neuronal/neurocytic and focal lipomatous differentiation, previously classified as lipomatous medulloblastoma ***Grades I or II***	Same definition as in previous edition , but assigned ***Grade II*** TP53 missense mutations in 20%[71]	
Papillary glioneuronal tumour				Tumour composed of GFAP-positive astrocytes lining hyalinised vascular pseudopapillae and synaptophysin-positive interpapillary collections of sheets of neurocytes, neurons and “ganglioid” cells SLC44A1-PRKCA fusion[72] ***Grade I***	
Rosette-forming glioneuronal tumour of the 4th ventricle				Neoplasms of fourth ventricular region composed of a) uniform neurocytes forming rosettes or perivascular pseudorosettes and b) component resembling pilocytic astrocytoma IDH1/IDH2 wildtype[73]	

				FGFR1 mutations[74] No KIAA1549-BRAF fusion or BRAF V600E mutations[75] ***Grade I***	
Multinodular and vacuolating neuronal tumour of the cerebrum					Accepted as a pattern of gangliocytoma, previously descibed in patients with epilepsy, predominately in temporal lobe[21, 22] ***Grade not assigned***
Diffuse leptomeningeal glioneuronal tumour					Predominant and widespread leptomeningeal growth, OLC and neuronal differentiation Deletion 1p in 59%[76, 77], IDH1/2 wildtype[76, 78] KIAA1549-BRAF fusion in 75%[77] ***Grade not assigned***

Legend to Table 2: BRAF = v-Raf murine sarcoma viral oncogene homolog B, DIA = Desmoplastic infantile astrocytoma, DIG = Desmoplastic infantile ganglioglioma, GC = Gangliocytoma, GG = ganglioglioma, GFAP = glial fibrillary acidic protein, IDH = isocitrate dehydrogenase, MAP2 = microtubule-associated protein 2, NFP = neurofilament protein, NOS = not otherwise specified, NSE = neuron-specific enolase, OLC = oligodendroglial-like cells, PTEN = phosphatase and tensin homolog, SYN = synaptophysin, TSC = tuberous sclerosis complex. * mentioned tumours were classified under “Neuronal Tumours” along with Ganglioneuroblastoma and Neuroblastoma in the WHO‘s 1979 classification. ** first appearence of the chapter entitled “Neuronal and mixed neuronal-glial tumours”, still including Olfactory Neuroblastoma and its variant Olfactory neuroepithelioma.