| **Core/**  **Non-core** | **Element name** | **Values** | **Commentary** | **Implementation notes** |
| --- | --- | --- | --- | --- |
| **HISTOLOGICAL ASSESSMENT REPORTING GUIDE** | | | | |
| Non-core | PRIOR THERAPY | Single selection value list:  • Not administered  • Prior treatment not known  • Administered, specify | Detail on prior treatment may not be available at the time of tumour diagnosis. Nonetheless, it can be helpful to know whether the patient has had specific therapies such as radiation therapy, chemotherapy, corticosteroid therapy, embolization, or radiosurgery. In particular, knowledge of such prior therapy may help to interpret changes such as necrosis, vasculature changes, cellular atypia and inflammatory cells. |  |
| Non-core | RELEVANT PATIENT/FAMILY HISTORY | Multi selection value list (select all that apply):  • Not provided  OR  • Previous history of cancer, specify  • History of neurological tumour syndrome, specify  • Relevant familial history, specify  • Other, specify | Several genetic conditions (such as neurofibromatosis 1 and 2, and Turcot/Lynch, tuberous sclerosis, von-Hippel-Lindau, Cowden, Li-Fraumeni and Gorlin syndromes) are known to predispose individuals to specific primary CNS tumours. Knowledge of this information may therefore help in differential diagnoses. In addition, the behaviour of tumours in such syndromes may differ from those of their sporadic counterparts, and thus knowledge of a genetic condition may inform prognostic estimation. |  |
| Non-core | OPERATIVE PROCEDURE | Single selection value list:  • Not provided  OR  • Biopsy, specify  • Resection, specify  • Other, specify | The physical size of tissue specimens submitted for pathological assessment varies greatly depending on the operative procedure. Specimens obtained by stereotactic or, less commonly, endoscopic biopsy are typically the smallest and may be crushed during handling. Those from open biopsy are more ample and typically less damaged than those of stereotactic biopsy. Resection specimens are largest, and require careful macroscopic inspection in order to sample properly. Importantly, the size of the submitted sample does not always reflect the procedure; use of ultrasonic surgical aspirators, for example, may decrease the size of the submitted material relative to the total amount of resected material. Because the reliability of pathological diagnosis depends heavily on the representative nature and adequacy of material assessed, it is important to pay attention to any discrepancy between submitted material and clinical information, including operative procedures and imaging findings. Doing so can help to minimise the influence of sampling and/or regional heterogeneity on the rendered diagnosis.  References  1 Fuller GN (2009). Intraoperative consultation and optimal processing In: Practical Surgical Neuropathology, Perry A and Brat DJ (eds), Churchill Livingstone, Philadelphia, 35-45. |  |
| Non-core | TUMOUR SITE(S) | Single selection value list  • No macroscopically visible tumour  • Indeterminate  OR  Multi selection value list (select all that apply):  • Skull, specify precise location, if known  • Dura, specify precise location, if known  • Leptomeninges, specify precise location, if known  • Brain  o Cerebral lobes, specify precise location, if known  o Deep grey matter, specify  o Ventricle, specify precise location, if known  • Pineal, specify  • Sellar/suprasellar/pituitary  • Brain stem, specify precise location, if known  • Cerebellum, specify site, if known  • Spine/vertebral column, specify precise location, if known  • Spinal cord, specify precise location, if known  • Spinal nerve root(s), specify precise location, if known  • Peripheral nerve, specify site, if known  • Other, specify | Imaging studies are crucial in guiding neurosurgical and radiotherapeutic management of brain tumours. 1 Knowledge of the specific anatomic area in which the tumour resides can aid in the differential diagnosis and may correlate with tumour type and outcome. If known, it should be recorded whether a tumour is intra-axial (cerebrum, deep white matter, cerebellum, brain stem, spinal cord), extra-axial (dural/leptomeningeal, cerebellopontine angle, intraventricular, intra- or extradurally in the spinal canal), or located in the skull, skull base, sellar/suprasellar region, pineal gland, spine, etc. When available, the pathologist should indicate the exact location (e.g. cerebral convexity/lobe, lateral versus third or fourth ventricle, etc.).  References  1 Vincentelli C, Hwang SN, Holder CA and Brat DJ (2012). The use of neuroimaging to guide the histologic diagnosis of central nervous system lesions. Adv Anat Pathol 19(2):97-107. |  |
| Non-core | TUMOUR LATERALITY | Single selection value list:  • Not specified  • Right  • Left  • Midline  • Bilateral  • Other, specify | Tumour laterality, as determined by imaging studies and as indicated by the surgeon, should be indicated as occurring on the right or left side of the CNS (e.g., right frontal lobe, left occipital convexity, right lateral ventricle, etc.). Midline tumours arising in the sellar, pineal, third or fourth ventricular, and spinal locations, among others, should be recorded as such. Occasionally, tumours may involve both sides of the brain and should be referred to as bilateral; a “butterfly” glioblastoma crossing the corpus callosum and involving both sides of the cerebrum is an example. |  |
| Non-core | TUMOUR FOCALITY | Single selection value list:  • Unifocal  • Indeterminate  • Multifocal, Specify number of lesions | While most CNS tumours are solitary (unifocal), multifocal examples exist, often representing malignant brain tumours (e.g., glioblastoma and CNS lymphoma). For tumours to be considered multifocal, they should be noncontiguous, as determined by neuroimaging studies—although it is recognised that histological autopsy studies of such radiologically multifocal tumours may reveal contiguity between lesions. Gliomatosis cerebri, previously recognised as a distinct diffuse glioma entity involving multiple cerebral lobes, is now recognised as a growth pattern in the 2016 WHO Classification of CNS Tumours. 1  References  1 Louis DN, Ohgaki H, Wiestler OD and Cavenee WK (eds) (2016). WHO Classification of Tumours of the Central Nervous System, Revised. Fourth Edition, IARC, Lyon. |  |
| Non-core | TUMOUR DIMENSIONS | Numeric:  • \_\_\_ mm x \_\_\_ mm x \_\_\_ mm | Radiologic tumour dimensions serve as approximate guidance as to whether tumours have been sampled adequately, particularly when dealing with small biopsies. Post-surgery, they also give information regarding how much of the tumour has been resected. For example, radiologicpathologic correlations can guard against making a diagnosis of low-grade glioma on a stereotactic biopsy sample obtained from the edge of a large, heterogeneously enhancing cerebral lesion. | Largest/dominant lesion |
| Non-core | RELATIONSHIP OF TUMOUR TO ADJACENT TISSUE | Single selection value list:  • Indeterminate  • Well demarcated  • Diffuse/infiltrative  • Mixed (both well-demarcated and diffuse in different areas)  **Peritumoral edema**  Single selection value list:  • Absent  • Present | The interface between tumour and adjacent brain as depicted by neuroimaging (MRI, CT) provides information on the growth pattern and on the dynamics of tumour growth. Hyperintensity on fluidattenuated inversion recovery (FLAIR) images may indicate an infiltrative tumour growth and reflect invasiveness of the tumour. This may also be reflected by diffuse or patchy contrast enhancement at the interface between tumour and normal brain (see below). Absence of peritumoural alterations on T2 and FLAIR sequences suggests a more benign nature of a lesion. The MRI patterns may also vary within one tumour with partly well-demarcated areas and partly infiltrative growth. Oedemais visualised as a hypointense signal alteration on T1-weighted sequences without contrast and, similar to infiltrative growth, as hyperintense signal on FLAIR sequences. Differentiation between infiltrative growth and oedema is often impossible, notably in gliomas. Slowly growing, more benign tumours induce relatively less oedema than fast growing, malignant tumours. Information provided by the surgeon on where the tissue specimens were collected relative to the MRI changes also aids the pathologist in interpreting the histological findings. |  |
| Non-core | CONTRAST ENHANCEMENT | Single selection value list:  • Information not available  •Non-enhancing  •Enhancing  o Diffuse/solid  o Patchy/heterogeneous  o Ring or rim | Contrast enhancement is commonly interpreted as reflecting blood-brain barrier disturbance. Extraaxial tumours growing outside the brain parenchyma (e.g., meningiomas) commonly take up contrast vividly. For intrinsic brain tumours such as gliomas, contrast enhancement is commonly interpreted as a sign of increasing malignancy, but this correlation is far from complete. For example, it is not uncommon for non-enhancing diffuse gliomas to be deemed anaplastic on histological examination. Moreover, pilocytic astrocytomas, gangliogliomas, and others are exceptions since they take up contrast, but are assigned to WHO grade I and carry a favourable prognosis. Ring enhancement is commonly associated with extensive central necrosis and reflects a high grade of histological malignancy, but is occasionally seen in benign tumours as well. Contrast enhancement is subject to pharmacological modification (e.g., by corticosteroids) or antiangiogenic agents, (e.g., bevacizumab). Thus, pharmacotherapy may be a challenge for MRI interpretation. Changes in contrast enhancement have traditionally played a central role in response assessment in neuro-oncology, (e.g., in the Macdonald criteria), but the additional consideration of T2 and FLAIR sequences has increasingly been implemented into response assessment in recent years. |  |
| Core | SPECIMEN SIZE | Numeric:  • \_\_\_ mm x \_\_\_ mm x \_\_\_ mm  • Cannot be assessed, specify | Intrinsic tumours grow diffusely within the brain and in many instances cannot be completely removed. Clinical factors (e.g., performance status) and tumour location often determine the extent of resection, ranging from a stereotactic biopsy to a resection of a lobe. Surgical technique may result in a discrepancy of the amount of tissue resected and received in the pathology department, in particular when a surgical ultrasonic aspirator is used and the collected tissue is partly discarded. It is important to record the volume of tissue arriving in the pathology department and thus the amount of tissue available for diagnosis (and where possible for frozen tissue banking for subsequent studies). If a tumour, for example a schwannoma or meningioma, arrives in one piece, it can be measured relatively accurately. Brain tumour surgery, however, often results in tissue fragments, making an accurate assessment difficult. Where possible, the size of large resection specimens should be recorded in three dimensions and piecemeal resections should be estimated by their aggregate size in three dimensions. Alternatively, an accurate and reproducible determination of the tissue volume may be achieved by weighing tissue fragments, compared to visual estimates in three dimensions, but this is not a common practice. | Record for each specimen submitted |
| Non-core | SPECIMEN DESCRIPTION | Text | The description of resection margins is generally not applicable for intra-axial CNS tumours as surgical technique results in fragmented specimens in most instances, except when complete resection of a lobe can be achieved. Therefore, staging and assessment of resection margins is generally not possible and thus not included in published protocols. Additionally, diffusely infiltrative tumours have often invaded well beyond designated surgical margins, even when tumour cells are not evident at that margin. Extra-axial tumours, such as meningiomas, schwannomas, and other well-demarcated tumours can often be resected and submitted intact. This allows a description of the lesion itself, and adherent structures, such as meninges, nerve roots, and CNS tissue. However, when arriving in fragmented state, the report may necessarily be limited to a description of individual components, and the degree of fragmentation. When applicable, description should also include the presence of other components, such as CNS tissue, dura mater, skin, bone, blood clot and extrinsic components such as haemostatic material, metal clips, synthetic bone, mesh, shunt ducts etc. Specimens may arrive fresh or in fixative. This should be indicated when describing the colour of the specimen as it changes with fixation. Specimens may also arrive in already processed forms, such as blocks or slides. In such situations, description should be given for blocks and slides, indicating the number of blocks and/or slides. Slides may be described in greater detail, e.g. total number of glass slides, comprising number of H&E and other slides (e.g., immunohistochemistry, smears, controls), as well as other materials (e.g., EM prints, neuroimaging files). |  |
| Non-core | ADEQUACY OF SPECIMEN FOR HISTOLOGICAL ASSESSMENT | Single selection value list:  • Specimen is adequate for analysis  • Specimen is adequate but limited by, specify  • Specimen is inadequate for analysis, specify (Multi-select -select all that apply)  o Crush  o Autolysis  o Cautery  o Necrosis  o Other, specify | The adequacy of a specimen for histological assessment can be affected by various intraoperative procedures, tissue fixation issues (duration in/volume of fixative), and technical processing issues in the histology laboratory. These include, but are not limited to, electrocautery/heat/laser treatment intraoperatively, distortion of tissue due to surgical instrumentation, delay in placing wet tissue into fixative by the surgeon/operating room technician, less than 10:1 fixative-to-tissue volume ratio, and excessive fracturing/knife chatter in tissue during cutting of the frozen tissue/paraffin block. Tiny size of a biopsy can lead to tissue exhaustion during processing. Highly necrotic, mucinous, fibrous, calcified, or ossified specimens may cause suboptimal processing/sectioning. Any of these conditions can obscure nuclear/nucleolar features, distort degree of cellularity, blur tumour margins, and or make mitotic activity impossible to assess. Prior freezing of the tissue for frozen section intraoperative diagnosis may negatively impact cytological assessment in the fixed, embedded tissues and immunohistochemistry for some antibodies. In each case, the pathologist should state which of these conditions make the tissue inadequate/suboptimal for histological assessment. |  |
| Non-core | ADEQUACY OF SPECIMEN FOR DIAGNOSTIC PURPOSES | Single selection value list:  • Specimen is adequate for diagnostic purposes  • Specimen is adequate but limited by, specify  • Specimen is inadequate for diagnostic purposes (e.g. not representative of likely clinic-radiological diagnosis), specify | Many intraparenchymal brain lesions are surgically assessed by either small open excisional biopsy or stereotactic biopsy. While navigational equipment is usually employed to optimise targeting, the known ability of brain tissue to swell during an operative procedure can cause shifting of brain tissue during the procedure, which can result in biopsies that are suboptimally centred on the area(s) of interest. Examples of suboptimally centred tissues include: biopsies from diffuse infiltrating gliomas taken from the edge, not centre, of the tumour; biopsies from infections in which the necrotic/purulent centre may be submitted by the surgeon for culture(s), leaving the pathologist with reactive, but not organism-containing, edges of the process. Occasionally, tissue lost to intraoperative suctioning or lesional tissues given in overly generous amounts to brain banks can render the tissue sent to the pathologist suboptimal for diagnosis. Any of these situations can leave the pathologist with tissue that can be misleading in terms of type of tumour, grade of tumour, or inability to detect organisms, if present. The diagnosis possible on the submitted tissues may be under-representative or misrepresentative of the lesion based on the neuroimaging studies. In a few instances, more sophisticated testing (e.g., molecular) may be required for full/correct diagnosis, but the small tissue size, tissue processing issues, or suboptimal targeting of biopsy materials may make this testing impossible. The pathologist should specify any, and all, limitations of the tissue in achieving optimal diagnosis. |  |
| Non-core | HISTOLOGICAL APPEARANCE | Text:  Describe the appearance from the WHO 2016 entities and variants based on histological appearance only  Single selection value list:  • Other, specify  • Cannot be determined | In nearly all pathology reports of CNS neoplasms, the diagnosis should ideally include one of the >150 entities and variants listed in the 2016 CNS WHO1,2 (see Table 1 below) and when additionally possible, the histological appearance should further be combined with signature molecular alterations to establish a more specific “integrated diagnosis” (e.g., diffuse astrocytoma, IDHmutant; see section on Integrated Diagnosis). When using such an approach, histological impressions such as “oligoastrocytoma” and “anaplastic oligoastrocytoma” will virtually always be altered to either astrocytoma or oligodendroglioma categories based on specific molecular patterns identified. Similar modifications also apply to the RELA-fusion positive supratentorial ependymomas, diffuse midline gliomas, the solitary fibrous tumours/haemangiopericytomas, and the overarching group of embryonal neoplasms, such as medulloblastoma variants, atypical teratoid/rhabdoid tumour, and embryonal tumour with multilayered rosettes, each of which require additional molecular (or surrogate immunohistochemical biomarker) testing before a definitive diagnosis can be made. However, in the majority of entities still lacking disease-defining molecular signatures, the final diagnosis will be based on classical histopathology alone. In either approach (histological or integrated), obtaining as precise a final diagnosis as possible is critically important, as this forms the basis for all subsequent patient management decisions, accruing patients to the appropriate clinical trials, epidemiologically assessing disease trends over time, and establishing valid research conclusions. 3-6 As such, the strict application of WHO 2016 diagnostic guidelines is required to enhance both accuracy and interobserver reproducibility across the globe and it is noteworthy that for many entities, criteria have changed dramatically from the earlier 2007 WHO classification. In the remaining cases that do not neatly conform to a well-recognised entity or variant (see last category listed as “Other (specify)”), a descriptive diagnosis should be rendered instead, providing as much information as possible including relevant molecular information (e.g., small round cell sarcoma of indeterminate type; low-grade neuroepithelial tumour with oligodendroglial-like histological features suggestive of dysembryoplastic neuroepithelial tumour or paediatric oligodendroglioma; high-grade glioneuronal neoplasm; poorly differentiated malignancy; etc.). Such cases can be considered Not Elsewhere Classified (NEC).7 It should be noted that in some cases the results are not clear cut and the addition of a secondary diagnosis may be of benefit to record in the report. This element should be considered CORE if it constitutes the final diagnosis.  Table 1 Histologically Defined Diagnostic Category (based on histological appearance only, i.e., not full 2016 CNS WHO diagnoses)  Diffuse glioma  Diffuse astrocytoma  Gemistocytic astrocytoma  Anaplastic astrocytoma  Glioblastoma  Giant cell glioblastoma  Gliosarcoma  Epithelioid glioblastoma  Oligodendroglioma  Anaplastic oligodendroglioma  Oligoastrocytoma  Anaplastic oligoastrocytoma  Pilocytic astrocytoma  Pilomyxoid astrocytoma  Subependymal giant cell astrocytoma  Pleomorphic xanthoastrocytoma  Anaplastic pleomorphic xanthoastrocytoma  Chordoid glioma of third ventricle  Angiocentric glioma  Astroblastoma  Subependymoma  Myxopapillary ependymoma  Ependymoma  Papillary ependymoma  Clear cell ependymoma  Tanycytic ependymoma  Anaplastic ependymoma  Choroid plexus papilloma  Atypical choroid plexus papilloma  Choroid plexus carcinoma  Dysembryoplastic neuroepithelial tumour  Gangliocytoma  Ganglioglioma  Anaplastic ganglioglioma  Dysplastic gangliocytoma of cerebellum (Lhermitte-Duclos disease)  Desmoplastic infantile astrocytoma or ganglioglioma (DIA or DIG)  Papillary glioneuronal tumour  Rosette-forming glioneuronal tumour  Diffuse leptomeningeal glioneuronal tumour  Central neurocytoma  Extraventricular neurocytoma  Cerebellar liponeurocytoma  Paraganglioma  Pineocytoma  Pineal parenchymal tumour of intermediate differentiation  Pineoblastoma  Papillary tumour of the pineal region  CNS Embryonal tumour  CNS Embryonal tumour with rhabdoid features  Medulloblastoma  Medulloblastoma, classic  Medulloblastoma, desmoplastic/nodular  Medulloblastoma with extensive nodularity  Medulloblastoma, large cell/anaplastic  Embryonal tumour with multilayered rosettes  Medulloepithelioma  CNS Neuroblastoma  CNS Ganglioneuroblastoma  Schwannoma  Cellular schwannoma  Plexiform schwannoma  Melanotic schwannoma  Neurofibroma  Plexiform neurofibroma  Perineurioma  Hybrid nerve sheath tumour  Malignant peripheral nerve sheath tumour (MPNST)  Epithelioid MPNST  Melanotic MPNST  MPNST with mesenchymal differentiation  MPNST with glandular differentiation  MPNST with perineurial differentiation  Meningioma  Meningothelial meningioma  Fibrous meningioma  Transitional meningioma  Psammomatous meningioma  Angiomatous meningioma  Microcystic meningioma  Secretory meningioma  Lymphoplasmacyte-rich meningioma  Metaplastic meningioma  Chordoid meningioma  Clear cell meningioma  Atypical meningioma  Papillary meningioma  Rhabdoid meningioma  Anaplastic (malignant) meningioma  Solitary fibrous tumour/haemangiopericytoma  Haemangioblastoma  Haemangioma  Epithelioid hemangioendothelioma  Angiosarcoma  Kaposi sarcoma  Ewing sarcoma-peripheral primitive neuroectodermal tumour  Lipoma  Angiolipoma  Liposarcoma  Desmoid-type fibromatosis  Myofibroblastoma  Inflammatory myofibroblastic tumour  Benign fibrous histiocytoma  Fibrosarcoma  Undifferentiated pleomorphic sarcoma (UPS)/malignant fibrous histiocytoma (MFH)  Leiomyoma  Leiomyosarcoma  Rhabdomyoma  Rhabdomyosarcoma  Chondroma  Chondrosarcoma  Osteoma  Osteochondroma  Osteosarcoma  Diffuse melanocytosis  Meningeal melanocytoma  Melanoma  Meningeal melanomatosis  Diffuse large B cell lymphoma (DLBCL) of the CNS  Immunodeficiency-associated lymphoproliferative disorders of the CNS  Low grade B cell lymphomas of the CNS  T-cell and NK/T-cell lymphomas of the CNS  Anaplastic large cell lymphoma  Lymphomatoid granulomatosis  Intravascular large B-cell lymphoma  MALT lymphoma of the dura  Langerhans cell histiocytosis  Erdheim-Chester disease  Rosai-Dorfman disease  Juvenile xanthogranuloma  Histiocytic sarcoma  Germinoma  Embryonal carcinoma  Yolk sac tumour  Choriocarcinoma  Teratoma  Mature teratoma  Immature teratoma  Teratoma with malignant transformation  Mixed germ cell tumour  Craniopharyngioma  Adamantinomatous craniopharyngioma  Papillary craniopharyngioma  Granular cell tumour  Pituicytoma  Spindle cell oncocytoma  Pituitary adenoma  Somatotroph adenoma  Lactotroph adenoma  Thyrotroph adenoma  Corticotroph adenoma  Gonadotroph adenoma  Null cell adenoma  Plurihormonal and double adenomas  Pituitary carcinoma  Pituitary blastoma  Gangliocytoma and mixed gangliocytoma-adenoma  Granular cell tumour  Pituicytoma  Spindle cell oncocytoma  Metastatic carcinoma  Metastatic melanoma  Metastatic sarcoma  Metastatic lymphoma/leukemia  References  1 Louis DN, Ohgaki H, Wiestler OD and Cavenee WK (eds) (2016). WHO Classification of Tumours of the Central Nervous System, Revised. Fourth Edition, IARC, Lyon.  2 Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, Ohgaki H, Wiestler OD, Kleihues P and Ellison DW (2016). The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathologica 131(6):803-820.  3 Louis DN, Perry A, Burger P, Ellison DW, Reifenberger G, von Deimling A, Aldape K, Brat D, Collins VP, Eberhart C, Figarella-Branger D, Fuller GN, Giangaspero F, Giannini C, Hawkins C, Kleihues P, Korshunov A, Kros JM, Beatriz Lopes M, Ng HK, Ohgaki H, Paulus W, Pietsch T, Rosenblum M, Rushing E, Soylemezoglu F, Wiestler O and Wesseling P (2014). International Society Of Neuropathology--Haarlem consensus guidelines for nervous system tumor classification and grading. Brain Pathol 24(5):429-435.  4 van den Bent MJ, Weller M, Wen PY, Kros JM, Aldape K and Chang S (2017). A clinical perspective on the 2016 WHO brain tumor classification and routine molecular diagnostics. Neuro Oncol 19(5):614-624.  5 Fuller CE, Jones DTW and Kieran MW (2017). New Classification for Central Nervous System Tumors: Implications for Diagnosis and Therapy. Am Soc Clin Oncol Educ Book 37:753-763.  6 Perry A and Wesseling P (2016). Histologic classification of gliomas. Handb Clin Neurol 134:71- 95.  7 Louis DN, Wesseling P, Paulus W, Giannini C, Batchelor TT, Cairncross JG, Capper D, FigarellaBranger D, Lopes MB, Wick W and van den Bent M (2018). cIMPACT-NOW update 1: Not Otherwise Specified (NOS) and Not Elsewhere Classified (NEC). Acta Neuropathol. 135(3):481- 484. | Value list from the WHO Classification of Tumours of the Central Nervous System, (2016).  Note that permission to publish the WHO classification of tumours may be needed in your implementation. It is advisable to check with the International Agency for Research on Cancer (IARC). |
| Core | HISTOLOGICAL GRADE | Single selection value list:  • Not applicable  • Cannot be determined, specify  • WHO grade I  • WHO grade II  • WHO grade III  • WHO grade IV | In as many pathology reports of CNS neoplasms as possible, the diagnosis should include a grade based on the WHO 2016 classification (see Table 2 below). 1,2 This scheme differs from the approaches in many other organ systems in that in most circumstances, the diagnosis dictates a given WHO grade rather than a range of grades within a diagnostic category. The scale of WHO grades from I to IV reflects the natural histories of various tumour types, rather than their shifting prognoses with changes in therapeutic practice over time. 3 Roughly speaking, a WHO grade I tumour is considered benign and potentially curable by surgery, although in unfavourable locations, such tumours may still create significant morbidity. WHO grade II tumours typically are slowly growing malignancies that often recur and are associated with significant mortality, albeit with survival times of many years in most cases. WHO grade III tumours are rapidly growing malignancies with typical survivals of only a few years if treated with surgery alone. Nearly all such tumours are designated as “anaplastic”. WHO grade IV neoplasms are highly aggressive malignancies with rapid mortality (typically in less than 2 years after diagnosis) in the absence of adjuvant therapies (e.g., glioblastomas and embryonal neoplasms). Progression from lower-grade malignancy to higher-grade forms occurs in some CNS neoplasms, most commonly the diffuse gliomas (Table 3) and to a lesser extent in the meningiomas (Table 4). There are exceptions to the automatic assignment of a single WHO grade based on diagnosis, mostly in entities for which definite parameters for histological grading have not been established yet. Other bone and soft tissue neoplasms occurring within the neural axis are classified and graded using the same criteria as in other parts of the body. Lastly, it should be noted that in some cases, assigning a WHO grade is not possible or could cause more confusion than clarification for clinical colleagues (e.g., when the exact tumour subtype remains unclear). In such cases, it is preferable to omit the WHO grade from the final diagnosis.  Table 2 WHO Grades Based on Histologically Defined Diagnostic Category (based on histological appearance only, i.e., not full 2016 CNS WHO diagnoses)#   |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | | **Tumour Group** | **Tumour Type** | **Grade**  **I** | **Grade**  **II** | **Grade**  **III** | **Grade**  **IV** | | Astrocytic tumours | Diffuse astrocytoma |  | **X** |  |  | |  | Anaplastic astrocytoma |  |  | **X** |  | |  | Glioblastoma (and variants) |  |  |  | **X** | |  | Pilocytic astrocytoma | **X** |  |  |  | |  | Pilomyxoid astrocytoma (grade not assigned) |  |  |  |  | |  | Subependymal giant cell astrocytoma | **X** |  |  |  | |  | Pleomorphic xanthoastrocytoma |  | **X** |  |  | |  | Anaplastic pleomorphic xanthoastrocytoma |  |  | **X** |  | | Oligodendrogliomas | Oligodendroglioma |  | **X** |  |  | | **Tumour Group** | **Tumour Type** | **Grade**  **I** | **Grade**  **II** | **Grade**  **III** | **Grade**  **IV** | | Oligoastrocytomas | Oligoastrocytoma |  | **X** |  |  | |  | Anaplastic oligoastrocytoma |  |  | **X** |  | | Ependymal tumours | Ependymoma |  | **X** |  |  | |  | Anaplastic ependymoma |  |  | **X** |  | |  | Subependymoma | **X** |  |  |  | |  | Myxopapillary ependymoma | **X** |  |  |  | | Choroid plexus tumours | Choroid plexus papilloma | **X** |  |  |  | | Atypical choroid plexus papilloma |  | **X** |  |  | | Choroid plexus carcinoma |  |  | **X** |  | | Other neuroepithelial tumours | Chordoid glioma of the third ventricle |  | **X** |  |  | | Angiocentric glioma | **X** |  |  |  | | Neuronal-glial tumours | Gangliocytoma | **X** |  |  |  | |  | Desmoplastic infantile ganglioglioma/ astrocytoma (DIG/DIA) | **X** |  |  |  | |  | Dysembryoplastic neuroepithelial tumour | **X** |  |  |  | |  | Ganglioglioma | **X** |  |  |  | |  | Anaplastic ganglioglioma |  |  | **X** |  | |  | Central neurocytoma |  | **X** |  |  | |  | Extraventricular neurocytoma |  | **X** |  |  | |  | Cerebellar liponeurocytoma |  | **X** |  |  | |  | Papillary glioneuronal tumour | **X** |  |  |  | |  | Rosette-forming glioneuronal tumour of the fourth ventricle | **X** |  |  |  | |  | Paraganglioma of the spinal cord | **X** |  |  |  | | Pineal parenchymal tumours | Pineocytoma | **X** |  |  |  | |  | Pineal parenchymal tumour of intermediate differentiation |  | **X** | **X** |  | | **Tumour Group** | **Tumour Type** | **Grade**  **I** | **Grade**  **II** | **Grade**  **III** | **Grade**  **IV** | |  | Pineoblastoma |  |  |  | **X** | |  | Papillary tumour of the pineal region |  | **X** | **X** |  | | Embryonal tumours | Medulloblastoma |  |  |  | **X** | |  | CNS embryonal tumour, NOS |  |  |  | **X** | |  | Medulloepithelioma |  |  |  | **X** | |  | CNS Neuroblastoma |  |  |  | **X** | |  | CNS Ganglioneuroblastoma |  |  |  | **X** | |  | Ependymoblastoma |  |  |  | **X** | |  | Atypical teratoid/rhabdoid tumour |  |  |  | **X** | | Cranial and peripheral nerve tumours | Schwannoma (and variants) | **X** |  |  |  | |  | Neurofibroma (and variants) | **X** |  |  |  | |  | Perineurioma | **X** |  |  |  | |  | Malignant peripheral nerve sheath tumours (MPNST) |  | **X** | **X** | **X** | | Meningeal tumours | Meningioma (and variants) | **X** |  |  |  | |  | Atypical meningioma |  | **X** |  |  | |  | Clear cell meningioma |  | **X** |  |  | |  | Chordoid meningioma |  | **X** |  |  | |  | Anaplastic meningioma |  |  | **X** |  | |  | Papillary meningioma |  |  | **X** |  | |  | Rhabdoid meningioma |  |  | **X** |  | | Mesenchymal tumours[4](#_ENREF_4),[5](#_ENREF_5) | (Named as soft tissue counterpart) | **X** | **X** | **X** | **X** | |  | Solitary fibrous tumour / Haemangiopericytoma | **X** | **X** | **X** |  | | Tumours of uncertain histogenesis | Haemangioblastoma | **X** |  |  |  |   Tumour histology and grade are strong predictors of clinical behaviour for different CNS tumours, including diffusely infiltrating astrocytomas and meningiomas. Tables 3 and 4 list the grading criteria for these common CNS tumour types.  Table 3 WHO Grading System for Diffuse, Infiltrating Astrocytomas#   |  |  |  | | --- | --- | --- | | WHO Grade | **WHO Designation** | **Histologic Criteria** | | II | Diffuse astrocytoma | Nuclear atypia | | III | Anaplastic astrocytoma | Nuclear atypia and mitotic figures | | IV | Glioblastoma | Nuclear atypia, mitotic figures, and microvascular proliferation and/or necrosis |   Table 4 WHO Grading of Meningiomas#   |  |  | | --- | --- | | **WHO grade I** | **Benign meningioma** (and variants)  None of the criteria below for WHO grades II or III | | **WHO grade II** | **Atypical meningioma**  Mitotic figures ≥ 4/10 high-power fields (HPF)  ***or***  At least 3 of 5 parameters:  Sheeting architecture (loss of whorling and/or fascicles)  Small cell formation  Macronucleoli  Hypercellularity  Spontaneous necrosis  ***or***  Brain invasion  ***or***  **Clear cell meningioma**  ***or***  **Chordoid meningioma** | | **WHO grade III** | **Anaplastic (malignant) meningioma**  Mitotic figures ≥ 20/10 HPF  ***or***  Frank anaplasia (sarcoma, carcinoma, or melanoma-like histology)  ***or***  **Papillary meningioma**  ***or***  **Rhabdoid meningioma** |   #Modified from the original versions in Brat DJ, Parisi JE, DeMasters BK et al. Protocol for the Examination of Specimens From Patients with Tumors of the Central Nervous System.  2014.  Available at [www.cap.org/cancerprotocols](http://www.cap.org/cancerprotocols).  References  1 Louis DN, Ohgaki H, Wiestler OD and Cavenee WK (eds) (2016). WHO Classification of Tumours of the Central Nervous System, Revised. Fourth Edition, IARC, Lyon.  2 Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, Ohgaki H, Wiestler OD, Kleihues P and Ellison DW (2016). The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathologica 131(6):803-820.  3 Louis DN, Perry A, Burger P, Ellison DW, Reifenberger G, von Deimling A, Aldape K, Brat D, Collins VP, Eberhart C, Figarella-Branger D, Fuller GN, Giangaspero F, Giannini C, Hawkins C, Kleihues P, Korshunov A, Kros JM, Beatriz Lopes M, Ng HK, Ohgaki H, Paulus W, Pietsch T, Rosenblum M, Rushing E, Soylemezoglu F, Wiestler O and Wesseling P (2014). International Society Of Neuropathology--Haarlem consensus guidelines for nervous system tumor classification and grading. Brain Pathol 24(5):429-435.  4 Perry A and Brat DJ (2010). Practical Surgical Pathology: A Diagnostic Approach. Elsevier, Philadelphia.  5 McLendon RE, Rosenblum MK and Bigner DD (eds) (2006). Russell and Rubinstein's Pathology of Tumors of the Nervous System. 7th ed, Hodder Arnold, New York. |  |
| Non-core | INVASION | Single selection value list:  • Not identified (i.e. tumour is well-demarcated from surrounding brain or other tissues)  • Cannot be assessed (e.g. only tumour is present), specify  •Present, specify type | Most neuroepithelial tumours, particularly infiltrating gliomas, demonstrate diffuse infiltration of tumour cells beyond grossly discernable margins. Isolated tumour cells are often present in grossly normal-appearing parenchyma surrounding the lesions. Involvement of leptomeninges and VirchowRobin spaces are also common in gliomas, even in benign examples such as pilocytic astrocytoma and ganglioglioma. These “invasions” provide no prognostic significance beyond the given biological malignancy of each tumour. Furthermore, direct invasion into adjacent structures, such as dura and skull, is quite exceptional in gliomas. Assessment of such features therefore, has not been included as an element for the dataset for intra-axial CNS tumours. On the other hand, invasion of adjacent structures may be relevant in some non-neuroepithelial tumours, meningioma in particular, and can be assessed if the interface between the tumour and the adjacent tissue is appropriately submitted for assessment. Brain invasion is a criterion for atypical meningioma in the 2016 CNS WHO Classification1 and is characterised by irregular, tonguelike protrusions of tumour tissue into underlying parenchyma without an intervening layer of leptomeninges; however, extension along Virchow-Robin spaces does not constitute brain invasion. Bone involvement has been associated with increased recurrence rates in the setting of atypical meningioma. 2  References  1 Perry A (2016). Meningiomas. In: WHO Classification of Tumours of the Central Nervous System, Revised. Fourth Edition, Louis DN, Ohgaki H, Wiestler OD and Cavenee WK (eds), ARC, Lyon, 232-245.  2 Gabeau-Lacet D, Aghi M, Betensky RA, Barker FG, Loeffler JS and Louis DN (2009). Bone involvement predicts poor outcome in atypical meningioma. J Neurosurg 111(3):464-471. |  |
| Non-core | HISTOLOGICAL EVIDENCE OF PRIOR THERAPY | • No evidence of prior therapy  • Positive response, specify type of response (Multi-select - select all that apply)  o Vascular changes  o Reactive glial changes  o Inflammatory changes  o Radiation type necrosis  o Granulation and/or scar tissue  o Ischemic type of necrosis  o Foreign material (e.g. embolisation/procoagulant material)  o Other, specify | Prior therapy, including prior surgery, intravascular embolization, chemotherapy and radiotherapy— may significantly alter the histological appearance of tissues and result in difficulties in tumour typing and grading. Information on prior therapy (see Prior Therapy) is, however, not always available to the pathologist and the absence of histological evidence does not necessarily imply absence of prior therapy. Therapy-associated histological findings are often non-specific, except for iatrogenically introduced foreign materials such as embolic agents, and are not always adequately distinguished form tumourassociated findings. In this regard, WHO grades may not be readily assigned to the specimens after adjuvant therapies. Histological changes of radiation damage are particularly common in specimens from recurrent diffuse gliomas. These include large foci of coagulative necrosis with hypocellular edges and microcalcifications; hyalinised or necrotic vessels with enlarged, atypical endothelial cells; and pale, rarefied parenchyma with fibrin deposits. The presence of such changes is highly suggestive of prior radiation therapy, even if a clear clinical history of prior radiation has not been provided.  References  1 Perry A (2009). Therapy-associated neuropathology. In: Practical Surgical Neuropathology, Perry A and Brat DJ (eds), Churchill Livingstone, Philadelphia, 35-45. |  |
| **MOLECULAR INFORMATION REPORTING GUIDE** | | | | |
|  | OVERVIEW OF SELECTED MOLECULAR MARKERS |  | **Refer to bottom of document.** |  |
| Non-core | ADEQUACY OF SPECIMEN FOR MOLECULAR ASSESSMENT | Single selection value list:  • Specimen is adequate for analysis • Specimen is inadequate for analysis, *give reason*, (Multi-select - select all that apply)  o Crush  o Autolysis  o Cautery  o Necrosis  o Decalcification  o Tumour cell quantity  o Fixation issues, *specify*  o Other, *specify* | The 2016 CNS WHO uses histology and molecular parameters to define many tumour entities.1 Procuring viable and adequate tumour tissue allows appropriate histological and molecular assessment. However, the requirements for an adequate specimen for molecular assessment are not always the same as those for histological assessment. For example, ischemic times are critical for the quality of nucleic acid in general; the sooner samples can be frozen or fixed, the better. If immediate freezing or immediate appropriate fixation is not possible, placement in refrigerator may reduce the degradation of nucleic acid.2 Crush or freezing artefacts may affect adequacy for immunohistochemical or FISH testing, but do not often affect adequacy for molecular assays. Samples embedded in OCT compound for cryostat sectioning can be a good source, and an advantage of using such samples is that one can evaluate tumour cell quantity as well as quality by checking histological sections of each sample. Formalin-fixed, paraffin-embedded (FFPE) tissue samples also often provide a valuable source of information for molecular assessment.3 FFPE samples, however, can sometimes be more difficult for molecular biology assays because of the fixation issues (such as overfixation and decalcification) that often cause nucleic acid degradation, resulting in fragmented DNA and RNA transcripts. Nonetheless, many laboratories have optimised molecular assays for FFPE tissue, given its commonplace nature. Histological examination of tissue specimens used for nucleic acid extraction and subsequent molecular testing is mandatory to assure that vital tumour tissue with sufficient neoplastic cell content is being analysed. In certain cases, microdissection of cellular tumour areas may be required to ensure sensitivity of molecular analysis.  References  1 Louis DN, Ohgaki H, Wiestler OD and Cavenee WK (eds) (2016). *WHO Classification of Tumours of the Central Nervous System, Revised. Fourth Edition*, IARC, Lyon.  2 Chung JY, Braunschweig T, Williams R, Guerrero N, Hoffmann KM, Kwon M, Song YK, Libutti SK and Hewitt SM (2008). Factors in tissue handling and processing that impact RNA obtained from formalin-fixed, paraffin-embedded tissue. *J Histochem Cytochem* 56(11):1033-1042.  3 Walter RF, Mairinger FD, Wohlschlaeger J, Worm K, Ting S, Vollbrecht C, Schmid KW and Hager T (2013). FFPE tissue as a feasible source for gene expression analysis--a comparison of three reference genes and one tumor marker. *Pathol Res Pract* 209(12):784-789. |  |
| Non-core | *ATRX* MUTATION | Single selection value list:  • Cannot be determined  • Negative  • Positive  TESTING METHOD (Multi-select - select all that apply)  • Sanger sequencing  • Next-generation sequencing  • PCR-based method  • Other, *specify*  ***ATRX* expression (immunohistochemistry)**  Single selection value list:  • Cannot be determined  • Intact nuclear expression  • Loss of nuclear expression | In the setting of a diffuse glioma with an IDH mutation, the diagnosis of an IDH-mutant astrocytoma (including diffuse astrocytoma, anaplastic astrocytoma, and glioblastoma) is supported by the presence of a *TP53* mutation or alteration (mutation or deletion) of the α-thalassemia/mental retardation syndrome X-linked gene (*ATRX*; chromosome Xq21.1).1-3 Evaluation for these two markers is also commonly used to rule out the possibility of an oligodendroglioma.  Among IDH-mutant tumours, inactivating mutations of *ATRX* appear restricted to those carrying *TP53* mutations and this combination is almost mutually exclusive with codeletion of 1p/19q.2,4-6 Nearly all diffuse gliomas with IDH and *ATRX* mutations are associated with the alternative lengthening of telomeres (ALT) phenotype. Less commonly, *ATRX* mutations co-occur with H3.3 mutations in paediatric high-grade gliomas, most often in those with G34R/V-mutations.7  Documentation of *ATRX* loss/mutations can be achieved in a number of ways, with a practical and cost-effective manner being immunohistochemistry. The loss of nuclear ATRX immunostaining in neoplastic cells, with its maintained expression in non-neoplastic cells, such as endothelial cells or normal glia, is strongly associated with *ATRX* deletion or mutation and can be reliably used as a surrogate of genetic alteration.6,8,9 Mosaic staining patterns have also been reported, but these are not always associated with ATRX mutation.10 In combination with immunohistochemistry for IDH1 R132H and p53, ATRX immunohistochemistry provides definitive results in the majority of cases, with the added benefit of preserving cytoarchitecture for microscopic examination.1,8  References  1 Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, Kos I, Batinic-Haberle I, Jones S, Riggins GJ, Friedman H, Friedman A, Reardon D, Herndon J, Kinzler KW, Velculescu VE, Vogelstein B and Bigner DD (2009). IDH1 and IDH2 mutations in gliomas. *N Engl J Med* 360(8):765-773.  2 Brat DJ, Verhaak RG, Aldape KD, Yung WK, Salama SR, Cooper LA, Rheinbay E, Miller CR, Vitucci M, Morozova O, Robertson AG, Noushmehr H, Laird PW, Cherniack AD, Akbani R, Huse JT, Ciriello G, Poisson LM, Barnholtz-Sloan JS, Berger MS, Brennan C, Colen RR, Colman H, Flanders AE, Giannini C, Grifford M, Iavarone A, Jain R, Joseph I, Kim J, Kasaian K, Mikkelsen T, Murray BA, O'Neill BP, Pachter L, Parsons DW, Sougnez C, Sulman EP, Vandenberg SR, Van Meir EG, von Deimling A, Zhang H, Crain D, Lau K, Mallery D, Morris S, Paulauskis J, Penny R, Shelton T, Sherman M, Yena P, Black A, Bowen J, Dicostanzo K, Gastier-Foster J, Leraas KM, Lichtenberg TM, Pierson CR, Ramirez NC, Taylor C, Weaver S, Wise L, Zmuda E, Davidsen T, Demchok JA, Eley G, Ferguson ML, Hutter CM, Mills Shaw KR, Ozenberger BA, Sheth M, Sofia HJ, Tarnuzzer R, Wang Z, Yang L, Zenklusen JC, Ayala B, Baboud J, Chudamani S, Jensen MA, Liu J, Pihl T, Raman R, Wan Y, Wu Y, Ally A, Auman JT, Balasundaram M, Balu S, Baylin SB, Beroukhim R, Bootwalla MS, Bowlby R, Bristow CA, Brooks D, Butterfield Y, Carlsen R, Carter S, Chin L, Chu A, Chuah E, Cibulskis K, Clarke A, Coetzee SG, Dhalla N, Fennell T, Fisher S, Gabriel S, Getz G, Gibbs R, Guin R, Hadjipanayis A, Hayes DN, Hinoue T, Hoadley K, Holt RA, Hoyle AP, Jefferys SR, Jones S, Jones CD, Kucherlapati R, Lai PH, Lander E, Lee S, Lichtenstein L, Ma Y, Maglinte DT, Mahadeshwar HS, Marra MA, Mayo M, Meng S, Meyerson ML, Mieczkowski PA, Moore RA, Mose LE, Mungall AJ, Pantazi A, Parfenov M, Park PJ, Parker JS, Perou CM, Protopopov A, Ren X, Roach J, Sabedot TS, Schein J, Schumacher SE, Seidman JG, Seth S, Shen H, Simons JV, Sipahimalani P, Soloway MG, Song X, Sun H, Tabak B, Tam A, Tan D, Tang J,  Thiessen N, Triche T, Jr., Van Den Berg DJ, Veluvolu U, Waring S, Weisenberger DJ, Wilkerson MD, Wong T, Wu J, Xi L, Xu AW, Yang L, Zack TI, Zhang J, Aksoy BA, Arachchi H, Benz C, Bernard B, Carlin D, Cho J, DiCara D, Frazer S, Fuller GN, Gao J, Gehlenborg N, Haussler D, Heiman DI, Iype L, Jacobsen A, Ju Z, Katzman S, Kim H, Knijnenburg T, Kreisberg RB, Lawrence MS, Lee W, Leinonen K, Lin P, Ling S, Liu W, Liu Y, Liu Y, Lu Y, Mills G, Ng S, Noble MS, Paull E, Rao A, Reynolds S, Saksena G, Sanborn Z, Sander C, Schultz N, Senbabaoglu Y, Shen R, Shmulevich I, Sinha R, Stuart J, Sumer SO, Sun Y, Tasman N, Taylor BS, Voet D, Weinhold N, Weinstein JN, Yang D, Yoshihara K, Zheng S, Zhang W, Zou L, Abel T, Sadeghi S, Cohen ML, Eschbacher J, Hattab EM, Raghunathan A, Schniederjan MJ, Aziz D, Barnett G, Barrett W, Bigner DD, Boice L, Brewer C, Calatozzolo C, Campos B, Carlotti CG, Jr., Chan TA, Cuppini L, Curley E, Cuzzubbo S, Devine K, DiMeco F, Duell R, Elder JB, Fehrenbach A, Finocchiaro G, Friedman W, Fulop J, Gardner J, Hermes B, Herold-Mende C, Jungk C, Kendler A, Lehman NL, Lipp E, Liu O, Mandt R, McGraw M, McLendon R, McPherson C, Neder L, Nguyen P, Noss A, Nunziata R, Ostrom QT, Palmer C, Perin A, Pollo B, Potapov A, Potapova O, Rathmell WK, Rotin D, Scarpace L, Schilero C, Senecal K, Shimmel K, Shurkhay V, Sifri S, Singh R, Sloan AE, Smolenski K, Staugaitis SM, Steele R, Thorne L, Tirapelli DP, Unterberg A, Vallurupalli M, Wang Y, Warnick R, Williams F, Wolinsky Y, Bell S, Rosenberg M, Stewart C, Huang F, Grimsby JL, Radenbaugh AJ and Zhang J (2015). Comprehensive, Integrative Genomic Analysis of Diffuse Lower-Grade Gliomas. *N Engl J Med* 372(26):2481-2498.  3 Ceccarelli M, Barthel FP, Malta TM, Sabedot TS, Salama SR, Murray BA, Morozova O, Newton Y, Radenbaugh A, Pagnotta SM, Anjum S, Wang J, Manyam G, Zoppoli P, Ling S, Rao AA, Grifford M, Cherniack AD, Zhang H, Poisson L, Carlotti CG, Jr., Tirapelli DP, Rao A, Mikkelsen T, Lau CC, Yung WK, Rabadan R, Huse J, Brat DJ, Lehman NL, Barnholtz-Sloan JS, Zheng S, Hess K, Rao G, Meyerson M, Beroukhim R, Cooper L, Akbani R, Wrensch M, Haussler D, Aldape KD, Laird PW, Gutmann DH, Noushmehr H, Iavarone A and Verhaak RG (2016). Molecular Profiling Reveals Biologically Discrete Subsets and Pathways of Progression in Diffuse Glioma. *Cell* 164(3):550-563.  4 Liu XY, Gerges N, Korshunov A, Sabha N, Khuong-Quang DA, Fontebasso AM, Fleming A, Hadjadj D, Schwartzentruber J, Majewski J, Dong Z, Siegel P, Albrecht S, Croul S, Jones DT, Kool M, Tonjes M, Reifenberger G, Faury D, Zadeh G, Pfister S and Jabado N (2012). Frequent ATRX mutations and loss of expression in adult diffuse astrocytic tumors carrying IDH1/IDH2 and TP53 mutations. *Acta Neuropathol* 124(5):615-625.  5 Kannan K, Inagaki A, Silber J, Gorovets D, Zhang J, Kastenhuber ER, Heguy A, Petrini JH, Chan TA and Huse JT (2012). Whole-exome sequencing identifies ATRX mutation as a key molecular determinant in lower-grade glioma. *Oncotarget* 3(10):1194-1203.  6 Reuss DE, Sahm F, Schrimpf D, Wiestler B, Capper D, Koelsche C, Schweizer L, Korshunov A, Jones DT, Hovestadt V, Mittelbronn M, Schittenhelm J, Herold-Mende C, Unterberg A, Platten M, Weller M, Wick W, Pfister SM and von Deimling A (2015). 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(1):133-146.  7 Khuong-Quang DA, Buczkowicz P, Rakopoulos P, Liu XY, Fontebasso AM, Bouffet E, Bartels U, Albrecht S, Schwartzentruber J, Letourneau L, Bourgey M, Bourque G, Montpetit A, Bourret G, Lepage P, Fleming A, Lichter P, Kool M, von Deimling A, Sturm D, Korshunov A, Faury D, Jones DT, Majewski J, Pfister SM, Jabado N and Hawkins C (2012). K27M mutation in histone H3.3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas. *Acta Neuropathol* 124(3):439-447.  8 Wiestler B, Capper D, Holland-Letz T, Korshunov A, von Deimling A, Pfister SM, Platten M, Weller M and Wick W (2013). ATRX loss refines the classification of anaplastic gliomas and identifies a subgroup of IDH mutant astrocytic tumors with better prognosis. *Acta Neuropathol* 126(3):443-451.  9 Nguyen DN, Heaphy CM, de Wilde RF, Orr BA, Odia Y, Eberhart CG, Meeker AK and Rodriguez FJ (2013). Molecular and morphologic correlates of the alternative lengthening of telomeres phenotype in high-grade astrocytomas. *Brain Pathol* 23(3):237-243.  10 Purkait S, Miller CA, Kumar A, Sharma V, Pathak P, Jha P, Sharma MC, Suri V, Suri A, Sharma BS, Fulton RS, Kale SS, Dahiya S and Sarkar C (2017). ATRX in Diffuse Gliomas With its Mosaic/Heterogeneous Expression in a Subset. *Brain Pathol* 27(2):138-145. |  |
| Non-core | *BRAF* ALTERATIONS | ***BRAF* mutation**  Single selection value list:  • Cannot be determined  • Absent  • BRAF V600E (c.1799T>A) mutation present  • Other BRAF mutation present, *specify*  MUTATIONS ASSESSED  (Multi-select - select all that apply)  • V600E  • Any mutation in exon 15  • Other, specify  TESTING METHOD  (Multi-select - select all that apply)  • Sanger sequencing  • Next-generation sequencing  • PCR-based method  • Other, *specify*  **BRAF V600E expression (immunohistochemistry)**  Single selection value list:  • Cannot be determined  • Negative  • Positive  ***BRAF* rearrangement/duplication**  Single selection value list:  • Cannot be determined  • Negative  • Positive, *specify*  MUTATIONS ASSESSED  (Multi-select - select all that apply)  • 7q34 tandem duplication  • KIAA-BRAF fusion  • BRAF-RAF1 fusion  • Other, *specify*  TESTING METHOD  (Multi-select - select all that apply)  • In situ hybridization (FISH)  • RT-PCR  • Array-based method  • RNA-sequencing  • Other, *specify* | ***BRAF* Mutation**  The *BRAF* V600E mutation in exon 15, which is the most common *BRAF* alteration, affects a large variety of CNS tumours. It has been reported in 96% of papillary craniopharyngiomas1, 65-75% of pleomorphic xanthoastrocytomas (PXA) with and without anaplasia2, 25-60% of gangliogliomas, 20–25% of dysembryoplastic neuroepithelial tumours (DNET), and 7% of pilocytic astrocytomas (PA), especially those in supratentorial locations.1,2,3,4 *BRAF* mutation has been also detected in about one-half of epithelioid glioblastomas and, in up to 25% of diffuse astrocytic gliomas in children and young adults.5 The detection of a *BRAF* mutation has diagnostic implications in specific tumours such as PXA, ganglioglioma, DNT, or epithelioid glioblastoma. Moreover, the detection of the mutation can help to distinguish a ganglioglioma from the cortical infiltration of a diffuse glioma. Besides its diagnostic value, *BRAF* mutation has therapeutic implications as targeted therapies against mutated BRAF V600 protein have been recently developed, including in settings such as *BRAF*-mutant craniopharyngioma.6 In paediatric low-grade gliomas, *BRAF* V600E mutation has been linked to poor response to conventional cytotoxic therapy and poor prognosis.7 In routine settings, BRAF V600E can be identified by IHC (see below) or by molecular approaches such as Sanger sequencing, high-resolution melting analysis, pyrosequencing, allele-specific quantitative polymerase chain reaction (ASQ-PCR), and next-generation sequencing (NGS).8 Although Sanger sequencing is a well-established tool to detect *BRAF* V600E and other rarer *BRAF* mutations, it has a detection threshold of 20% (of mutated alleles). This high threshold reduces the relevance of this technique in samples that contain a minority of mutated cells. Molecular methods with much lower thresholds, such as ASQ-PCR, digital PCR, or NGS, are more sensitive although precise cut-offs for mutant allele frequency have not been defined.  **BRAF V600E Expression (Immunohistochemistry)**9  Immunohistochemistry is a commonly used method to detect the BRAF V600E protein in FFPE tissue in CNS tumours.10,11 Two monoclonal antibodies (clone VE1 and clone V600E) against BRAF V600E are commercially available. Clone VE1 is the most widely used and is sensitive and specific.12 The concordance between immunohistochemistry and detection of *BRAF* V600E mutation by molecular genetic techniques demonstrates variability between studies in different types of neoplasms, but the overall concordance is strong.12 Immunohistochemistry plays a key role when FFPE material available is not sufficient for molecular genetic analysis and when low tumour cell content may lead to false-negative results. The presence of nonspecific staining is a potential pitfall, which could lead to false-positive results, and light staining can lead to false-negative interpretations.  ***BRAF* Rearrangement/Duplication**  Circumscribed duplication of the *BRAF* locus is a common copy number variation that occurs in PAs of the cerebellum, hypothalamus, or optic chiasm, but may occur in PAs from other sites as well. Chromosome 7q34 gain has been characterised as a *BRAF* duplication with a tandem insertion in the *KIAA1549* gene.13 Fusion genes containing *BRAF* variants activate the MAPK signalling pathway, which appears to be the key signalling pathway in the development of PA. The major alterations leading to constitutive activation of MAPK in PAs are gene fusions and point mutations involving *BRAF*. Fusions between *KIAA1549* and *BRAF* are the most frequent genetic change in PAs (>70 %) and occur in almost all anatomical locations, although most frequently in the cerebellum and less frequently at other sites. The most common fusion is between *KIAA1549*-exon 16 and exon 9 of *BRAF*, followed by 15-9, and 16-11. Much rarer fusions involving *BRAF* or *RAF1* have also been found. Identification of the *KIAA1549*-*BRAF* fusions has been used as a diagnostic marker for PAs. It has been observed in pilomyxoid astrocytoma, ganglioglioma and in the recently described diffuse leptomeningeal glioneuronal tumour (DLGNT).14 15 *KIAA1549*-*BRAF* fusions, while all coding for a fusion protein that includes the activating *BRAF* kinase domain, can be derived from at least nine different fusion site combinations. This makes reverse transcriptase polymerase chain reaction (RT-PCR) a difficult method to identify or exclude all variants of the fusion gene. Fluorescence in situ hybridisation (FISH) analysis, which demonstrates the tandem duplication at 7q34, is an indirect way to indicate the presence of a *KIAA1549*-*BRAF* fusion. However, *BRAF* copy number gains due to trisomy 7 or whole 7q gains are common in diffusely infiltrating astrocytomas including glioblastomas, and should not be mistaken as circumscribed *BRAF* duplication or *BRAF* fusion. A method that may identify all types of *BRAF* and *RAF1* fusion variants in a single experiment is RNA sequencing by NGS.  References  1 Brastianos PK, Taylor-Weiner A, Manley PE, Jones RT, Dias-Santagata D, Thorner AR, Lawrence MS, Rodriguez FJ, Bernardo LA, Schubert L, Sunkavalli A, Shillingford N, Calicchio ML, Lidov HG, Taha H, Martinez-Lage M, Santi M, Storm PB, Lee JY, Palmer JN, Adappa ND, Scott RM, Dunn IF, Laws ER, Jr., Stewart C, Ligon KL, Hoang MP, Van Hummelen P, Hahn WC, Louis DN, Resnick AC, Kieran MW, Getz G and Santagata S (2014). Exome sequencing identifies BRAF mutations in papillary craniopharyngiomas. *Nat Genet* 46(2):161-165.  2 Dias-Santagata D, Lam Q, Vernovsky K, Vena N, Lennerz JK, Borger DR, Batchelor TT, Ligon KL, Iafrate AJ, Ligon AH, Louis DN and Santagata S (2011). BRAF V600E mutations are common in pleomorphic xanthoastrocytoma: diagnostic and therapeutic implications. *PLoS ONE* 6(3):e17948.  3 Chappe C, Padovani L, Scavarda D, Forest F, Nanni-Metellus I, Loundou A, Mercurio S, Fina F, Lena G, Colin C and Figarella-Branger D (2013). Dysembryoplastic neuroepithelial tumors share with pleomorphic xanthoastrocytomas and gangliogliomas BRAF(V600E) mutation and expression. *Brain Pathol* 23(5):574-583.  4 Schindler G, Capper D, Meyer J, Janzarik W, Omran H, Herold-Mende C, Schmieder K, Wesseling P, Mawrin C, Hasselblatt M, Louis DN, Korshunov A, Pfister S, Hartmann C, Paulus W, Reifenberger G and von Deimling A (2011). 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 Neuropathol* 121(3):397-405.  5 Kleinschmidt-DeMasters BK, Aisner DL, Birks DK and Foreman NK (2013). Epithelioid GBMs show a high percentage of BRAF V600E mutation. *Am J Surg Pathol* 37(5):685-698.  6 Brastianos PK, Shankar GM, Gill CM, Taylor-Weiner A, Nayyar N, Panka DJ, Sullivan RJ, Frederick DT, Abedalthagafi M, Jones PS, Dunn IF, Nahed BV, Romero JM, Louis DN, Getz G, Cahill DP, Santagata S, Curry WT, Jr. and Barker FG, 2nd (2016). Dramatic Response of BRAF V600E Mutant Papillary Craniopharyngioma to Targeted Therapy. *J Natl Cancer Inst* 108(2).  7 Lassaletta A, Zapotocky M, Mistry M, Ramaswamy V, Honnorat M, Krishnatry R, Guerreiro Stucklin A, Zhukova N, Arnoldo A, Ryall S, Ling C, McKeown T, Loukides J, Cruz O, de Torres C, Ho CY, Packer RJ, Tatevossian R, Qaddoumi I, Harreld JH, Dalton JD, Mulcahy-Levy J, Foreman N, Karajannis MA, Wang S, Snuderl M, Nageswara Rao A, Giannini C, Kieran M, Ligon KL, Garre ML, Nozza P, Mascelli S, Raso A, Mueller S, Nicolaides T, Silva K, Perbet R, Vasiljevic A, Faure Conter C, Frappaz D, Leary S, Crane C, Chan A, Ng HK, Shi ZF, Mao Y, Finch E, Eisenstat D, Wilson B, Carret AS, Hauser P, Sumerauer D, Krskova L, Larouche V, Fleming A, Zelcer S, Jabado N, Rutka JT, Dirks P, Taylor MD, Chen S, Bartels U, Huang A, Ellison DW, Bouffet E, Hawkins C and Tabori U (2017). Therapeutic and Prognostic Implications of BRAF V600E in Pediatric Low-Grade Gliomas. *J Clin Oncol* 35(25):2934-2941.  8 Ihle MA, Fassunke J, Konig K, Grunewald I, Schlaak M, Kreuzberg N, Tietze L, Schildhaus HU, Buttner R and Merkelbach-Bruse S (2014). Comparison of high resolution melting analysis, pyrosequencing, next generation sequencing and immunohistochemistry to conventional Sanger sequencing for the detection of p.V600E and non-p.V600E BRAF mutations. *BMC Cancer* 14:13.  9 Ida CM, Vrana JA, Rodriguez FJ, Jentoft ME, Caron AA, Jenkins SM and Giannini C (2013). Immunohistochemistry is highly sensitive and specific for detection of BRAF V600E mutation in pleomorphic xanthoastrocytoma. *Acta Neuropathol Commun* 1:20.  10 Capper D, Preusser M, Habel A, Sahm F, Ackermann U, Schindler G, Pusch S, Mechtersheimer G, Zentgraf H and von Deimling A (2011). Assessment of BRAF V600E mutation status by immunohistochemistry with a mutation-specific monoclonal antibody. *Acta Neuropathol* 122(1):11-19.  11 Breton Q, Plouhinec H, Prunier-Mirebeau D, Boisselier B, Michalak S, Menei P and Rousseau A (2017). BRAF-V600E immunohistochemistry in a large series of glial and glial-neuronal tumors. *Brain Behav* 7(3):e00641.  12 Ritterhouse LL and Barletta JA (2015). BRAF V600E mutation-specific antibody: A review. *Semin Diagn Pathol* 32(5):400-408.  13 Collins VP, Jones DT and Giannini C (2015). Pilocytic astrocytoma: pathology, molecular mechanisms and markers. *Acta Neuropathol* 129(6):775-788.  14 Ida CM, Lambert SR, Rodriguez FJ, Voss JS, Mc Cann BE, Seys AR, Halling KC, Collins VP and Giannini C (2012). BRAF alterations are frequent in cerebellar low-grade astrocytomas with diffuse growth pattern. *J Neuropathol Exp Neurol* 71(7):631-639.  15 Rodriguez FJ, Schniederjan MJ, Nicolaides T, Tihan T, Burger PC and Perry A (2015). High rate of concurrent BRAF-KIAA1549 gene fusion and 1p deletion in disseminated oligodendroglioma-like leptomeningeal neoplasms (DOLN). *Acta Neuropathol* 129(4):609-610. |  |
| Non-core | *CDKN2A/B* HOMOZYGOUS DELETION | Single selection value list:  • Cannot be determined  • Absent  • Homozygous deletion  • Heterozygous deletion  TESTING METHOD  (Multi-select - select all that apply)  • In situ hybridization (FISH, CISH)  • Array-based method  • Next-generation sequencing  • Other, *specify* | Homozygous deletion of the *CDKN2A/B* genes on the short arm of chromosome 9 is associated with higher-grade diffuse gliomas and has been suggested as a marker for assessing likely behaviour (and grading) of IDH-mutant diffuse astrocytic tumours, with those harbouring homozygous *CDKN2A/B* deletions following more aggressive courses.1 On the other hand, *CDKN2A/B* deletions have been shown to be a characteristic genetic feature in pleomorphic xanthoastrocytomas, occurring in up to 87% of cases in one series; in this situation, along with *BRAF* V600E mutation, the *CDKN2A/B* deletions do not connote more aggressive behaviour.2 In neuropathological practice, FISH or high-resolution cytogenetic techniques (e.g., array-CGH, SNP arrays, methylation arrays) can be used to detect homozygous *CDKN2A/B* deletions.  The *CDKN2A* gene encodes the p16 protein, which can be detected using immunohistochemistry. However, whether loss of p16 nuclear staining has similar prognostic information to homozygous *CDKN2A/B* deletion remains to be determined and, at the present time, p16 immunohistochemistry cannot be recommended as a substitute for assessing homozygous *CDKN2A/B* deletion.1  References  1 Shirahata M, Ono T, Stichel D, Schrimpf D, Reuss DE, Sahm F, Koelsche C, Wefers A, Reinhardt A, Huang K, Sievers P, Shimizu H, Nanjo H, Kobayashi Y, Miyake Y, Suzuki T, Adachi JI, Mishima K, Sasaki A, Nishikawa R, Bewerunge-Hudler M, Ryzhova M, Absalyamova O, Golanov A, Sinn P, Platten M, Jungk C, Winkler F, Wick A, Hanggi D, Unterberg A, Pfister SM, Jones DTW, van den Bent M, Hegi M, French P, Baumert BG, Stupp R, Gorlia T, Weller M, Capper D, Korshunov A, Herold-Mende C, Wick W, Louis DN and von Deimling A (2018). Novel, improved grading system(s) for IDH-mutant astrocytic gliomas. *Acta Neuropathol*.  2 Vaubel RA, Caron AA, Yamada S, Decker PA, Eckel Passow JE, Rodriguez FJ, Nageswara Rao AA, Lachance D, Parney I, Jenkins R and Giannini C (2018). Recurrent copy number alterations in low-grade and anaplastic pleomorphic xanthoastrocytoma with and without BRAF V600E mutation. *Brain Pathol* 28(2):172-182. |  |
| Non-core | **C19MC ALTERATION** | Single selection value list:  • Cannot be determined  • Absent  • Absent with low level gain  • Present, *specify, including copy number*  TESTING METHOD  (Multi-select - select all that apply)  • In situ hybridization (FISH, CISH)  • Array-based method  • Next-generation sequencing  •Other,*specify* | **C19MC alteration1-7**  Demonstration of C19MC alteration is required for the diagnosis of embryonal tumour with multilayered rosettes (ETMR), C19MC-altered. This alteration consists of C19MC amplification or fusion, typically a focal high-level amplicon of chromosome 19q13.42 covering a large, poorly characterised microRNA cluster (hence C19MC) and the miR-371-373 locus, which map about 100 kb apart. The width and the level of gains at this locus, as assessed by array-CGH, is variable but always encompasses the same miRNA cluster. Even in the absence of multilayered rosettes, a CNS embryonal tumour with C19MC-alteration is diagnosed as ETMR, C19MC-altered. In routine neuropathological practice, FISH or chromogenic in situ hybridisation (CISH), or high-resolution cytogenetic techniques (e.g. array-CGH, SNP arrays, methylation arrays) can be used to detect amplification of the C19MC region. ETMRs lacking C19MC alterations and those that are not tested for this alteration or in which the test results are inconclusive are designated as ETMR, NOS (not otherwise specified), or with a medulloepithelioma phenotype as medulloepithelioma. LIN28A immunohistochemistry (see **LIN28A expression (immunohistochemistry)**) has also been used in the diagnosis of ETMR.  References  1 Ceccom J, Bourdeaut F, Loukh N, Rigau V, Milin S, Takin R, Richer W, Uro-Coste E, Couturier J, Bertozzi AI, Delattre O and Delisle MB (2014). Embryonal tumor with multilayered rosettes: diagnostic tools update and review of the literature. *Clin Neuropathol* 33(1):15-22.  2 Korshunov A, Remke M, Gessi M, Ryzhova M, Hielscher T, Witt H, Tobias V, Buccoliero AM, Sardi I, Gardiman MP, Bonnin J, Scheithauer B, Kulozik AE, Witt O, Mork S, von Deimling A, Wiestler OD, Giangaspero F, Rosenblum M, Pietsch T, Lichter P and Pfister SM (2010). Focal genomic amplification at 19q13.42 comprises a powerful diagnostic marker for embryonal tumors with ependymoblastic rosettes. *Acta Neuropathol* 120(2):253-260.  3 Korshunov A, Sturm D, Ryzhova M, Hovestadt V, Gessi M, Jones DT, Remke M, Northcott P, Perry A, Picard D, Rosenblum M, Antonelli M, Aronica E, Schuller U, Hasselblatt M, Woehrer A, Zheludkova O, Kumirova E, Puget S, Taylor MD, Giangaspero F, Peter Collins V, von Deimling A, Lichter P, Huang A, Pietsch T, Pfister SM and Kool M (2014). Embryonal tumor with abundant neuropil and true rosettes (ETANTR), ependymoblastoma, and medulloepithelioma share molecular similarity and comprise a single clinicopathological entity. *Acta Neuropathol* 128(2):279-289.  4 Li M, Lee KF, Lu Y, Clarke I, Shih D, Eberhart C, Collins VP, Van Meter T, Picard D, Zhou L, Boutros PC, Modena P, Liang ML, Scherer SW, Bouffet E, Rutka JT, Pomeroy SL, Lau CC, Taylor MD, Gajjar A, Dirks PB, Hawkins CE and Huang A (2009). Frequent amplification of a chr19q13.41 microRNA polycistron in aggressive primitive neuroectodermal brain tumors. *Cancer Cell* 16(6):533-546.  5 Nobusawa S, Orimo K, Horiguchi K, Ikota H, Yokoo H, Hirato J and Nakazato Y (2014). Embryonal tumor with abundant neuropil and true rosettes with only one structure suggestive of an ependymoblastic rosette. *Pathol Int* 64(9):472-477.  6 Pfister S, Remke M, Castoldi M, Bai AH, Muckenthaler MU, Kulozik A, von Deimling A, Pscherer A, Lichter P and Korshunov A (2009). Novel genomic amplification targeting the microRNA cluster at 19q13.42 in a pediatric embryonal tumor with abundant neuropil and true rosettes. *Acta Neuropathol* 117(4):457-464.  7 Spence T, Sin-Chan P, Picard D, Barszczyk M, Hoss K, Lu M, Kim SK, Ra YS, Nakamura H, Fangusaro J, Hwang E, Kiehna E, Toledano H, Wang Y, Shi Q, Johnston D, Michaud J, La Spina M, Buccoliero AM, Adamek D, Camelo-Piragua S, Peter Collins V, Jones C, Kabbara N, Jurdi N, Varlet P, Perry A, Scharnhorst D, Fan X, Muraszko KM, Eberhart CG, Ng HK, Gururangan S, Van Meter T, Remke M, Lafay-Cousin L, Chan JA, Sirachainan N, Pomeroy SL, Clifford SC, Gajjar A, Shago M, Halliday W, Taylor MD, Grundy R, Lau CC, Phillips J, Bouffet E, Dirks PB, Hawkins CE and Huang A (2014). CNS-PNETs with C19MC amplification and/or LIN28 expression comprise a distinct histogenetic diagnostic and therapeutic entity. *Acta Neuropathol* 128(2):291-303. |  |
| Non-core | CHROMOSOMAL ARM 1p/19q CODELETION | Single selection value list:  • Cannot be determined  • None detected  • 1p/19q codeletion  • 1p only deletion  • 19q only deletion  • Polysomy, *specify*  TESTING METHOD  (Multi-select - select all that apply)  • In situ hybridization (FISH, CISH)  • Array-based method  • PCR/Loss of heterozygosity assay  • Next-generation sequencing  • Other, *specify* | This cytogenetic alteration refers to whole-arm codeletion of chromosome arms 1p and 19q that together with IDH mutation constitutes the diagnostic molecular criteria for *oligodendroglioma, IDH-mutant and 1p/19q-codeleted, WHO grade II*, as well as *anaplastic oligodendroglioma, IDH -mutant and 1p/19q-codeleted, WHO grade III*.1 The whole-arm codeletion in oligodendroglial tumours is caused by an unbalanced t(1;19)(q10;p10) translocation.2,3 Of note, only whole-arm 1p/19q codeletion combined with IDH mutation is the diagnostically relevant marker; partial deletions on either chromosome arm may be found in other types of diffuse gliomas, including IDH-wildtype glioblastomas, and are neither diagnostic for IDH-mutant and 1p/19q-codeleted oligodendroglial tumours1 nor associated with favourable patient outcome.4 Moreover, detection of 1p/19q codeletion in the absence of IDH mutation is suspicious of partial deletions, and by definition is not sufficient for a diagnosis of an IDH-mutant and 1p/19q-codeleted oligodendroglial tumour.  Various techniques are being used for the diagnostic assessment of 1p/19q codeletion. Commonly used methods include microsatellite analysis for loss of heterozygosity (LOH), FISH or CISH, and multiplex ligation-dependent probe amplification (MLPA). FISH/CISH can be applied on routine FFPE sections. However, analysis is often restricted to single loci on each chromosome arm, which may not reliably distinguish whole-arm losses from partial deletions. There is no standardized cut-off for determination of codeletion by FISH/CISH, with each laboratory needing to validate its assay. In addition, polysomies of chromosomes 1 or 19 may complicate diagnostic assessment and have been associated with less favourable outcome.5-7 LOH analysis and MLPA assess multiple loci along each chromosome arm and thereby reduce the risk of false-positive findings due to partial deletions. However, extraction of tumour DNA (for MLPA) as well as tumour and leukocyte DNA (for LOH analysis) is required for these techniques. Microarray-based approaches may also be used for diagnostic purposes, including DNA methylation bead arrays that allow for simultaneous detection of 1p/19q codeletion, *MGMT* promoter methylation, and G-CIMP status indicative of IDH mutation.8 Most recently, panel-based NGS approaches have been used for 1p/19q detection and simultaneous mutational analyses of *IDH1* and *IDH2*, a well as other alterations commonly associated with 1p/19q codeletion, such as *TERT* promoter mutation and *CIC* mutation.9,10 Immunostaining for the proneural α-internexin protein11,12 or NOGO-A13 cannot substitute as a surrogate marker for 1p/19q codeletion.  References  1 Louis DN, Ohgaki H, Wiestler OD and Cavenee WK (eds) (2016). *WHO Classification of Tumours of the Central Nervous System, Revised. Fourth Edition*, IARC, Lyon.  2 Griffin CA, Burger P, Morsberger L, Yonescu R, Swierczynski S, Weingart JD and Murphy KM (2006). Identification of der(1;19)(q10;p10) in five oligodendrogliomas suggests mechanism of concurrent 1p and 19q loss. *J Neuropathol Exp Neurol* 65(10):988-994.  3 Jenkins RB, Blair H, Ballman KV, Giannini C, Arusell RM, Law M, Flynn H, Passe S, Felten S, Brown PD, Shaw EG and Buckner JC (2006). 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(20):9852-9861.  4 Vogazianou AP, Chan R, Backlund LM, Pearson DM, Liu L, Langford CF, Gregory SG, Collins VP and Ichimura K (2010). Distinct patterns of 1p and 19q alterations identify subtypes of human gliomas that have different prognoses. *Neuro Oncol* 12(7):664-678.  5 Reddy KS (2008). Assessment of 1p/19q deletions by fluorescence in situ hybridization in gliomas. *Cancer Genet Cytogenet* 184(2):77-86.  6 Snuderl M, Eichler AF, Ligon KL, Vu QU, Silver M, Betensky RA, Ligon AH, Wen PY, Louis DN and Iafrate AJ (2009). Polysomy for chromosomes 1 and 19 predicts earlier recurrence in anaplastic oligodendrogliomas with concurrent 1p/19q loss. *Clin Cancer Res* 15(20):6430-6437.  7 Wiens AL, Cheng L, Bertsch EC, Johnson KA, Zhang S and Hattab EM (2012). Polysomy of chromosomes 1 and/or 19 is common and associated with less favorable clinical outcome in oligodendrogliomas: fluorescent in situ hybridization analysis of 84 consecutive cases. *J Neuropathol Exp Neurol* 71(7):618-624.  8 Wiestler B, Capper D, Hovestadt V, Sill M, Jones DT, Hartmann C, Felsberg J, Platten M, Feiden W, Keyvani K, Pfister SM, Wiestler OD, Meyermann R, Reifenberger G, Pietsch T, von Deimling A, Weller M and Wick W (2014). Assessing CpG island methylator phenotype, 1p/19q codeletion, and MGMT promoter methylation from epigenome-wide data in the biomarker cohort of the NOA-04 trial. *Neuro Oncol* 16(12):1630-1638.  9 Sahm F, Schrimpf D, Jones DT, Meyer J, Kratz A, Reuss D, Capper D, Koelsche C, Korshunov A, Wiestler B, Buchhalter I, Milde T, Selt F, Sturm D, Kool M, Hummel M, Bewerunge-Hudler M, Mawrin C, Schuller U, Jungk C, Wick A, Witt O, Platten M, Herold-Mende C, Unterberg A, Pfister SM, Wick W and von Deimling A (2016). Next-generation sequencing in routine brain tumor diagnostics enables an integrated diagnosis and identifies actionable targets. *Acta Neuropathol* 131(6):903-910.  10 Zacher A, Kaulich K, Stepanow S, Wolter M, Kohrer K, Felsberg J, Malzkorn B and Reifenberger G (2017). Molecular Diagnostics of Gliomas Using Next Generation Sequencing of a Glioma-Tailored Gene Panel. *Brain Pathol* 27(2):146-159.  11 Ducray F, Criniere E, Idbaih A, Mokhtari K, Marie Y, Paris S, Navarro S, Laigle-Donadey F, Dehais C, Thillet J, Hoang-Xuan K, Delattre JY and Sanson M (2009). alpha-Internexin expression identifies 1p19q codeleted gliomas. *Neurology* 72(2):156-161.  12 Eigenbrod S, Roeber S, Thon N, Giese A, Krieger A, Grasbon-Frodl E, Egensperger R, Tonn JC, Kreth FW and Kretzschmar HA (2011). alpha-Internexin in the diagnosis of oligodendroglial tumors and association with 1p/19q status. *J Neuropathol Exp Neurol* 70(11):970-978.  13 Marucci G, Di Oto E, Farnedi A, Panzacchi R, Ligorio C and Foschini MP (2012). Nogo-A: a useful marker for the diagnosis of oligodendroglioma and for identifying 1p19q codeletion. *Hum Pathol* 43(3):374-380. |  |
| Non-core | CHROMOSOME 7 GAIN (combined with chromosome 10 loss) | Single selection value list:  • Cannot be determined  • Absent  • Present  TESTING METHOD  (Multi-select - select all that apply)  • In situ hybridization  • Array-based method  • Next-generation sequencing  • Other, *specify* | Partial or complete chromosome 7 gain and 10 loss are often found in glioblastoma, particularly glioblastoma, IDH-wildtype, but chromosome 7 or 7q gain can also be found in other glial brain tumours. In one study,1 81/136 glioblastomas and 123/136 glioblastomas had partial alterations or combined complete 7 gain and 10 loss, respectively. In contrast, no chromosome 7 gains or 10 losses were identified in only 11/136 glioblastomas. Chromosome 7 gain may be trisomy, tetrasomy or even higher polysomy. It is unclear whether extent of polysomy/ degree of gain impacts prognosis. Gain of chromosome 7 is more frequent than *EGFR* amplification in glioblastoma, IDH-wildtype.  Recent studies have advocated for testing of these markers as part of prognostic stratification.2,3 The most likely significance of these changes, given their association with glioblastomas, is in the setting of an IDH-wildtype diffuse astrocytoma or anaplastic astrocytoma, in which 7 or 7q gain/10 or 10q loss may be associated with a course and outcome paralleling that of glioblastoma, IDH-wildtype, WHO grade IV. Of note, some subtypes of glioblastoma, such as gliosarcoma and giant cell glioblastoma, tend to have considerably less frequent *EGFR* amplification (5-6%) than IDH-wildtype glioblastoma, but still may show gains of chromosome 7 and losses of chromosome 10.  References  1 Reuss DE, Sahm F, Schrimpf D, Wiestler B, Capper D, Koelsche C, Schweizer L, Korshunov A, Jones DT, Hovestadt V, Mittelbronn M, Schittenhelm J, Herold-Mende C, Unterberg A, Platten M, Weller M, Wick W, Pfister SM and von Deimling A (2015). 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(1):133-146.  2 van den Bent MJ, Weller M, Wen PY, Kros JM, Aldape K and Chang S (2017). A clinical perspective on the 2016 WHO brain tumor classification and routine molecular diagnostics. *Neuro Oncol* 19(5):614-624.  3 Weller M, Weber RG, Willscher E, Riehmer V, Hentschel B, Kreuz M, Felsberg J, Beyer U, Loffler-Wirth H, Kaulich K, Steinbach JP, Hartmann C, Gramatzki D, Schramm J, Westphal M, Schackert G, Simon M, Martens T, Bostrom J, Hagel C, Sabel M, Krex D, Tonn JC, Wick W, Noell S, Schlegel U, Radlwimmer B, Pietsch T, Loeffler M, von Deimling A, Binder H and Reifenberger G (2015). Molecular classification of diffuse cerebral WHO grade II/III gliomas using genome- and transcriptome-wide profiling improves stratification of prognostically distinct patient groups. *Acta Neuropathol* 129(5):679-693. | combined with chromosome 10 loss |
| Non-core | CHROMOSOME 10q23 (PTEN LOCUS) DELETION AND  PTEN MUTATION | **Chromosome 10q23 (*PTEN* Locus) deletion**  Single selection value list:  • Cannot be determined  • None detected  • Interstitial deletion present  • Monosomy*, specify*  • Polysomy*, specify*  TESTING METHOD  (Multi-select - select all that apply)  • In situ hybridization  • Array-based method  • PCR/Loss of heterozygosity assay  • Other, *specify*  ***PTEN* mutation**  Single selection value list:  • Cannot be determined  • Absent  • Present, *specify*  TESTING METHOD  (Multi-select - select all that apply)  • Sanger sequencing  • Next-generation sequencing  • PCR-based method  • Other, *specify* | **Chromosome band 10q23 (*PTEN* Locus) Deletion**  Hemizygous deletions affecting the *PTEN* gene locus at band 10q23 are detectable in the vast majority of glioblastomas, IDH-wildtype and IDH-mutant, due to monosomy 10 or deletion of 10q.1,2 Losses of chromosome 10 or chromosome arm 10q have also been reported in smaller fractions of WHO grade II and III diffuse gliomas.2-4 However, when detected in an IDH-wildtype astrocytic glioma of WHO grade II or III, monosomy 10 or 10q23 deletion may indicate a glioblastoma, IDH-wildtype, in particular when associated with gain of chromosome 7 and other glioblastoma-associated genetic alterations, like *EGFR* amplification and *TERT* promoter mutation.3-5 Homozygous *PTEN* deletion is less common than hemizygous deletion, and mostly restricted to a small fraction of IDH-wildtype glioblastomas.1 Detection of 10q23 (*PTEN* locus) deletion is commonly accomplished by FISH or CISH on routine FFPE tissue sections. Other diagnostically useful methods include MLPA, microarray-based DNA copy number profiling, and NGS-based analyses.  ***PTEN* Mutation**  Mutations in the *PTEN* tumour suppressor gene at 10q23 are found in approximately 30% of glioblastomas, IDH-wildtype.1 *PTEN* mutation in IDH-wildtype glioblastomas is usually accompanied by loss of the second allele due to monosomy 10 or deletion of 10q. Mutations are distributed across the entire gene with the highest frequency of mutations seen in exons 5 and 6, which encode the catalytic domain of the PTEN protein.6 Therefore, diagnostic investigation for *PTEN* mutations requires sequencing of all exons including the flanking intronic regions for detection of splice site mutations. NGS-based approaches represent the most convenient way to detect *PTEN* mutations, while Sanger sequencing is also possible but more laborious.7-9 Immunohistochemical demonstration of loss of PTEN protein expression does not correlate well with *PTEN* mutation or *PTEN* promoter methylation in glioblastomas, and thus cannot serve as a surrogate marker.10  References  1 Brennan CW, Verhaak RG, McKenna A, Campos B, Noushmehr H, Salama SR, Zheng S, Chakravarty D, Sanborn JZ, Berman SH, Beroukhim R, Bernard B, Wu CJ, Genovese G, Shmulevich I, Barnholtz-Sloan J, Zou L, Vegesna R, Shukla SA, Ciriello G, Yung WK, Zhang W, Sougnez C, Mikkelsen T, Aldape K, Bigner DD, Van Meir EG, Prados M, Sloan A, Black KL, Eschbacher J, Finocchiaro G, Friedman W, Andrews DW, Guha A, Iacocca M, O'Neill BP, Foltz G, Myers J, Weisenberger DJ, Penny R, Kucherlapati R, Perou CM, Hayes DN, Gibbs R, Marra M, Mills GB, Lander E, Spellman P, Wilson R, Sander C, Weinstein J, Meyerson M, Gabriel S, Laird PW, Haussler D, Getz G and Chin L (2013). The somatic genomic landscape of glioblastoma. *Cell* 155(2):462-477.  2 Ceccarelli M, Barthel FP, Malta TM, Sabedot TS, Salama SR, Murray BA, Morozova O, Newton Y, Radenbaugh A, Pagnotta SM, Anjum S, Wang J, Manyam G, Zoppoli P, Ling S, Rao AA, Grifford M, Cherniack AD, Zhang H, Poisson L, Carlotti CG, Jr., Tirapelli DP, Rao A, Mikkelsen T, Lau CC, Yung WK, Rabadan R, Huse J, Brat DJ, Lehman NL, Barnholtz-Sloan JS, Zheng S, Hess K, Rao G, Meyerson M, Beroukhim R, Cooper L, Akbani R, Wrensch M, Haussler D, Aldape KD, Laird PW, Gutmann DH, Noushmehr H, Iavarone A and Verhaak RG (2016). Molecular Profiling Reveals Biologically Discrete Subsets and Pathways of Progression in Diffuse Glioma. *Cell* 164(3):550-563.  3 Weller M, Weber RG, Willscher E, Riehmer V, Hentschel B, Kreuz M, Felsberg J, Beyer U, Loffler-Wirth H, Kaulich K, Steinbach JP, Hartmann C, Gramatzki D, Schramm J, Westphal M, Schackert G, Simon M, Martens T, Bostrom J, Hagel C, Sabel M, Krex D, Tonn JC, Wick W, Noell S, Schlegel U, Radlwimmer B, Pietsch T, Loeffler M, von Deimling A, Binder H and Reifenberger G (2015). Molecular classification of diffuse cerebral WHO grade II/III gliomas using genome- and transcriptome-wide profiling improves stratification of prognostically distinct patient groups. *Acta Neuropathol* 129(5):679-693.  4 Brat DJ, Verhaak RG, Aldape KD, Yung WK, Salama SR, Cooper LA, Rheinbay E, Miller CR, Vitucci M, Morozova O, Robertson AG, Noushmehr H, Laird PW, Cherniack AD, Akbani R, Huse JT, Ciriello G, Poisson LM, Barnholtz-Sloan JS, Berger MS, Brennan C, Colen RR, Colman H, Flanders AE, Giannini C, Grifford M, Iavarone A, Jain R, Joseph I, Kim J, Kasaian K, Mikkelsen T, Murray BA, O'Neill BP, Pachter L, Parsons DW, Sougnez C, Sulman EP, Vandenberg SR, Van Meir EG, von Deimling A, Zhang H, Crain D, Lau K, Mallery D, Morris S, Paulauskis J, Penny R, Shelton T, Sherman M, Yena P, Black A, Bowen J, Dicostanzo K, Gastier-Foster J, Leraas KM, Lichtenberg TM, Pierson CR, Ramirez NC, Taylor C, Weaver S, Wise L, Zmuda E, Davidsen T, Demchok JA, Eley G, Ferguson ML, Hutter CM, Mills Shaw KR, Ozenberger BA, Sheth M, Sofia HJ, Tarnuzzer R, Wang Z, Yang L, Zenklusen JC, Ayala B, Baboud J, Chudamani S, Jensen MA, Liu J, Pihl T, Raman R, Wan Y, Wu Y, Ally A, Auman JT, Balasundaram M, Balu S, Baylin SB, Beroukhim R, Bootwalla MS, Bowlby R, Bristow CA, Brooks D, Butterfield Y, Carlsen R, Carter S, Chin L, Chu A, Chuah E, Cibulskis K, Clarke A, Coetzee SG, Dhalla N, Fennell T, Fisher S, Gabriel S, Getz G, Gibbs R, Guin R, Hadjipanayis A, Hayes DN, Hinoue T, Hoadley K, Holt RA, Hoyle AP, Jefferys SR, Jones S, Jones CD, Kucherlapati R, Lai PH, Lander E, Lee S, Lichtenstein L, Ma Y, Maglinte DT, Mahadeshwar HS, Marra MA, Mayo M, Meng S, Meyerson ML, Mieczkowski PA, Moore RA, Mose LE, Mungall AJ, Pantazi A, Parfenov M, Park PJ, Parker JS, Perou CM, Protopopov A, Ren X, Roach J, Sabedot TS, Schein J, Schumacher SE, Seidman JG, Seth S, Shen H, Simons JV, Sipahimalani P, Soloway MG, Song X, Sun H, Tabak B, Tam A, Tan D, Tang J, Thiessen N, Triche T, Jr., Van Den Berg DJ, Veluvolu U, Waring S, Weisenberger DJ, Wilkerson MD, Wong T, Wu J, Xi L, Xu AW, Yang L, Zack TI, Zhang J, Aksoy BA, Arachchi H, Benz C, Bernard B, Carlin D, Cho J, DiCara D, Frazer S, Fuller GN, Gao J, Gehlenborg N, Haussler D, Heiman DI, Iype L, Jacobsen A, Ju Z, Katzman S, Kim H, Knijnenburg T, Kreisberg RB, Lawrence MS, Lee W, Leinonen K, Lin P, Ling S, Liu W, Liu Y, Liu Y, Lu Y, Mills G, Ng S, Noble MS, Paull E, Rao A, Reynolds S, Saksena G, Sanborn Z, Sander C, Schultz N, Senbabaoglu Y, Shen R, Shmulevich I, Sinha R, Stuart J, Sumer SO, Sun Y, Tasman N, Taylor BS, Voet D, Weinhold N, Weinstein JN, Yang D, Yoshihara K, Zheng S, Zhang W, Zou L, Abel T, Sadeghi S, Cohen ML, Eschbacher J, Hattab EM, Raghunathan A, Schniederjan MJ, Aziz D, Barnett G, Barrett W, Bigner DD, Boice L, Brewer C, Calatozzolo C, Campos B, Carlotti CG, Jr., Chan TA, Cuppini L, Curley E, Cuzzubbo S, Devine K, DiMeco F, Duell R, Elder JB, Fehrenbach A, Finocchiaro G, Friedman W, Fulop J, Gardner J, Hermes B, Herold-Mende C, Jungk C, Kendler A, Lehman NL, Lipp E, Liu O, Mandt R, McGraw M, McLendon R, McPherson C, Neder L, Nguyen P, Noss A, Nunziata R, Ostrom QT, Palmer C, Perin A, Pollo B, Potapov A, Potapova O, Rathmell WK, Rotin D, Scarpace L, Schilero C, Senecal K, Shimmel K, Shurkhay V, Sifri S, Singh R, Sloan AE,  Smolenski K, Staugaitis SM, Steele R, Thorne L, Tirapelli DP, Unterberg A, Vallurupalli M, Wang Y, Warnick R, Williams F, Wolinsky Y, Bell S, Rosenberg M, Stewart C, Huang F, Grimsby JL, Radenbaugh AJ and Zhang J (2015). Comprehensive, Integrative Genomic Analysis of Diffuse Lower-Grade Gliomas. *N Engl J Med* 372(26):2481-2498.  5 Wijnenga MMJ, Dubbink HJ, French PJ, Synhaeve NE, Dinjens WNM, Atmodimedjo PN, Kros JM, Dirven CMF, Vincent A and van den Bent MJ (2017). Molecular and clinical heterogeneity of adult diffuse low-grade IDH wild-type gliomas: assessment of TERT promoter mutation and chromosome 7 and 10 copy number status allows superior prognostic stratification. *Acta Neuropathol* 134(6):957-959.  6 Knobbe CB, Merlo A and Reifenberger G (2002). Pten signaling in gliomas. *Neuro Oncol* 4(3):196-211.  7 Nikiforova MN, Wald AI, Melan MA, Roy S, Zhong S, Hamilton RL, Lieberman FS, Drappatz J, Amankulor NM, Pollack IF, Nikiforov YE and Horbinski C (2016). Targeted next-generation sequencing panel (GlioSeq) provides comprehensive genetic profiling of central nervous system tumors. *Neuro Oncol* 18(3):379-387.  8 Sahm F, Schrimpf D, Jones DT, Meyer J, Kratz A, Reuss D, Capper D, Koelsche C, Korshunov A, Wiestler B, Buchhalter I, Milde T, Selt F, Sturm D, Kool M, Hummel M, Bewerunge-Hudler M, Mawrin C, Schuller U, Jungk C, Wick A, Witt O, Platten M, Herold-Mende C, Unterberg A, Pfister SM, Wick W and von Deimling A (2016). Next-generation sequencing in routine brain tumor diagnostics enables an integrated diagnosis and identifies actionable targets. *Acta Neuropathol* 131(6):903-910.  9 Zacher A, Kaulich K, Stepanow S, Wolter M, Kohrer K, Felsberg J, Malzkorn B and Reifenberger G (2017). Molecular Diagnostics of Gliomas Using Next Generation Sequencing of a Glioma-Tailored Gene Panel. *Brain Pathol* 27(2):146-159.  10 Baeza N, Weller M, Yonekawa Y, Kleihues P and Ohgaki H (2003). PTEN methylation and expression in glioblastomas. *Acta Neuropathol* 106(5):479-485. |  |
| Non-core | *EGFR* AMPLIFICATION AND EGFRvIII MUTATION | ***EGFR* amplification**  Single selection value list:  • Cannot be determined  • Absent  • Absent with low level gain  • Present, *specify, including copy number*  TESTING METHOD  (Multi-select - select all that apply)  • In situ hybridization(FISH, CISH)  • Array-based method  • Next-generation sequencing  • Other, *specify*  **EGFRvIII mutation**  Single selection value list:  • Cannot be determined  • Absent  • Present  TESTING METHOD  (Multi-select - select all that apply)  • Next-generation sequencing  • PCR-based method  • Immunohistochemistry  • Other, *specify* | ***EGFR* amplification and EGFRvIII mutation1**  The epidermal growth factor receptor (*EGFR*) gene at 7p12 is the most commonly amplified proto-oncogene in gliomas.2 *EGFR* amplification is detectable in approximately 40% of IDH-wildtype glioblastomas, WHO grade IV, and is particularly common in tumours from adult patients with the classic or receptor tyrosine kinase (RTK) type 2 molecular subtype of glioblastoma.3,4 *EGFR* amplification is commonly associated with point mutations and other genetic rearrangements, the most common of which, EGFRvIII, being detectable in about 50% of *EGFR*-amplified glioblastomas.5,6 EGFRvIII is caused by an 801-bp in-frame deletion of exons 2 to 7 that results in a constitutively active protein lacking major parts of the extracellular receptor domain including the ligand binding site.6 Moreover, EGFRvIII carries a unique peptide encoded by the fusion site of exons 1 and 8 that has served as a tumour-specific epitope for anti-EGFRvIII immunotherapy.7 As *EGFR* amplification and positivity for EGFRvIII are virtually restricted to glioblastoma, IDH-wildtype, their diagnostic detection in an IDH-wildtype diffuse astrocytic glioma may support a glioblastoma diagnosis even in the absence of characteristic histological features like microvascular proliferation and/or necrosis. Detection of *EGFR* amplification or EGFRvIII positivity also may be clinically relevant as a predictive marker of response to molecularly-guided therapies targeting *EGFR* and/or EGFRvIII.8,9  *EGFR* amplification is usually seen in the majority of neoplastic cells in a given tumour and can be readily detected by FISH or CISH on routine FFPE tissue sections, although amplification levels may be heterogeneous from cell to cell. Targeted molecular techniques based on extracted tumour DNA, such as quantitative real-time PCR and MLPA, are also suitable for diagnostic detection of *EGFR* amplification. More recently, microarray-based genomic or epigenetic analyses as well as NGS approaches are increasingly being used.10 Gene amplification (defined by a circumscribed high-level copy number gain of the *EGFR* gene at 7p12) needs to be distinguished from low-level copy number gains of chromosome 7 caused by numerical chromosomal abnormalities, in particular trisomy 7, which are not restricted to IDH-wildtype glioblastoma but also common in diffuse and anaplastic astrocytomas11 (see also **Chromosome 7 Gain**). To date, there is no evidence that different levels of *EGFR* gene amplification (e.g., increases in copy number of 10-fold versus 100-fold) have distinct diagnostic or prognostic impact.  Detection of EGFRvIII in *EGFR*-amplified glioblastomas also can be performed at the DNA level, e.g., by MLPA, microarray-based techniques and NGS. However, detection at the mRNA or protein level using RT-PCR or immunohistochemistry with EGFRvIII-specific antibodies appears to be more sensitive.5 This is due to the fact that EGFRvIII positivity usually shows regional heterogeneity and sometimes affects only a minor subset of the tumour cells.5 Thus, representative sampling of tumour tissue is an important issue to avoid false-negative testing for EGFRvIII. Unfortunately, precise cut-off values for distinction between high- and low-level copy number gains have not been defined and may need to be adjusted for each testing method.  References  1 Maire CL and Ligon KL (2014). Molecular pathologic diagnosis of epidermal growth factor receptor. *Neuro Oncol* 16 Suppl 8:viii1-6.  2 Aldape K, Zadeh G, Mansouri S, Reifenberger G and von Deimling A (2015). Glioblastoma: pathology, molecular mechanisms and markers. *Acta Neuropathol* 129(6):829-848.  3 Sturm D, Witt H, Hovestadt V, Khuong-Quang DA, Jones DT, Konermann C, Pfaff E, Tonjes M, Sill M, Bender S, Kool M, Zapatka M, Becker N, Zucknick M, Hielscher T, Liu XY, Fontebasso AM, Ryzhova M, Albrecht S, Jacob K, Wolter M, Ebinger M, Schuhmann MU, van Meter T, Fruhwald MC, Hauch H, Pekrun A, Radlwimmer B, Niehues T, von Komorowski G, Durken M, Kulozik AE, Madden J, Donson A, Foreman NK, Drissi R, Fouladi M, Scheurlen W, von Deimling A, Monoranu C, Roggendorf W, Herold-Mende C, Unterberg A, Kramm CM, Felsberg J, Hartmann C, Wiestler B, Wick W, Milde T, Witt O, Lindroth AM, Schwartzentruber J, Faury D, Fleming A, Zakrzewska M, Liberski PP, Zakrzewski K, Hauser P, Garami M, Klekner A, Bognar L, Morrissy S, Cavalli F, Taylor MD, van Sluis P, Koster J, Versteeg R, Volckmann R, Mikkelsen T, Aldape K, Reifenberger G, Collins VP, Majewski J, Korshunov A, Lichter P, Plass C, Jabado N and Pfister SM (2012). Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. *Cancer Cell* 22(4):425-437.  4 Brennan CW, Verhaak RG, McKenna A, Campos B, Noushmehr H, Salama SR, Zheng S, Chakravarty D, Sanborn JZ, Berman SH, Beroukhim R, Bernard B, Wu CJ, Genovese G, Shmulevich I, Barnholtz-Sloan J, Zou L, Vegesna R, Shukla SA, Ciriello G, Yung WK, Zhang W, Sougnez C, Mikkelsen T, Aldape K, Bigner DD, Van Meir EG, Prados M, Sloan A, Black KL, Eschbacher J, Finocchiaro G, Friedman W, Andrews DW, Guha A, Iacocca M, O'Neill BP, Foltz G, Myers J, Weisenberger DJ, Penny R, Kucherlapati R, Perou CM, Hayes DN, Gibbs R, Marra M, Mills GB, Lander E, Spellman P, Wilson R, Sander C, Weinstein J, Meyerson M, Gabriel S, Laird PW, Haussler D, Getz G and Chin L (2013). The somatic genomic landscape of glioblastoma. *Cell* 155(2):462-477.  5 Weller M, Kaulich K, Hentschel B, Felsberg J, Gramatzki D, Pietsch T, Simon M, Westphal M, Schackert G, Tonn JC, von Deimling A, Davis T, Weiss WA, Loeffler M and Reifenberger G (2014). Assessment and prognostic significance of the epidermal growth factor receptor vIII mutation in glioblastoma patients treated with concurrent and adjuvant temozolomide radiochemotherapy. *Int J Cancer* 134(10):2437-2447.  6 Gan HK, Cvrljevic AN and Johns TG (2013). The epidermal growth factor receptor variant III (EGFRvIII): where wild things are altered. *Febs j* 280(21):5350-5370.  7 Weller M, Butowski N, Tran DD, Recht LD, Lim M, Hirte H, Ashby L, Mechtler L, Goldlust SA, Iwamoto F, Drappatz J, O'Rourke DM, Wong M, Hamilton MG, Finocchiaro G, Perry J, Wick W, Green J, He Y, Turner CD, Yellin MJ, Keler T, Davis TA, Stupp R and Sampson JH (2017). Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial. *Lancet Oncol* 18(10):1373-1385.  8 Desai R, Suryadevara CM, Batich KA, Farber SH, Sanchez-Perez L and Sampson JH (2016). Emerging immunotherapies for glioblastoma. *Expert Opin Emerg Drugs* 21(2):133-145.  9 Thorne AH, Zanca C and Furnari F (2016). Epidermal growth factor receptor targeting and challenges in glioblastoma. *Neuro Oncol* 18(7):914-918.  10 Masui K, Mischel PS and Reifenberger G (2016). Molecular classification of gliomas. *Handb Clin Neurol* 134:97-120.  11 Ceccarelli M, Barthel FP, Malta TM, Sabedot TS, Salama SR, Murray BA, Morozova O, Newton Y, Radenbaugh A, Pagnotta SM, Anjum S, Wang J, Manyam G, Zoppoli P, Ling S, Rao AA, Grifford M, Cherniack AD, Zhang H, Poisson L, Carlotti CG, Jr., Tirapelli DP, Rao A, Mikkelsen T, Lau CC, Yung WK, Rabadan R, Huse J, Brat DJ, Lehman NL, Barnholtz-Sloan JS, Zheng S, Hess K, Rao G, Meyerson M, Beroukhim R, Cooper L, Akbani R, Wrensch M, Haussler D, Aldape KD, Laird PW, Gutmann DH, Noushmehr H, Iavarone A and Verhaak RG (2016). Molecular Profiling Reveals Biologically Discrete Subsets and Pathways of Progression in Diffuse Glioma. *Cell* 164(3):550-563. |  |
| Non-core | HISTONE H3 MUTATION AND H3 K27 TRIMETHYLATION (me3) | **Histone H3 gene family mutation**  Single selection value list:  • Cannot be determined  • Negative  • Positive for K27M  • Positive for G34R or G34V  • Positive, for other H3 mutation, *specify*  TESTING METHOD  (Multi-select - select all that apply)  • Sanger sequencing  • Next-generation sequencing  • PCR-based method  • Other, *specify*  **Histone H3 K27M expression (immunohistochemistry)**  Single selection value list:  • Cannot be determined  • Negative  • Positive  **Histone H3 G34R expression (immunohistochemistry)**  Single selection value list:  • Cannot be determined  • Negative  • Positive  **Histone H3 K27me3 expression (immunohistochemistry)**  Single selection value list:  • Cannot be determined  • Intact expression  • Loss of expression | Any standard sequencing method can be used to detect the H3 K27M mutation, including pyrosequencing, Taq Man PCR, droplet-digital PCR, Sanger sequencing, and NGS. A similar array of sequencing methods can be used for H3 G34 mutations, however due to the GC rich nature of this region, targeted methods can be more difficult to set up. For detection of both mutations using targeted methods (and alignment of non-targeted methods), consideration needs to be given to the high degree of homology among the H3 genes (human H3 variants include H3.3, H3.1, H3.2, CENP-A, H3t, H3.X and H3.Y) and the number of genes encoding each protein (H3.3 is encoded by two genes, *H3F3A* and *H3F3B*, while H3.1 and H3.2 are each encoded by multiple genes found within gene clusters). The exact gene being tested and the method used should be provided in the report.  **Histone H3 K27M Mutation (Sequencing) and Expression (Immunohistochemistry)**  Recurrent mutations in *H3F3A* (H3.3) and *HIST1H3B/C/I* (H3.1) with lysine 27 substituted for methionine (H3 K27M) are characteristic of paediatric high-grade astrocytomas with a predilection for a midline location; less commonly, these mutations are found in adult midline diffuse gliomas.1-3 These tumours have a poor prognosis. The H3.3 K27M mutation is found in approximately 70% of diffuse intrinsic pontine gliomas and H3.1 K27M in a further 15%. Furthermore, in the paediatric age group, H3.3 K27M is also found in approximately 50% of high-grade diffuse gliomas involving the thalamus and spinal cord. H3 K27M mutations also occur in a broader range of patient ages, morphologies, and locations; the median age to date is the third decade for spinal cord and thalamic tumours with patients as old as 65 years being reported with the alteration. Other locations include third ventricle, hypothalamus, pineal region and cerebellum.4 H3 K27M mutation can also be found in diffuse astrocytomas without classic high-grade features that generally behave more aggressively than their wild type counterparts. In occasional cases, the mutation has been found in other tumour types, including ganglioglioma,5 pilocytic astrocytoma6 and ependymoma.7 Testing for this alteration should be considered, at a minimum, in all midline diffuse gliomas in patients under the age of 30. These alterations can be identified by sequencing or a mutation-specific antibody. Detection of the mutation by either immunohistochemistry or sequencing is required for the diagnosis of *Diffuse midline glioma, H3 K27M mutant*. Lack of H3 K27-me3 is not a specific marker of H3 K27M status.  Immunohistochemistry with an antibody against the N-terminus of the mutant protein is highly sensitive and specific for detection of the H3K27M protein from either H3.3 or H3.1.8,9 In practice, the antibody can produce a fair amount of background cytoplasmic staining in non-tumour cells and only diffuse strong nuclear staining in most (or all) tumour cells should be considered positive. Further, poorly fixed tissue or tissue from post-mortem or older blocks may be false negative. If equivocal, a sequencing-based method (see below) should be considered as the standard of care.  **Histone H3 G34 Mutations (Sequencing) and Expression (Immunohistochemistry)**  Recurrent mutations in *H3F3A* (H3.3) with glycine 34 substituted for arginine (H3 G34R) or infrequently valine (H3 G34V) are found most commonly in hemispheric high-grade gliomas of the adolescent and young adult population (median age 15 years; range 9-51 years).10 The H3G34R mutation is found in ~15-20% of hemispheric high-grade glioma cases in the pediatric age group.11 Outcome is slightly better than in H3K27M-mutant tumours in a midline location, with a median survival of approximately 18 months. Testing for this alteration should be considered, at a minimum, in hemispheric, IDH-wildtype, high-grade gliomas in patients under the age of 30, particularly if ATRX  is lost and p53 is diffusely immunopositive. These alterations can be identified by sequencing or a mutation-specific (H3 G34R) antibody.  Immunohistochemistry with an antibody against the mutant protein is specific for detection of the H3G34R protein.12 In practice, the antibody works well on FFPE tissue with specific nuclear staining but does not stain every tumour cell; as a result, sensitivity may prove to be an issue as more experience is gained with the antibody. If immunohistochemical results are equivocal or if suspicion for mutation is high, a sequencing-based method should be considered as the standard of care.  **Histone H3 K27me3 Expression (Immunohistochemistry)**  The presence of the H3 K27M mutant protein is associated with a fairly widespread (and thus detectable on Western blot or immunohistochemistry) loss of the repressive trimethyl (me3) mark on lysine 27 (K27me3). Tumour cells harbouring the H3 K27M mutation (either H3.1 or H3.3 K27M) will typically show loss of nuclear expression of this protein on immunohistochemistry with retention of staining in entrapped non-neoplastic cells, e.g., endothelial cells (similar to the pattern seen with ATRX or INI1). However, it should be noted, that while loss of H3K27me3 is sensitive for detection of H3 K27M mutant tumours, it is not specific. Other tumours, notably some posterior fossa ependymomas,13 will also show loss of H3 K27me3; in ependymomas this lack of immunoreactivity aligns with the posterior fossa group A (PFA) tumours.13,14 Similarly, in some H3-wildtype cases, partial loss may be seen. Thus, while helpful for confirmation when combined with an H3 K27M stain, loss of H3 K27me3 staining by itself should be considered a non-specific surrogate marker for identifying H3 K27M-mutant diffuse midline gliomas.  References  1 Khuong-Quang DA, Buczkowicz P, Rakopoulos P, Liu XY, Fontebasso AM, Bouffet E, Bartels U, Albrecht S, Schwartzentruber J, Letourneau L, Bourgey M, Bourque G, Montpetit A, Bourret G, Lepage P, Fleming A, Lichter P, Kool M, von Deimling A, Sturm D, Korshunov A, Faury D, Jones DT, Majewski J, Pfister SM, Jabado N and Hawkins C (2012). K27M mutation in histone H3.3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas. *Acta Neuropathol* 124(3):439-447.  2 Schwartzentruber J, Korshunov A, Liu XY, Jones DT, Pfaff E, Jacob K, Sturm D, Fontebasso AM, Quang DA, Tonjes M, Hovestadt V, Albrecht S, Kool M, Nantel A, Konermann C, Lindroth A, Jager N, Rausch T, Ryzhova M, Korbel JO, Hielscher T, Hauser P, Garami M, Klekner A, Bognar L, Ebinger M, Schuhmann MU, Scheurlen W, Pekrun A, Fruhwald MC, Roggendorf W, Kramm C, Durken M, Atkinson J, Lepage P, Montpetit A, Zakrzewska M, Zakrzewski K, Liberski PP, Dong Z, Siegel P, Kulozik AE, Zapatka M, Guha A, Malkin D, Felsberg J, Reifenberger G, von Deimling A, Ichimura K, Collins VP, Witt H, Milde T, Witt O, Zhang C, Castelo-Branco P, Lichter P, Faury D, Tabori U, Plass C, Majewski J, Pfister SM and Jabado N (2012). Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature* 482(7384):226-231.  3 Wu G, Broniscer A, McEachron TA, Lu C, Paugh BS, Becksfort J, Qu C, Ding L, Huether R, Parker M, Zhang J, Gajjar A, Dyer MA, Mullighan CG, Gilbertson RJ, Mardis ER, Wilson RK, Downing JR, Ellison DW, Zhang J and Baker SJ (2012). Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. *Nat Genet* 44(3):251-253.  4 Solomon DA, Wood MD, Tihan T, Bollen AW, Gupta N, Phillips JJ and Perry A (2016). Diffuse Midline Gliomas with Histone H3-K27M Mutation: A Series of 47 Cases Assessing the Spectrum of Morphologic Variation and Associated Genetic Alterations. *Brain Pathol* 26(5):569-580.  5 Kleinschmidt-DeMasters BK, Donson A, Foreman NK and Dorris K (2017). H3 K27M Mutation in Gangliogliomas can be Associated with Poor Prognosis. *Brain Pathol* 27(6):846-850.  6 Morita S, Nitta M, Muragaki Y, Komori T, Masui K, Maruyama T, Ichimura K, Nakano Y, Sawada T, Koriyama S, Tsuzuki S, Yasuda T, Hashimoto K, Niwa A and Kawamata T (2017). Brainstem pilocytic astrocytoma with H3 K27M mutation: case report. *J Neurosurg*:1-5.  7 Gessi M, Capper D, Sahm F, Huang K, von Deimling A, Tippelt S, Fleischhack G, Scherbaum D, Alfer J, Juhnke BO, von Hoff K, Rutkowski S, Warmuth-Metz M, Chavez L, Pfister SM, Pietsch T, Jones DT and Sturm D (2016). Evidence of H3 K27M mutations in posterior fossa ependymomas 132(4):635-637.  8 Venneti S, Santi M, Felicella MM, Yarilin D, Phillips JJ, Sullivan LM, Martinez D, Perry A, Lewis PW, Thompson CB and Judkins AR (2014). A sensitive and specific histopathologic prognostic marker for H3F3A K27M mutant pediatric glioblastomas. *Acta Neuropathol* 128(5):743-753.  9 Bechet D, Gielen GG, Korshunov A, Pfister SM, Rousso C, Faury D, Fiset PO, Benlimane N, Lewis PW, Lu C, David Allis C, Kieran MW, Ligon KL, Pietsch T, Ellezam B, Albrecht S and Jabado N (2014). Specific detection of methionine 27 mutation in histone 3 variants (H3K27M) in fixed tissue from high-grade astrocytomas. *Acta Neuropathol* 128(5):733-741.  10 Korshunov A, Capper D, Reuss D, Schrimpf D, Ryzhova M, Hovestadt V, Sturm D, Meyer J, Jones C, Zheludkova O, Kumirova E, Golanov A, Kool M, Schuller U, Mittelbronn M, Hasselblatt M, Schittenhelm J, Reifenberger G, Herold-Mende C, Lichter P, von Deimling A, Pfister SM and Jones DT (2016). Histologically distinct neuroepithelial tumors with histone 3 G34 mutation are molecularly similar and comprise a single nosologic entity. *Acta Neuropathol* 131(1):137-146.  11 Mackay A, Burford A, Carvalho D, Izquierdo E, Fazal-Salom J, Taylor KR, Bjerke L, Clarke M, Vinci M, Nandhabalan M, Temelso S, Popov S, Molinari V, Raman P, Waanders AJ, Han HJ, Gupta S, Marshall L, Zacharoulis S, Vaidya S, Mandeville HC, Bridges LR, Martin AJ, Al-Sarraj S, Chandler C, Ng HK, Li X, Mu K, Trabelsi S, Brahim DH, Kisljakov AN, Konovalov DM, Moore AS, Carcaboso AM, Sunol M, de Torres C, Cruz O, Mora J, Shats LI, Stavale JN, Bidinotto LT, Reis RM, Entz-Werle N, Farrell M, Cryan J, Crimmins D, Caird J, Pears J, Monje M, Debily MA, Castel D, Grill J, Hawkins C, Nikbakht H, Jabado N, Baker SJ, Pfister SM, Jones DTW, Fouladi M, von Bueren AO, Baudis M, Resnick A and Jones C (2017). Integrated Molecular Meta-Analysis of 1,000 Pediatric High-Grade and Diffuse Intrinsic Pontine Glioma. *Cancer Cell* 32(4):520-537.e525.  12 Haque F, Varlet P, Puntonet J, Storer L, Bountali A, Rahman R, Grill J, Carcaboso AM, Jones C, Layfield R and Grundy RG (2017). Evaluation of a novel antibody to define histone 3.3 G34R mutant brain tumours. *Acta Neuropathol Commun* 5(1):45.  13 Bayliss J, Mukherjee P, Lu C, Jain SU, Chung C, Martinez D, Sabari B, Margol AS, Panwalkar P, Parolia A, Pekmezci M, McEachin RC, Cieslik M, Tamrazi B, Garcia BA, La Rocca G, Santi M, Lewis PW, Hawkins C, Melnick A, David Allis C, Thompson CB, Chinnaiyan AM, Judkins AR and Venneti S (2016). Lowered H3K27me3 and DNA hypomethylation define poorly prognostic pediatric posterior fossa ependymomas. *Sci Transl Med* 8(366):366ra161.  14 Panwalkar P, Clark J, Ramaswamy V, Hawes D, Yang F, Dunham C, Yip S, Hukin J, Sun Y, Schipper MJ, Chavez L, Margol A, Pekmezci M, Chung C, Banda A, Bayliss JM, Curry SJ, Santi M, Rodriguez FJ, Snuderl M, Karajannis MA, Saratsis AM, Horbinski CM, Carret AS, Wilson B, Johnston D, Lafay-Cousin L, Zelcer S, Eisenstat D, Silva M, Scheinemann K, Jabado N, McNeely PD, Kool M, Pfister SM, Taylor MD, Hawkins C, Korshunov A, Judkins AR and Venneti S (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(5):705-714. |  |
| Non-core | *IDH1/IDH2* MUTATION | ***IDH1/IDH2* mutation**  Single selection value list:  • Cannot be determined  • Absent  • Present, *specify*  TESTING METHOD  (Multi-select - select all that apply)  • Sanger sequencing  • Next-generation sequencing  • PCR-based method  • Other, *specify*  **IDH1 R132H expression (immunohistochemistry)**  Single selection value list:  • Cannot be determined  • Negative  • Positive | ***IDH1/IDH2* Mutation and IDH1 R132H Expression (Immunohistochemistry)**  Isocitrate dehydrogenase (IDH) is an enzyme that exists in five isoforms, each of which catalyses the reaction of isocitrate to α-ketoglutarate.1 Mutations in *IDH1/IDH2* are frequent (greater than 80%) in WHO grades II and III astrocytomas but are found in only about 10% of the glioblastomas. Most glioblastomas that have progressed from lower-grade astrocytomas ('secondary glioblastomas) are IDH-mutant tumours.2 The finding of IDH mutations in an infiltrating astrocytoma is associated with better prognosis, grade for grade. The 2016 CNS WHO divides diffuse astrocytoma, anaplastic astrocytoma, and glioblastoma into classes that are IDH-mutant and IDH-wildtype. Oligodendrogliomas are now defined as diffuse gliomas with *IDH1/IDH2* mutations and whole arm deletions of chromosomes 1p and 19q. The mutant forms of *IDH1* and *IDH2* lead to the production of the oncometabolite 2-hydroxyglutarate, which inhibits the function of numerous α-ketoglutarate–dependent enzymes.3 Inhibition of the family of histone demethylases and the ten-eleven translocation (TET) family of 5-methylcytosine hydroxylases has profound effects on the epigenetic status of mutated cells and leads directly to a hypermethylator phenotype that has been referred to as the glioma CpG island methylator phenotype (G-CIMP).4  *IDH1* and *IDH2* mutations target the enzyme’s active site and result in a substitution for a key arginine at codons R132 and R172, respectively.2,5,6 The most frequent mutation, representing 92.7%, occurs at codon 132 of the IDH1 gene, and results in the substitution of arginine for histidine (R132H).5 Less frequent *IDH1* mutations include R132C (4.2%), R132S (1.5%), R132G (1.4%), and R132L (0.2%).5 Residue R172 in exon 4 of the *IDH2* gene is homologous to R132 in the *IDH1* gene, with R172K representing 64.5% of all *IDH2* mutations followed by R172M (19.3%), and R172W (16.2%).5 *IDH2* mutations are much less frequent than *IDH1* mutations among diffuse gliomas (approximately 3%), but are slightly more common in oligodendrogliomas than astrocytomas.5  A monoclonal antibody has been developed to the mutant IDH1 R132H protein, allowing its use in FFPE specimens (mIDH1 R132H).7 The ability of the antibody to detect a small number of cells as mutant makes this method more sensitive than sequencing for identifying R132H-mutant gliomas.8,9 However, mutations in *IDH2* and other *IDH1* mutations will not be detected using immunohistochemistry with this antibody, and in the proper clinical setting, it may be necessary to test for other *IDH1* or *IDH2* mutations by sequencing analysis. It has been suggested that sequencing may not be warranted in the setting of a negative R132H immunostain in glioblastomas arising in patients older than 55 years due to the rarity of non-R132H *IDH1* and *IDH2* mutations in patients in this age group.10,11 On the other hand, all diffusely infiltrating gliomas of WHO grade II and III that lack IDH1 R132H positivity by immunohistochemistry should be assessed for less common *IDH1* or *IDH2* mutations by sequencing or other appropriate methods.  References  1 Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, Olivi A, McLendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz LA, Jr., Hartigan J, Smith DR, Strausberg RL, Marie SK, Shinjo SM, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE and Kinzler KW (2008). An integrated genomic analysis of human glioblastoma multiforme. *Science* 321(5897):1807-1812.  2 Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, Kos I, Batinic-Haberle I, Jones S, Riggins GJ, Friedman H, Friedman A, Reardon D, Herndon J, Kinzler KW, Velculescu VE, Vogelstein B and Bigner DD (2009). IDH1 and IDH2 mutations in gliomas. *N Engl J Med* 360(8):765-773.  3 Turcan S, Rohle D, Goenka A, Walsh LA, Fang F, Yilmaz E, Campos C, Fabius AW, Lu C, Ward PS, Thompson CB, Kaufman A, Guryanova O, Levine R, Heguy A, Viale A, Morris LG, Huse JT, Mellinghoff IK and Chan TA (2012). IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. *Nature* 483(7390):479-483.  4 Noushmehr H, Weisenberger DJ, Diefes K, Phillips HS, Pujara K, Berman BP, Pan F, Pelloski CE, Sulman EP, Bhat KP, Verhaak RG, Hoadley KA, Hayes DN, Perou CM, Schmidt HK, Ding L, Wilson RK, Van Den Berg D, Shen H, Bengtsson H, Neuvial P, Cope LM, Buckley J, Herman JG, Baylin SB, Laird PW and Aldape K (2010). Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. *Cancer Cell* 17(5):510-522.  5 Hartmann C, Meyer J, Balss J, Capper D, Mueller W, Christians A, Felsberg J, Wolter M, Mawrin C, Wick W, Weller M, Herold-Mende C, Unterberg A, Jeuken JW, Wesseling P, Reifenberger G and von Deimling A (2009). Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1,010 diffuse gliomas. *Acta Neuropathol* 118(4):469-474.  6 Balss J, Meyer J, Mueller W, Korshunov A, Hartmann C and von Deimling A (2008). Analysis of the IDH1 codon 132 mutation in brain tumors. *Acta Neuropathol* 116(6):597-602.  7 Capper D, Weissert S, Balss J, Habel A, Meyer J, Jager D, Ackermann U, Tessmer C, Korshunov A, Zentgraf H, Hartmann C and von Deimling A (2010). Characterization of R132H mutation-specific IDH1 antibody binding in brain tumors. *Brain Pathol* 20(1):245-254.  8 Capper D, Zentgraf H, Balss J, Hartmann C and von Deimling A (2009). Monoclonal antibody specific for IDH1 R132H mutation. *Acta Neuropathol* 118(5):599-601.  9 Brandner S and von Deimling A (2015). Diagnostic, prognostic and predictive relevance of molecular markers in gliomas. *Neuropathol Appl Neurobiol* 41(6):694-720.  10 Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, Ohgaki H, Wiestler OD, Kleihues P and Ellison DW (2016). The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathologica* 131(6):803-820.  11 Chen L, Voronovich Z, Clark K, Hands I, Mannas J, Walsh M, Nikiforova MN, Durbin EB, Weiss H and Horbinski C (2014). Predicting the likelihood of an isocitrate dehydrogenase 1 or 2 mutation in diagnoses of infiltrative glioma. *Neuro Oncol* 16(11):1478-1483. |  |
| Non-core | Ki-67 IMMUNOHISTOCHEMISTRY | Numeric:  • Percentage of positive nuclei \_\_\_%  OR  • Cannot be determined | **Ki-67 immunohistochemistry1-4**  The protein detected by the Ki-67 antibody is a marker of cell proliferation that is present in the nucleus during all active phases of the cell cycle (G1, S, G2, M), but absent in resting cells (G0). In general, there is a progressive increase in Ki-67 labelling index associated with more aggressive behaviour of CNS tumours. Ki-67 immunohistochemistry can be useful for assessment of malignancy grade, especially when only small biopsies are available and for selection of areas for counting mitoses in large specimens. Moreover, Ki-67 labelling indices have been used to predict behaviour in lower-grade tumours such as WHO grade I meningiomas, pituitary adenomas, and WHO grade II oligodendrogliomas, among others—but are not universally used for these purposes.  Because of methodological variation, however, unequivocal Ki-67 labelling index cut-off levels for assigning WHO grade to CNS tumours are not available. For example, assessment of precise cut-off levels is difficult because of gradual increase in nuclear content of Ki-67 protein (marked increase in especially S phase of the cell cycle), staining of proliferating non-neoplastic cells in a tumour, considerable regional variation of the labelling index within a tumour, and substantial variability in staining results between institutions. In many centres, the MIB-1 antibody is used to determine the Ki-67 labelling index, one of its primary advantages over the original Ki-67 antibody being that it can be used on sections of FFPE tissue.  References  1 Louis DN, Ohgaki H, Wiestler OD and Cavenee WK (eds) (2016). *WHO Classification of Tumours of the Central Nervous System, Revised. Fourth Edition*, IARC, Lyon.  2 Bruno S and Darzynkiewicz Z (1992). Cell cycle dependent expression and stability of the nuclear protein detected by Ki-67 antibody in HL-60 cells. *Cell Prolif* 25(1):31-40.  3 Gerdes J, Schwab U, Lemke H and Stein H (1983). Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. *Int J Cancer* 31(1):13-20.  4 Darzynkiewicz Z, Zhao H, Zhang S, Lee MY, Lee EY and Zhang Z (2015). Initiation and termination of DNA replication during S phase in relation to cyclins D1, E and A, p21WAF1, Cdt1 and the p12 subunit of DNA polymerase delta revealed in individual cells by cytometry. *Oncotarget* 6(14):11735-11750. |  |
| Non-core | L1CAM EXPRESSION (IMMUNOHISTOCHEMISTRY) | Single selection value list:  • Cannot be determined  • Negative  • Positive | **L1CAM expression (immunohistochemistry)1,2**  Strong and diffuse cytoplasmic L1CAM (L1 Cell Adhesion Molecule) immunostaining of tumour cells is a sensitive surrogate marker for *RELA* fusion–positive ependymomas (see ***RELA* fusion**); these tumours are the majority of paediatric ependymomas in the supratentorial compartment, generally present in children, and carry a *C11orf95-RELA* fusion. However, L1CAM immunopositivity is not a specific marker as it can also be expressed by other types of tumours. Nonetheless, L1CAM immunohistochemistry is recommended for indicating that a supratentorial ependymoma likely belongs to the *RELA* fusion–positive category when *RELA* fusion testing is not possible or yields equivocal results.  References  1 Parker M, Mohankumar KM, Punchihewa C, Weinlich R, Dalton JD, Li Y, Lee R, Tatevossian RG, Phoenix TN, Thiruvenkatam R, White E, Tang B, Orisme W, Gupta K, Rusch M, Chen X, Li Y, Nagahawhatte P, Hedlund E, Finkelstein D, Wu G, Shurtleff S, Easton J, Boggs K, Yergeau D, Vadodaria B, Mulder HL, Becksfort J, Gupta P, Huether R, Ma J, Song G, Gajjar A, Merchant T, Boop F, Smith AA, Ding L, Lu C, Ochoa K, Zhao D, Fulton RS, Fulton LL, Mardis ER, Wilson RK, Downing JR, Green DR, Zhang J, Ellison DW and Gilbertson RJ (2014). C11orf95-RELA fusions drive oncogenic NF-kappaB signalling in ependymoma. *Nature* 506(7489):451-455.  2 Figarella-Branger D, Lechapt-Zalcman E, Tabouret E, Junger S, de Paula AM, Bouvier C, Colin C, Jouvet A, Forest F, Andreiuolo F, Quintin-Roue I, Machet MC, Heitzmann A, Milin S, Sevestre H, Godfraind C, Labrousse F, Metellus P, Scavarda D and Pietsch T (2016). Supratentorial clear cell ependymomas with branching capillaries demonstrate characteristic clinicopathological features and pathological activation of nuclear factor-kappaB signaling. *Neuro Oncol* 18(7):919-927. |  |
| Non-core | LIN28A EXPRESSION (IMMUNOHISTOCHEMISTRY) | Single selection value list:  • Cannot be determined  • Negative  • Positive | **LIN28A expression (immunohistochemistry)1-5**  Strong LIN28A cytoplasmic immunostaining of tumour cells is a highly sensitive marker for embryonal tumours with multilayered rosettes (ETMR), C19MC-altered (see **C19MC alteration**). However, LIN28A immunostaining is not specific to these tumours as it can also be present in medulloepitheliomas lacking the C19MC alteration, as well as in some gliomas, atypical teratoid/rhabdoid tumours (AT/RT), germ cell tumours, and non-CNS neoplasms. LIN28A immunohistochemistry is recommended as a surrogate marker for ETMR, C19MC-altered when testing for C19MC -alteration is not available. In these tumours, LIN28A immunoreactivity is generally prominent in multilayered rosettes, in poorly differentiated small-cell areas, and in the papillary and tubular structures of the medulloepithelioma pattern. Nonetheless, molecular testing for C19MC status is required for the diagnosis of ETMR, C19MC-altered. Therefore, although LIN28 immunopositivity is a useful surrogate marker for recognition of ETMR, C19MC-altered, when no C19MC testing is done or the results of such testing are inconclusive, an ETMR should be diagnosed as ETMR, NOS.  References  1 Korshunov A, Sturm D, Ryzhova M, Hovestadt V, Gessi M, Jones DT, Remke M, Northcott P, Perry A, Picard D, Rosenblum M, Antonelli M, Aronica E, Schuller U, Hasselblatt M, Woehrer A, Zheludkova O, Kumirova E, Puget S, Taylor MD, Giangaspero F, Peter Collins V, von Deimling A, Lichter P, Huang A, Pietsch T, Pfister SM and Kool M (2014). Embryonal tumor with abundant neuropil and true rosettes (ETANTR), ependymoblastoma, and medulloepithelioma share molecular similarity and comprise a single clinicopathological entity. *Acta Neuropathol* 128(2):279-289.  2 Korshunov A, Ryzhova M, Jones DT, Northcott PA, van Sluis P, Volckmann R, Koster J, Versteeg R, Cowdrey C, Perry A, Picard D, Rosenblum M, Giangaspero F, Aronica E, Schuller U, Hasselblatt M, Collins VP, von Deimling A, Lichter P, Huang A, Pfister SM and Kool M (2012). LIN28A immunoreactivity is a potent diagnostic marker of embryonal tumor with multilayered rosettes (ETMR). *Acta Neuropathol* 124(6):875-881.  3 Picard D, Miller S, Hawkins CE, Bouffet E, Rogers HA, Chan TS, Kim SK, Ra YS, Fangusaro J, Korshunov A, Toledano H, Nakamura H, Hayden JT, Chan J, Lafay-Cousin L, Hu P, Fan X, Muraszko KM, Pomeroy SL, Lau CC, Ng HK, Jones C, Van Meter T, Clifford SC, Eberhart C, Gajjar A, Pfister SM, Grundy RG and Huang A (2012). Markers of survival and metastatic potential in childhood CNS primitive neuro-ectodermal brain tumours: an integrative genomic analysis. *Lancet Oncol* 13(8):838-848.  4 Spence T, Sin-Chan P, Picard D, Barszczyk M, Hoss K, Lu M, Kim SK, Ra YS, Nakamura H, Fangusaro J, Hwang E, Kiehna E, Toledano H, Wang Y, Shi Q, Johnston D, Michaud J, La Spina M, Buccoliero AM, Adamek D, Camelo-Piragua S, Peter Collins V, Jones C, Kabbara N, Jurdi N, Varlet P, Perry A, Scharnhorst D, Fan X, Muraszko KM, Eberhart CG, Ng HK, Gururangan S, Van Meter T, Remke M, Lafay-Cousin L, Chan JA, Sirachainan N, Pomeroy SL, Clifford SC, Gajjar A, Shago M, Halliday W, Taylor MD, Grundy R, Lau CC, Phillips J, Bouffet E, Dirks PB, Hawkins CE and Huang A (2014). CNS-PNETs with C19MC amplification and/or LIN28 expression comprise a distinct histogenetic diagnostic and therapeutic entity. *Acta Neuropathol* 128(2):291-303.  5 Weingart MF, Roth JJ, Hutt-Cabezas M, Busse TM, Kaur H, Price A, Maynard R, Rubens J, Taylor I, Mao XG, Xu J, Kuwahara Y, Allen SJ, Erdreich-Epstein A, Weissman BE, Orr BA, Eberhart CG, Biegel JA and Raabe EH (2015). Disrupting LIN28 in atypical teratoid rhabdoid tumors reveals the importance of the mitogen activated protein kinase pathway as a therapeutic target. *Oncotarget* 6(5):3165-3177. |  |
| Non-core | MEDULLOBLASTOMA IMMUNOHISTOCHEMISTRY | Single selection value list:  **ß-catenin expression (immunohistochemistry)**  • Cannot be determined  • Absence of nuclear expression  • Positive nuclear expression  **GAB1 expression (immunohistochemistry)**  • Cannot be determined  • Negative  • Positive  **YAP1 expression (immunohistochemistry)**  • Cannot be determined  • Negative  • Positive | 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 -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).  **β-catenin Nuclear Expression (Immunohistochemistry)**  Upon WNT activation, -catenin, encoded by the *CTNNB1* gene, translocates to the nucleus, where it interacts with transcription factors. Thus, nuclear -catenin immunopositivity reflects activation of the WNT signalling pathway.  In the clinically relevant WNT-activated group of medulloblastoma, immunohistochemistry for -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).  **Immunohistochemistry with antibodies to β-catenin, GAB1, and YAP1 in the determination of medulloblastoma molecular groups**  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.    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. |  |
| Non-core | *MGMT* PROMOTER METHYLATION | Single selection value list:  • Cannot be determined  • Absent  • Present  TESTING METHOD  (Multi-select - select all that apply)  • Methylation-specific PCR  • Other, *specify* | O6 methylguanine-DNA methyl transferase (MGMT) is a DNA repair protein that facilitates repair of DNA damage induced by chemotherapeutic alkylating agents, and has therefore been associated with chemoresistance.1 Epigenetic silencing of the *MGMT* gene by promoter methylation plays an important role in regulating MGMT expression in gliomas.2-4 *MGMT* promoter methylation has been reported as a predictive marker for temozolomide sensitivity in clinical trials.4-6 Promoter methylation correlates with better progression-free and overall survival in IDH-wildtype glioblastoma patients treated with temozolomide. In IDH-mutant anaplastic (WHO grade III) gliomas, MGMT status is a prognostic factor irrespective of treatment but is not predictive for outcome to alkylating chemotherapy versus radiotherapy.7,8 The impact of *MGMT* promoter methylation on clinical care is still being established.  The optimal method to carry out MGMT analysis and interpretation of the results has yet to be determined. Pyrosequencing is a commonly used method9-11 that has proved to be reproducible between different laboratories.9-12 Methylation-specific PCR is semi-quantitative and has also been widely used including in pivotal clinical trials,4,5 but may not be as reproducible as pyrosequencing.11  References  1 Esteller M, Garcia-Foncillas J, Andion E, Goodman SN, Hidalgo OF, Vanaclocha V, Baylin SB and Herman JG (2000). Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. *N Engl J Med* 343(19):1350-1354.  2 Esteller M, Hamilton SR, Burger PC, Baylin SB and Herman JG (1999). Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. *Cancer Res* 59(4):793-797.  3 Brennan CW, Verhaak RG, McKenna A, Campos B, Noushmehr H, Salama SR, Zheng S, Chakravarty D, Sanborn JZ, Berman SH, Beroukhim R, Bernard B, Wu CJ, Genovese G, Shmulevich I, Barnholtz-Sloan J, Zou L, Vegesna R, Shukla SA, Ciriello G, Yung WK, Zhang W, Sougnez C, Mikkelsen T, Aldape K, Bigner DD, Van Meir EG, Prados M, Sloan A, Black KL, Eschbacher J, Finocchiaro G, Friedman W, Andrews DW, Guha A, Iacocca M, O'Neill BP, Foltz G, Myers J, Weisenberger DJ, Penny R, Kucherlapati R, Perou CM, Hayes DN, Gibbs R, Marra M, Mills GB, Lander E, Spellman P, Wilson R, Sander C, Weinstein J, Meyerson M, Gabriel S, Laird PW, Haussler D, Getz G and Chin L (2013). The somatic genomic landscape of glioblastoma. *Cell* 155(2):462-477.  4 Hegi ME, Diserens AC, Gorlia T, Hamou MF, De Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L, Bromberg JE, Hau P, Mirimanoff RO, Cairncross JG, Janzer RC and Stupp R (2005). MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* 352:997-1003.  5 Malmstrom A, Gronberg BH, Marosi C, Stupp R, Frappaz D, Schultz H, Abacioglu U, Tavelin B, Lhermitte B, Hegi ME, Rosell J and Henriksson R (2012). Temozolomide versus standard 6-week radiotherapy versus hypofractionated radiotherapy in patients older than 60 years with glioblastoma: the Nordic randomised, phase 3 trial. *Lancet Oncol* 13(9):916-926.  6 Wick W, Platten M, Meisner C, Felsberg J, Tabatabai G, Simon M, Nikkhah G, Papsdorf K, Steinbach JP, Sabel M, Combs SE, Vesper J, Braun C, Meixensberger J, Ketter R, Mayer-Steinacker R, Reifenberger G and Weller M (2012). Temozolomide chemotherapy alone versus radiotherapy alone for malignant astrocytoma in the elderly: the NOA-08 randomised, phase 3 trial. *Lancet Oncol* 13(7):707-715.  7 Wick W, Meisner C, Hentschel B, Platten M, Schilling A, Wiestler B, Sabel MC, Koeppen S, Ketter R, Weiler M, Tabatabai G, von Deimling A, Gramatzki D, Westphal M, Schackert G, Loeffler M, Simon M, Reifenberger G and Weller M (2013). Prognostic or predictive value of MGMT promoter methylation in gliomas depends on IDH1 mutation. *Neurology* 81(17):1515-1522.  8 Wick W, Hartmann C, Engel C, Stoffels M, Felsberg J, Stockhammer F, Sabel MC, Koeppen S, Ketter R, Meyermann R, Rapp M, Meisner C, Kortmann RD, Pietsch T, Wiestler OD, Ernemann U, Bamberg M, Reifenberger G, von Deimling A and Weller M (2009). NOA-04 randomized phase III trial of sequential radiochemotherapy of anaplastic glioma with procarbazine, lomustine, and vincristine or temozolomide. *J Clin Oncol* 27(35):5874-5880.  9 Wick W, Weller M, van den Bent M, Sanson M, Weiler M, von Deimling A, Plass C, Hegi M, Platten M and Reifenberger G (2014). MGMT testing--the challenges for biomarker-based glioma treatment. *Nat Rev Neurol* 10(7):372-385.  10 Zhao H, Wang S, Song C, Zha Y and Li L (2016). The prognostic value of MGMT promoter status by pyrosequencing assay for glioblastoma patients' survival: a meta-analysis. *World J Surg Oncol* 14(1):261.  11 Bienkowski M, Berghoff AS, Marosi C, Wohrer A, Heinzl H, Hainfellner JA and Preusser M (2015). Clinical Neuropathology practice guide 5-2015: MGMT methylation pyrosequencing in glioblastoma: unresolved issues and open questions. *Clin Neuropathol* 34(5):250-257.  12 Quillien V, Lavenu A, Ducray F, Joly MO, Chinot O, Fina F, Sanson M, Carpentier C, Karayan-Tapon L, Rivet P, Entz-Werle N, Legrain M, Zalcman EL, Levallet G, Escande F, Ramirez C, Chiforeanu D, Vauleon E and Figarella-Branger D (2016). Validation of the high-performance of pyrosequencing for clinical MGMT testing on a cohort of glioblastoma patients from a prospective dedicated multicentric trial. *Oncotarget* 7(38):61916-61929. |  |
| Non-core | MONOSOMY 6 | Single selection value list:  • Cannot be determined  • Absent  • Present, *specify*  TESTING METHOD  (Multi-select - select all that apply)  • In situ hybridization  • Multiplex ligation-dependend probe amplification (MLPA)  • Array-based method  • Microsatellite analysis | Monosomy 6 is a chromosomal alteration present in approximately 85% of WNT-activated medulloblastomas. Its detection, together with the presence of -catenin nuclear immunoreactivity and/or *CTNNB1* mutation, facilitates identification of this prognostically favourable molecular group.  Monosomy 6 can be detected by array CGH or microsatellite analysis using fresh-frozen material. MLPA (with probes covering the short and long arms of chromosome 6) can be a robust method to analyze even small amounts of FFPE-derived degraded DNA. Detection of monosomy 6 can also be undertaken by interphase FISH.  References  1 Clifford SC, Lusher ME, Lindsey JC, Langdon JA, Gilbertson RJ, Straughton D and Ellison DW (2006). Wnt/Wingless pathway activation and chromosome 6 loss characterize a distinct molecular sub-group of medulloblastomas associated with a favorable prognosis. *Cell Cycle* 5(22):2666-2670.  2 Goschzik T, Zur Muhlen A, Kristiansen G, Haberler C, Stefanits H, Friedrich C, von Hoff K, Rutkowski S, Pfister SM and Pietsch T (2015). Molecular stratification of medulloblastoma: comparison of histological and genetic methods to detect Wnt activated tumours. *Neuropathol Appl Neurobiol* 41(2):135-144. |  |
| Non-core | MYC GENE FAMILY AMPLIFICATION (*MYC* and/or *MYCN*) | Single selection value list:  • Cannot be determined  • Absent  • Absent with low level gain  • Present, *specify, including copy number*  TESTING METHOD  (Multi-select - select all that apply)  • In situ hybridization (FISH, CISH)  • Array-based method  • Next-generation sequencing  • Other, *specify* | The c-Myc protein (MYC) has a fundamental role in cell proliferation, cell size, differentiation, stem cell self-renewal, and apoptosis. Its deregulation occurs in many cancers including a range of brain tumours. The MYC transcription factor family also includes its paralogues MYCN and MYCL.1 *MYC*, *MYCN,* and *MYCL* amplifications are prognostically relevant in medulloblastomas.2 *MYC* and *MYCN* gene amplification and fusions are seen in the SHH group, and non-WNT/non-SHH, but almost never in WNT-activated medulloblastomas.2,3  A commonly used laboratory method to detect MYC gene family amplifications is *in situ* hybridisation, either using FISH or CISH.4 Other approaches include PCR-based methods such as real-time PCR, NGS, MLPA, or array technologies.5,6,7  References  1 Dang CV (2013). MYC, metabolism, cell growth, and tumorigenesis. *Cold Spring Harb Perspect Med* 3(8).  2 Northcott PA, Shih DJ, Peacock J, Garzia L, Morrissy AS, Zichner T, Stutz AM, Korshunov A, Reimand J, Schumacher SE, Beroukhim R, Ellison DW, Marshall CR, Lionel AC, Mack S, Dubuc A, Yao Y, Ramaswamy V, Luu B, Rolider A, Cavalli FM, Wang X, Remke M, Wu X, Chiu RY, Chu A, Chuah E, Corbett RD, Hoad GR, Jackman SD, Li Y, Lo A, Mungall KL, Nip KM, Qian JQ, Raymond AG, Thiessen NT, Varhol RJ, Birol I, Moore RA, Mungall AJ, Holt R, Kawauchi D, Roussel MF, Kool M, Jones DT, Witt H, Fernandez LA, Kenney AM, Wechsler-Reya RJ, Dirks P, Aviv T, Grajkowska WA, Perek-Polnik M, Haberler CC, Delattre O, Reynaud SS, Doz FF, Pernet-Fattet SS, Cho BK, Kim SK, Wang KC, Scheurlen W, Eberhart CG, Fevre-Montange M, Jouvet A, Pollack IF, Fan X, Muraszko KM, Gillespie GY, Di Rocco C, Massimi L, Michiels EM, Kloosterhof NK, French PJ, Kros JM, Olson JM, Ellenbogen RG, Zitterbart K, Kren L, Thompson RC, Cooper MK, Lach B, McLendon RE, Bigner DD, Fontebasso A, Albrecht S, Jabado N, Lindsey JC, Bailey S, Gupta N, Weiss WA, Bognar L, Klekner A, Van Meter TE, Kumabe T, Tominaga T, Elbabaa SK, Leonard JR, Rubin JB, Liau LM, Van Meir EG, Fouladi M, Nakamura H, Cinalli G, Garami M, Hauser P, Saad AG, Iolascon A, Jung S, Carlotti CG, Vibhakar R, Ra YS, Robinson S, Zollo M, Faria CC, Chan JA, Levy ML, Sorensen PH, Meyerson M, Pomeroy SL, Cho YJ, Bader GD, Tabori U, Hawkins CE, Bouffet E, Scherer SW, Rutka JT, Malkin D, Clifford SC, Jones SJ, Korbel JO, Pfister SM, Marra MA and Taylor MD (2012). Subgroup-specific structural variation across 1,000 medulloblastoma genomes. *Nature* 488(7409):49-56.  3 Roussel MF and Robinson GW (2013). Role of MYC in Medulloblastoma. *Cold Spring Harb Perspect Med* 3(11).  4 Fernandez AP, Sun Y, Tubbs RR, Goldblum JR and Billings SD (2012). FISH for MYC amplification and anti-MYC immunohistochemistry: useful diagnostic tools in the assessment of secondary angiosarcoma and atypical vascular proliferations. *J Cutan Pathol* 39(2):234-242.  5 Iwakawa R, Kohno T, Kato M, Shiraishi K, Tsuta K, Noguchi M, Ogawa S and Yokota J (2011). MYC amplification as a prognostic marker of early-stage lung adenocarcinoma identified by whole genome copy number analysis. *Clin Cancer Res* 17(6):1481-1489.  6 Mehrotra M, Luthra R, Abraham R, Mishra BM, Virani S, Chen H, Routbort MJ, Patel KP, Medeiros LJ and Singh RR (2017). Validation of quantitative PCR-based assays for detection of gene copy number aberrations in formalin-fixed, paraffin embedded solid tumor samples. *Cancer Genet* 212-213:24-31.  7 Verschuur-Maes AH, Moelans CB, de Bruin PC and van Diest PJ (2014). Analysis of gene copy number alterations by multiplex ligation-dependent probe amplification in columnar cell lesions of the breast. *Cell Oncol (Dordr)* 37(2):147-154. |  |
| Non-core | *NAB2-STAT6* FUSION | ***NAB2-STAT6* fusion**  Single selection value list:  • Cannot be determined  • Negative  • Positive  TESTING METHOD  (Multi-select - select all that apply)  • FISH  • Next generation sequencing  • Other, *specify*  **STAT6 expression (immunohistochemistry)**  Single selection value list:  • Cannot be determined  • Absence of nuclear expression  • Positive nuclear expression | In-frame *NAB2-STAT6* gene fusions result from chromosome 12q13 inversions and represent highly sensitive and specific signature alterations of meningeal solitary fibrous tumour/haemangiopericytoma (SFT/HPC) of grade 1, 2, or 3; these fusions are also characteristic of the analogous soft tissue/extracranial counterparts, which are referred to as SFT or malignant SFT. Given the relative ease of detecting this genetic alteration using a STAT6 immunohistochemical surrogate (see **STAT6 expression (immunohistochemistry)**), diagnostic confirmation is highly recommended in the WHO 2016 classification scheme before a diagnosis of SFT/HPC is rendered.1,2  ***NAB2-STAT6* Gene Fusion**  *NAB2-STAT6* gene fusions are detectable using RT-PCR or various other sequencing techniques, including NGS if designed appropriately.3,4 Over 40 fusion variants have been detected to date, with the most common meningeal SFT/HPC subtypes fusing exon 6 of *NAB2* with exons 16, 17, or 18 of *STAT6* (roughly one-half of all cases).4 Preliminary data also suggests that the *NAB2* exon 4-*STAT6* exon 2/3 fusions are more common in the lower grade and clinically less aggressive SFT/HPC, though larger studies are needed for further validation.4,5  **STAT6 Nuclear Expression (Immunohistochemistry)**  The STAT6 protein is normally expressed in the cytoplasm of cells, whereas NAB2 is expressed in nuclei; however, the *NAB2-STAT6* fusions cause the STAT6 protein to translocate to the nucleus. As such, STAT6 immunohistochemistry represents a highly reliable and practical surrogate for detecting this signature alteration, with nearly 100% sensitivity and specificity regardless of the fusion variant.3,6 Nearly all meningeal SFT/HPC and extracranial SFTs display strong and extensive/diffuse nuclear positivity, whereas other diagnostic considerations, such as meningiomas, nerve sheath tumours, and various sarcomas, either lack expression or show only cytoplasmic staining. As such, the pathologist is cautioned against rendering a diagnosis of SFT/HPC in the absence of nuclear STAT6 immunoreactivity.  References  1 Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, Ohgaki H, Wiestler OD, Kleihues P and Ellison DW (2016). The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathologica* 131(6):803-820.  2 Louis DN, Ohgaki H, Wiestler OD and Cavenee WK (eds) (2016). *WHO Classification of Tumours of the Central Nervous System, Revised. Fourth Edition*, IARC, Lyon.  3 Schweizer L, Koelsche C, Sahm F, Piro RM, Capper D, Reuss DE, Pusch S, Habel A, Meyer J, Gock T, Jones DT, Mawrin C, Schittenhelm J, Becker A, Heim S, Simon M, Herold-Mende C, Mechtersheimer G, Paulus W, Konig R, Wiestler OD, Pfister SM and von Deimling A (2013). Meningeal hemangiopericytoma and solitary fibrous tumors carry the NAB2-STAT6 fusion and can be diagnosed by nuclear expression of STAT6 protein. *Acta Neuropathol* 125(5):651-658.  4 Nakada S, Minato H and Nojima T (2016). Clinicopathological differences between variants of the NAB2-STAT6 fusion gene in solitary fibrous tumors of the meninges and extra-central nervous system. *Brain Tumor Pathol* 33(3):169-174.  5 Fritchie KJ, Jin L, Rubin BP, Burger PC, Jenkins SM, Barthelmess S, Moskalev EA, Haller F, Oliveira AM and Giannini C (2016). NAB2-STAT6 Gene Fusion in Meningeal Hemangiopericytoma and Solitary Fibrous Tumor. *J Neuropathol Exp Neurol* 75(3):263-271.  6 Koelsche C, Schweizer L, Renner M, Warth A, Jones DT, Sahm F, Reuss DE, Capper D, Knosel T, Schulz B, Petersen I, Ulrich A, Renker EK, Lehner B, Pfister SM, Schirmacher P, von Deimling A and Mechtersheimer G (2014). Nuclear relocation of STAT6 reliably predicts NAB2-STAT6 fusion for the diagnosis of solitary fibrous tumour. *Histopathology* 65(5):613-622. |  |
| Non-core | PITUITARY HORMONES AND TRANSCRIPTION FACTORS IMMUNOHISTOCHEMISTRY | Tumour cells are reactive for  • Cannot be determined  OR  (Multi-select - select all that apply)  • Prolactin  • Human growth hormone  • ß-TSH  • ß-FSH  • ß-LH  • Alpha subunit  • ACTH  • PIT1  • TPIT  • SF1  • Other, *specify* | Standard immunohistochemical evaluation of pituitary adenomas includes specific anterior pituitary hormones (prolactin, growth hormone, follicle stimulating hormone, luteinizing hormone, thyroid stimulating hormone, adrenocorticotrophic hormone (PRL, GH, FSH, LH, TSH, ACTH, respectively) and/or pituitary transcription factors (PIT1, TPIT, steroidogenic factor 1/SF1).2 Immunohistochemistry for these proteins, coupled with keratin (AE1/AE3 or CAM5.2) staining, for presence or absence of rounded cytoplasmic inclusions known as fibrous bodies, allows classification of adenomas for prognosis and medical treatment purposes. Antibodies directed against the pituitary transcription factor for corticotroph lineage adenoma (TPIT) are not as widely available as the other antibodies listed above.  For diagnostic purposes, some advocate first screening with three antibodies (PIT1, SF1, and ACTH) and then using the other anterior pituitary hormone assays based on initial results.3 Others utilise the full panel initially and may variably supplement the panel with additional reticulin histochemical stain and/or a cell cycle labelling marker (MIB1). There appears to be little, if any, utility for p53 immunohistochemistry.  The new WHO 2017 Classification system4 notes that: “Special adenoma subtypes that commonly show aggressive behaviour…include sparsely granulated somatotroph adenoma, lactotroph adenomas in men, Crooke cell adenoma and silent corticotroph adenoma, and plurihormonal PIT1-positive adenoma (previously called "silent subtype 3 adenoma").  For tumours of the posterior pituitary gland (granular cell tumour of the sellar region, pituicytoma, spindle cell oncocytoma), nuclear staining for the transcription factor TTF-1 is diagnostic.4  References  1 Lopes MBS (2017). The 2017 World Health Organization classification of tumors of the pituitary gland: a summary. *Acta Neuropathol*.  2 Nose V, Ezzat S, Horvath E, Kovacs K, Laws ER, Lloyd R, Lopes MB and Asa SL (2011). Protocol for the examination of specimens from patients with primary pituitary tumors. *Arch Pathol Lab Med* 135(5):640-646.  3 McDonald WC, Banerji N, McDonald KN, Ho B, Macias V and Kajdacsy-Balla A (2017). Steroidogenic Factor 1, Pit-1, and Adrenocorticotropic Hormone: A Rational Starting Place for the Immunohistochemical Characterization of Pituitary Adenoma. *Arch Pathol Lab Med* 141(1):104-112.  4 Louis DN, Ohgaki H, Wiestler OD and Cavenee WK (eds) (2016). *WHO Classification of Tumours of the Central Nervous System, Revised. Fourth Edition*, IARC, Lyon. |  |
| Non-core | *RELA* FUSION | Single selection value list:  • Cannot be determined  • Negative  • Positive  TESTING METHOD  (Multi-select - select all that apply)  • FISH  • Next generation sequencing  • Other, *specify* | Approximately two-thirds of supratentorial ependymomas in children are characterised by fusions between *C11orf95* and the *RELA* genes.1,2 Detection of these fusions is essential for making the diagnosis of ependymoma, *RELA* fusion positive. These fusions can be identified clinically using RNA sequencing, RT-PCR based techniques, or FISH; whole genome sequencing can also detect the fusion. Targeted RNA sequencing and RT-PCR design should take into consideration the complex nature of the fusion events generated by chromothripsis on chromosome 11. FISH probes overlying either *RELA* or *C11orf95* may be used to detect the rearrangements on chromosome 11.1 These are designed using a break-apart strategy with red and green probes lying close to one another and producing a yellow signal in the wildtype situation; rearrangements will result in distancing of the probes from one another and distinct red and green signals. There are correlations between the presence of L1CAM positivity and RELA fusion in this type of this tumour (see **L1CAM expression (immunohistochemistry)**). There may also be other surrogate markers for *RELA* fusion–positive tumours and therefore other validated equivalents can be used to guide diagnosis; however, to date none of these is specific for *RELA* fusion as defined by FISH or sequencing.  **References**  1 Parker M, Mohankumar KM, Punchihewa C, Weinlich R, Dalton JD, Li Y, Lee R, Tatevossian RG, Phoenix TN, Thiruvenkatam R, White E, Tang B, Orisme W, Gupta K, Rusch M, Chen X, Li Y, Nagahawhatte P, Hedlund E, Finkelstein D, Wu G, Shurtleff S, Easton J, Boggs K, Yergeau D, Vadodaria B, Mulder HL, Becksfort J, Gupta P, Huether R, Ma J, Song G, Gajjar A, Merchant T, Boop F, Smith AA, Ding L, Lu C, Ochoa K, Zhao D, Fulton RS, Fulton LL, Mardis ER, Wilson RK, Downing JR, Green DR, Zhang J, Ellison DW and Gilbertson RJ (2014). C11orf95-RELA fusions drive oncogenic NF-kappaB signalling in ependymoma. *Nature* 506(7489):451-455.  2 Pajtler KW, Witt H, Sill M, Jones DT, Hovestadt V, Kratochwil F, Wani K, Tatevossian R, Punchihewa C, Johann P, Reimand J, Warnatz HJ, Ryzhova M, Mack S, Ramaswamy V, Capper D, Schweizer L, Sieber L, Wittmann A, Huang Z, van Sluis P, Volckmann R, Koster J, Versteeg R, Fults D, Toledano H, Avigad S, Hoffman LM, Donson AM, Foreman N, Hewer E, Zitterbart K, Gilbert M, Armstrong TS, Gupta N, Allen JC, Karajannis MA, Zagzag D, Hasselblatt M, Kulozik AE, Witt O, Collins VP, von Hoff K, Rutkowski S, Pietsch T, Bader G, Yaspo ML, von Deimling A, Lichter P, Taylor MD, Gilbertson R, Ellison DW, Aldape K, Korshunov A, Kool M and Pfister SM (2015). Molecular Classification of Ependymal Tumors across All CNS Compartments, Histopathological Grades, and Age Groups. *Cancer Cell* 27(5):728-743. |  |
| Non-core | ***SMARCA4/BRG1* ALTERATION** | ***SMARCA4/BRG1* mutation**  Single selection value list:  • Cannot be determined  • Absent  • Present, *specify*  TESTING METHOD  (Multi-select - select all that apply)  • Sanger sequencing  • Next-generation sequencing  • PCR-based method  • Other, *specify*  **BRG1 loss of expression (immunohistochemistry)**  • Cannot be determined  • Intact nuclear expression  • Loss of nuclear expression | AT/RT is defined as a CNS embryonal tumour that frequently (but not invariably) contains rhabdoid cells and demonstrates inactivation of *SMARCB1* (INI1) or *SMARCA4* (BRG1). AT/RTs with *SMARCA4* loss are extremely rare, but loss of BRG1 expression (and retention of INI1 expression) in these tumours can be readily demonstrated by immunohistochemistry.1 Associated genetic alterations of *SMARCA4*, whether copy number alterations or mutations, can be detected by a variety of array or sequencing methods.  References  1 Hasselblatt M, Gesk S, Oyen F, Rossi S, Viscardi E, Giangaspero F, Giannini C, Judkins AR, Fruhwald MC, Obser T, Schneppenheim R, Siebert R and Paulus W (2011). Nonsense mutation and inactivation of SMARCA4 (BRG1) in an atypical teratoid/rhabdoid |  |
| Non-core | ***SMARCB1/INI1/HSNF5* ALTERATION** | ***SMARCB1/INI1/HSNF5* mutation**  Single selection value list:  • Cannot be determined  • Absent  • Present, *specify*  TESTING METHOD  (Multi-select - select all that apply)  • Sanger sequencing  • Next-generation sequencing  • PCR-based method  • Other, *specify*  **INI1 (BAF47) loss of expression (immunohistochemistry)**  • Cannot be determined  • Intact nuclear expression  • Loss of nuclear expression | Inactivation of the *SMARCB1* (*INI1*, *BAF47*, *SNF5*) gene is present in almost all cases of AT/RT, resulting in nuclear loss of SMARCB1 protein which can be evaluated immunohistochemically. Genetic aberrations of the *SMARCB1* locus may include homozygous or heterozygous deletions and a variety of coding sequence mutations, leading to inactivation of both alleles. However, genetic testing is usually not required for making the diagnosis of AT/RT because immunohistochemistry is highly sensitive. SMARCB1 is a constitutively expressed protein, and therefore immunohistochemical staining for SMARCB1 is present in nuclei of non-neoplastic cells, such as vascular cells, residual brain cells, or inflammatory infiltrates, serving as internal positive control for neoplasms that have lost tumour cell staining. Some AT/RTs with nuclear loss of SMARCB1 exhibit cytoplasmic staining, possibly representing dysfunctional truncated protein. In tumours with histological features of AT/RTs but without demonstration of SMARCB1 inactivation (and without SMARCA4 inactivation), only a diagnosis of "CNS embryonal tumour with rhabdoid features" can be made.  A variety of other tumour types that may involve the nervous system exhibit loss of nuclear SMARCB1, including cribriform neuroepithelial tumour,1 poorly differentiated chordoma,2 rhabdoid tumour of the sellar region,3 myxoid meningeal tumours,4 and sinonasal carcinoma1,2,3,4,5 The molecular and nosologic relationship of these tumours to AT/RT is unclear to date. Furthermore, complete or incomplete (reduced, mosaic) loss of SMARCB1 protein has been found in some cases of choroid plexus carcinoma, synovial sarcoma, epithelioid schwannoma, and schwannoma associated with schwannomatosis.6  References  1 Johann PD, Hovestadt V, Thomas C, Jeibmann A, Hess K, Bens S, Oyen F, Hawkins C, Pierson CR, Aldape K, Kim SP, Widing E, Sumerauer D, Hauser P, van Landeghem F, Ryzhova M, Korshunov A, Capper D, Jones DTW, Pfister SM, Schneppenheim R, Siebert R, Paulus W, Fruhwald MC, Kool M and Hasselblatt M (2017). Cribriform neuroepithelial tumor: molecular characterization of a SMARCB1-deficient non-rhabdoid tumor with favorable long-term outcome. *Brain Pathol* 27(4):411-418.  2 Hasselblatt M, Thomas C, Hovestadt V, Schrimpf D, Johann P, Bens S, Oyen F, Peetz-Dienhart S, Crede Y, Wefers A, Vogel H, Riemenschneider MJ, Antonelli M, Giangaspero F, Bernardo MC, Giannini C, Ud Din N, Perry A, Keyvani K, van Landeghem F, Sumerauer D, Hauser P, Capper D, Korshunov A, Jones DT, Pfister SM, Schneppenheim R, Siebert R, Fruhwald MC and Kool M (2016). Poorly differentiated chordoma with SMARCB1/INI1 loss: a distinct molecular entity with dismal prognosis. *Acta Neuropathol* 132(1):149-151.  3 Nakata S, Nobusawa S, Hirose T, Ito S, Inoshita N, Ichi S, Amatya VJ, Takeshima Y, Sugiyama K, Sonoda Y, Haga H, Hirato J, Nakazato Y and Yokoo H (2017). Sellar Atypical Teratoid/Rhabdoid Tumor (AT/RT): A Clinicopathologically and Genetically Distinct Variant of AT/RT. *Am J Surg Pathol* 41(7):932-940.  4 Dadone B, Fontaine D, Mondot L, Cristofari G, Jouvet A, Godfraind C, Varlet P, Ranchere-Vince D, Coindre JM, Gastaud L, Baudoin C, Peyron AC, Thyss A, Coutts M, Michiels JF, Pedeutour F and Burel-Vandenbos F (2017). Meningeal SWI/SNF related, matrix-associated, actin-dependent regulator of chromatin, subfamily B member 1 (SMARCB1)-deficient tumours: an emerging group of meningeal tumours. *Neuropathol Appl Neurobiol* 43(5):433-449.  5 Agaimy A, Hartmann A, Antonescu CR, Chiosea SI, El-Mofty SK, Geddert H, Iro H, Lewis JS, Jr., Markl B, Mills SE, Riener MO, Robertson T, Sandison A, Semrau S, Simpson RH, Stelow E, Westra WH and Bishop JA (2017). SMARCB1 (INI-1)-deficient Sinonasal Carcinoma: A Series of 39 Cases Expanding the Morphologic and Clinicopathologic Spectrum of a Recently Described Entity. *Am J Surg Pathol* 41(4):458-471.  6 Caltabiano R, Magro G, Polizzi A, Pratico AD, Ortensi A, D'Orazi V, Panunzi A, Milone P, Maiolino L, Nicita F, Capone GL, Sestini R, Paganini I, Muglia M, Cavallaro S, Lanzafame S, Papi L and Ruggieri M (2017). A mosaic pattern of INI1/SMARCB1 protein expression distinguishes Schwannomatosis and NF2-associated peripheral schwannomas from solitary peripheral schwannomas and NF2-associated vestibular schwannomas. *Childs Nerv Syst* 33(6):933-940. |  |
| Non-core | *TERT* PROMOTER MUTATION | Single selection value list:  • Cannot be determined  • Absent  • Hotspot mutation (C228T or C250T)  • Other mutation, *specify*  TESTING METHOD  (Multi-select - select all that apply)  • Sanger sequencing  • Next-generation sequencing  • PCR-based method  • Other, *specify* | The *TERT* gene encodes telomerase reverse transcriptase, which is a major component of the protein complex telomerase and contributes to maintain telomere length. *TERT* promoter mutations create new binding sites for ETS transcription factors and subsequently increase expression and activity of telomerase. ***TERT*** promoter mutations occur in 55–80% of glioblastomas (far more commonly in IDH-wildtype glioblastomas), 70–80% of oligodendrogliomas, and 10–35% of diffuse astrocytomas.1,2 They provide independent prognostic information for diffuse gliomas. Thus, in oligodendroglioma, IDH-mutant and 1p/19q-codeleted, *TERT*-mutant tumours are associated with better prognosis than *TERT*-wildtype tumours, while in diffuse astrocytoma, IDH-wildtype, *TERT*-mutant tumours are associated with worse prognosis than *TERT*-wildtype tumours.3-5 About 20% of medulloblastomas carry *TERT* promoter mutations, and they are more common in adult patients and in the SHH-activated molecular type.1 In meningiomas, *TERT* promoter mutations have been found in 6% of tumours where they represent a marker of poor prognosis independent of WHO grading.6 About 50% of solitary fibrous tumours/hemangiopericytomas carry *TERT* promoter mutation while other tumours of the CNS only uncommonly exhibit these mutations.1  Two hotspot missense mutations (abbreviated as C228T and C250T) represent the vast majority of *TERT* promoter mutations. Other mutations have been rarely detected in brain tumours, such as C228A and C249T in gliomas.1 Mutations can be detected by Sanger sequencing or by NGS.  References  1 Koelsche C, Sahm F, Capper D, Reuss D, Sturm D, Jones DT, Kool M, Northcott PA, Wiestler B, Bohmer K, Meyer J, Mawrin C, Hartmann C, Mittelbronn M, Platten M, Brokinkel B, Seiz M, Herold-Mende C, Unterberg A, Schittenhelm J, Weller M, Pfister S, Wick W, Korshunov A and von Deimling A (2013). Distribution of TERT promoter mutations in pediatric and adult tumors of the nervous system. *Acta Neuropathol* 126(6):907-915.  2 Arita H, Narita Y, Takami H, Fukushima S, Matsushita Y, Yoshida A, Miyakita Y, Ohno M, Shibui S and Ichimura K (2013). TERT promoter mutations rather than methylation are the main mechanism for TERT upregulation in adult gliomas. *Acta Neuropathol* 126(6):939-941.  3 Reuss DE, Kratz A, Sahm F, Capper D, Schrimpf D, Koelsche C, Hovestadt V, Bewerunge-Hudler M, Jones DT, Schittenhelm J, Mittelbronn M, Rushing E, Simon M, Westphal M, Unterberg A, Platten M, Paulus W, Reifenberger G, Tonn JC, Aldape K, Pfister SM, Korshunov A, Weller M, Herold-Mende C, Wick W, Brandner S and von Deimling A (2015). Adult IDH wild type astrocytomas biologically and clinically resolve into other tumor entities. *Acta Neuropathol* 130(3):407-417.  4 Arita H, Yamasaki K, Matsushita Y, Nakamura T, Shimokawa A, Takami H, Tanaka S, Mukasa A, Shirahata M, Shimizu S, Suzuki K, Saito K, Kobayashi K, Higuchi F, Uzuka T, Otani R, Tamura K, Sumita K, Ohno M, Miyakita Y, Kagawa N, Hashimoto N, Hatae R, Yoshimoto K, Shinojima N, Nakamura H, Kanemura Y, Okita Y, Kinoshita M, Ishibashi K, Shofuda T, Kodama Y, Mori K, Tomogane Y, Fukai J, Fujita K, Terakawa Y, Tsuyuguchi N, Moriuchi S, Nonaka M, Suzuki H, Shibuya M, Maehara T, Saito N, Nagane M, Kawahara N, Ueki K, Yoshimine T, Miyaoka E, Nishikawa R, Komori T, Narita Y and Ichimura K (2016). A combination of TERT promoter mutation and MGMT methylation status predicts clinically relevant subgroups of newly diagnosed glioblastomas. *Acta Neuropathol Commun* 4(1):79.  5 Pekmezci M, Rice T, Molinaro AM, Walsh KM, Decker PA, Hansen H, Sicotte H, Kollmeyer TM, McCoy LS, Sarkar G, Perry A, Giannini C, Tihan T, Berger MS, Wiemels JL, Bracci PM, Eckel-Passow JE, Lachance DH, Clarke J, Taylor JW, Luks T, Wiencke JK, Jenkins RB and Wrensch MR (2017). Adult infiltrating gliomas with WHO 2016 integrated diagnosis: additional prognostic roles of ATRX and TERT. *Acta Neuropathol* 133(6):1001-1016.  6 Sahm F, Schrimpf D, Olar A, Koelsche C, Reuss D, Bissel J, Kratz A, Capper D, Schefzyk S, Hielscher T, Wang Q, Sulman EP, Adeberg S, Koch A, Okuducu AF, Brehmer S, Schittenhelm J, Becker A, Brokinkel B, Schmidt M, Ull T, Gousias K, Kessler AF, Lamszus K, Debus J, Mawrin C, Kim YJ, Simon M, Ketter R, Paulus W, Aldape KD, Herold-Mende C and von Deimling A (2016). TERT Promoter Mutations and Risk of Recurrence in Meningioma. *J Natl Cancer Inst* 108(5). |  |
| Non-core | *TP53* MUTATION | ***TP53* mutation**  Single selection value list:  • Cannot be determined  • Absent  • Present, *specify*  EXONS ANALYSED  • Exons 5-8  • All exons  • Other, *specify*  TESTING METHOD  (Multi-select - select all that apply)  • Sanger sequencing  • Next-generation sequencing  • PCR-based method  • Other, *specify*  ***p53 expression (immunohistochemistry)***  Single selection value list:  • Cannot be determined  • Negative or rare, lightly positive cells  • Intermediate (intermediate numbers of predominantly lightly positive cells)  • Positive (diffuse and strong nuclear positivity) | Mutations of the *TP53* gene, which encodes the p53 protein, are found in approximately two-thirds of all diffuse astrocytic gliomas1 and in over 80% of IDH-mutant diffuse astrocytic gliomas.2 *TP53* mutations are less common in IDH-wildtype glioblastomas (23-28%), and are notably uncommon in oligodendrogliomas, showing a strong inverse relationship with 1p/19q codeletion. *TP53* mutations are thus used as diagnostic markers for diffuse astrocytic gliomas, and have been used to distinguish low-cellularity diffuse astrocytic gliomas from reactive gliosis.3 Evaluation of *TP53* mutation may also be used to rule out the possibility of oligodendroglial tumours among IDH-mutant gliomas. Furthermore, *TP53* mutations are important for subclassifying medulloblastomas with SHH pathway activation, dividing them into high-risk *TP53*-mutant cases in older children versus lower-risk *TP53*-wildtype cases in young children and adults. *TP53* mutations are common in some other types of brain tumours, but are not used diagnostically as in the above situations.  Different DNA sequencing techniques may be used for detecting *TP53* mutations. Screening can be accomplished via sequencing of all exons or just exons 5 through 8, where most mutations occur; the great majority of mutations are missense.  p53 Expression (Immunohistochemistry)  Immunohistochemistry is a useful screening tool, given that most missense *TP53* mutations result in increased p53 protein half-life that produces strong immunoreactivity in the majority of tumour cell nuclei (rather than scattered positivity and/or light nuclear staining). Strong p53 positivity in >10% of the tumour cell nuclei has been found to have a sensitivity of 77.4-78.8% and a specificity of 78.6-96.7% when compared to sequencing.4,5 Positive nuclear p53 staining correlates well with missense mutations with a sensitivity of 92% and a specificity of 79.4%, whereas only 33% of tumours with truncating mutations show p53 positivity,5 with such mutations typically leading to negative staining.6  References  1 Ohgaki H and Kleihues P (2005). Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas. *J Neuropathol Exp Neurol* 64(6):479-489.  2 Brat DJ, Verhaak RG, Aldape KD, Yung WK, Salama SR, Cooper LA, Rheinbay E, Miller CR, Vitucci M, Morozova O, Robertson AG, Noushmehr H, Laird PW, Cherniack AD, Akbani R, Huse JT, Ciriello G, Poisson LM, Barnholtz-Sloan JS, Berger MS, Brennan C, Colen RR, Colman H, Flanders AE, Giannini C, Grifford M, Iavarone A, Jain R, Joseph I, Kim J, Kasaian K, Mikkelsen T, Murray BA, O'Neill BP, Pachter L, Parsons DW, Sougnez C, Sulman EP, Vandenberg SR, Van Meir EG, von Deimling A, Zhang H, Crain D, Lau K, Mallery D, Morris S, Paulauskis J, Penny R, Shelton T, Sherman M, Yena P, Black A, Bowen J, Dicostanzo K, Gastier-Foster J, Leraas KM, Lichtenberg TM, Pierson CR, Ramirez NC, Taylor C, Weaver S, Wise L, Zmuda E, Davidsen T, Demchok JA, Eley G, Ferguson ML, Hutter CM, Mills Shaw KR, Ozenberger BA, Sheth M, Sofia HJ, Tarnuzzer R, Wang Z, Yang L, Zenklusen JC, Ayala B, Baboud J, Chudamani S, Jensen MA, Liu J, Pihl T, Raman R, Wan Y, Wu Y, Ally A, Auman JT, Balasundaram M, Balu S, Baylin SB, Beroukhim R, Bootwalla MS, Bowlby R, Bristow CA, Brooks D, Butterfield Y, Carlsen R, Carter S, Chin L, Chu A, Chuah E, Cibulskis K, Clarke A, Coetzee SG, Dhalla N, Fennell T, Fisher S, Gabriel S, Getz G, Gibbs R, Guin R, Hadjipanayis A, Hayes DN, Hinoue T, Hoadley K, Holt RA, Hoyle AP, Jefferys SR, Jones S, Jones CD, Kucherlapati R, Lai PH, Lander E, Lee S, Lichtenstein L, Ma Y, Maglinte DT, Mahadeshwar HS, Marra MA, Mayo M, Meng S, Meyerson ML, Mieczkowski PA, Moore RA, Mose LE, Mungall AJ, Pantazi A, Parfenov M, Park PJ, Parker JS, Perou CM, Protopopov A, Ren X, Roach J, Sabedot TS, Schein J, Schumacher SE, Seidman JG, Seth S, Shen H, Simons JV, Sipahimalani P, Soloway MG, Song X, Sun H, Tabak B, Tam A, Tan D, Tang J, Thiessen N, Triche T, Jr., Van Den Berg DJ, Veluvolu U, Waring S, Weisenberger DJ, Wilkerson MD, Wong T, Wu J, Xi L, Xu AW, Yang L, Zack TI, Zhang J, Aksoy BA, Arachchi H, Benz C, Bernard B, Carlin D, Cho J, DiCara D, Frazer S, Fuller GN, Gao J, Gehlenborg N, Haussler D, Heiman DI, Iype L, Jacobsen A, Ju Z, Katzman S, Kim H, Knijnenburg T, Kreisberg RB, Lawrence MS, Lee W, Leinonen K, Lin P, Ling S, Liu W, Liu Y, Liu Y, Lu Y, Mills G, Ng S, Noble MS, Paull E, Rao A, Reynolds S, Saksena G, Sanborn Z, Sander C, Schultz N, Senbabaoglu Y, Shen R, Shmulevich I, Sinha R, Stuart J, Sumer SO, Sun Y, Tasman N, Taylor BS, Voet D, Weinhold N, Weinstein JN, Yang D, Yoshihara K, Zheng S, Zhang W, Zou L, Abel T, Sadeghi S, Cohen ML, Eschbacher J, Hattab EM, Raghunathan A, Schniederjan MJ, Aziz D, Barnett G, Barrett W, Bigner DD, Boice L, Brewer C, Calatozzolo C, Campos B, Carlotti CG, Jr., Chan TA, Cuppini L, Curley E, Cuzzubbo S, Devine K, DiMeco F, Duell R, Elder JB, Fehrenbach A, Finocchiaro G, Friedman W, Fulop J, Gardner J, Hermes B, Herold-Mende C, Jungk C, Kendler A, Lehman NL, Lipp E, Liu O, Mandt R, McGraw M, McLendon R, McPherson C, Neder L, Nguyen P, Noss A, Nunziata R, Ostrom QT, Palmer C, Perin A, Pollo B, Potapov A, Potapova O, Rathmell WK, Rotin D, Scarpace L, Schilero C, Senecal K, Shimmel K, Shurkhay V, Sifri S, Singh R, Sloan AE, Smolenski K, Staugaitis SM, Steele R, Thorne L, Tirapelli DP, Unterberg A, Vallurupalli M, Wang Y, Warnick R, Williams F, Wolinsky Y, Bell S, Rosenberg M, Stewart C, Huang F, Grimsby JL, Radenbaugh AJ and Zhang J (2015). Comprehensive, Integrative Genomic Analysis of Diffuse Lower-Grade Gliomas. *N Engl J Med* 372(26):2481-2498.  3 Camelo-Piragua S, Jansen M, Ganguly A, Kim JC, Cosper AK, Dias-Santagata D, Nutt CL, Iafrate AJ and Louis DN (2011). A sensitive and specific diagnostic panel to distinguish diffuse astrocytoma from astrocytosis: chromosome 7 gain with mutant isocitrate dehydrogenase 1 and p53. *J Neuropathol Exp Neurol* 70(2):110-115.  4 Takami H, Yoshida A, Fukushima S, Arita H, Matsushita Y, Nakamura T, Ohno M, Miyakita Y, Shibui S, Narita Y and Ichimura K (2015). Revisiting TP53 Mutations and Immunohistochemistry--A Comparative Study in 157 Diffuse Gliomas. *Brain Pathol* 25(3):256-265.  5 Gillet E, Alentorn A, Doukoure B, Mundwiller E, van Thuijl HF, Reijneveld JC, Medina JA, Liou A, Marie Y, Mokhtari K, Hoang-Xuan K, Sanson M, Delattre JY and Idbaih A (2014). TP53 and p53 statuses and their clinical impact in diffuse low grade gliomas. *J Neurooncol* 118(1):131-139.  6 Tanboon J, Williams EA and Louis DN (2016). The Diagnostic Use of Immunohistochemical Surrogates for Signature Molecular Genetic Alterations in Gliomas. *J Neuropathol Exp Neurol* 75(1):4-18. |  |
| Non-core | *YAP1* FUSION | Single selection value list:  • Cannot be determined  • Negative  • Positive  TESTING METHOD  (Multi-select - select all that apply)  • FISH  • Next generation sequencing  • Other, *specify* | Classifying ependymomas by molecular genetic alterations is beginning to find clinical utility. Currently, the *RELA* fusion-positive ependymoma is listed in the WHO classification, but any update would be expected to include other genetically defined entities, on the basis of recent studies describing the clinicopathological attributes of the varied molecular groups of ependymomas.1,2 *RELA* fusions are found only in supratentorial ependymomas, not those in the posterior fossa or spinal compartments, and they are present in the majority of paediatric ependymomas at this site.3 Among supratentorial ependymomas without a *RELA* fusion are those with a *YAP1* fusion, but these are rare and mostly restricted to young children.2  A *YAP1* fusion can be detected by a variety of methods, although an immunohistochemical approach is currently not available. Transcriptome sequencing can detect *YAP1* fused to several gene partners, such as *MAMLD1.2* This approach has some utility with derivatives from FFPE tissue, but methods using RT-PCR or interphase FISH are alternatives.2  References  1 Pajtler KW, Mack SC, Ramaswamy V, Smith CA, Witt H, Smith A, Hansford JR, von Hoff K, Wright KD, Hwang E, Frappaz D, Kanemura Y, Massimino M, Faure-Conter C, Modena P, Tabori U, Warren KE, Holland EC, Ichimura K, Giangaspero F, Castel D, von Deimling A, Kool M, Dirks PB, Grundy RG, Foreman NK, Gajjar A, Korshunov A, Finlay J, Gilbertson RJ, Ellison DW, Aldape KD, Merchant TE, Bouffet E, Pfister SM and Taylor MD (2017). The current consensus on the clinical management of intracranial ependymoma and its distinct molecular variants. *Acta Neuropathol* 133(1):5-12.  2 Pajtler KW, Witt H, Sill M, Jones DT, Hovestadt V, Kratochwil F, Wani K, Tatevossian R, Punchihewa C, Johann P, Reimand J, Warnatz HJ, Ryzhova M, Mack S, Ramaswamy V, Capper D, Schweizer L, Sieber L, Wittmann A, Huang Z, van Sluis P, Volckmann R, Koster J, Versteeg R, Fults D, Toledano H, Avigad S, Hoffman LM, Donson AM, Foreman N, Hewer E, Zitterbart K, Gilbert M, Armstrong TS, Gupta N, Allen JC, Karajannis MA, Zagzag D, Hasselblatt M, Kulozik AE, Witt O, Collins VP, von Hoff K, Rutkowski S, Pietsch T, Bader G, Yaspo ML, von Deimling A, Lichter P, Taylor MD, Gilbertson R, Ellison DW, Aldape K, Korshunov A, Kool M and Pfister SM (2015). Molecular Classification of Ependymal Tumors across All CNS Compartments, Histopathological Grades, and Age Groups. *Cancer Cell* 27(5):728-743.  3 Parker M, Mohankumar KM, Punchihewa C, Weinlich R, Dalton JD, Li Y, Lee R, Tatevossian RG, Phoenix TN, Thiruvenkatam R, White E, Tang B, Orisme W, Gupta K, Rusch M, Chen X, Li Y, Nagahawhatte P, Hedlund E, Finkelstein D, Wu G, Shurtleff S, Easton J, Boggs K, Yergeau D, Vadodaria B, Mulder HL, Becksfort J, Gupta P, Huether R, Ma J, Song G, Gajjar A, Merchant T, Boop F, Smith AA, Ding L, Lu C, Ochoa K, Zhao D, Fulton RS, Fulton LL, Mardis ER, Wilson RK, Downing JR, Green DR, Zhang J, Ellison DW and Gilbertson RJ (2014). C11orf95-RELA fusions drive oncogenic NF-kappaB signalling in ependymoma. *Nature* 506(7489):451-455. |  |
| Non-core | OTHER FINDINGS | **Text:**  **Other immunohistochemical findings,** *specify*  **Other molecular findings,** *specify test, testing method and findings* | These sections should be used for documenting findings for other genetic alterations and/or for other tumour types, such as metastases and haematological lesions. |  |
| **INTEGRATED FINAL DIAGNOSIS REPORTING GUIDE** | | | | |
| Core | INTEGRATED FINAL DIAGNOSIS | Text  OR  • Diagnosis not elsewhere classified | All reports should strive to render a diagnosis from the 2016 World Health Organization (WHO) Classification of Tumours of the Central Nervous System (2016 CNS WHO)1, although it is recognized that this may not be possible in all instances (i.e., that more descriptive diagnoses may be needed for tumours that do not meet criteria for 2016 CNS WHO entities).1,2  In many situations, 2016 CNS WHO diagnoses “integrate” histological and molecular information and have been referred to as “integrated” diagnoses; for these entities, both histological and molecular information is needed. (In this context, “molecular information” refers to data from any type of molecule, e.g., DNA, protein, etc., so that a immunohistochemical test provides “molecular information.”) In some scenarios, there may be differences between histological appearance and 2016 CNS WHO diagnosis (e.g., a diffuse glioma without overt oligodendroglial features but with IDH mutation and 1p/19q codeletion). Moreover, in other scenarios, necessary molecular information may not be available, leading to one of the “not otherwise specified” (“NOS”) 2016 CNS WHO diagnoses. Nonetheless, it is important to keep in mind that *the majority of 2016 CNS WHO entities can be diagnosed solely on the basis of histological features*.  To capture this nosological heterogeneity and to provide as much clinically relevant information in each report, it is recommended that layered diagnostic formatting be utilized in reports, typically with four layers:   * 2016 CNS WHO diagnosis (as per this dataset) * Histological appearance (as per “Histological assessment of CNS specimens” dataset) * WHO (histological) grade (as per “Histological assessment of CNS specimens” dataset) * Molecular parameters (as per “Molecular information for CNS specimens” dataset)   As mentioned above, for some entities, the 2016 CNS WHO diagnosis may be identical to the histological appearance (e.g., choroid plexus tumours), but for others there may be differences such as the following:   * 2016 CNS WHO diagnosis: Diffuse astrocytoma, IDH-mutant * Histological appearance: Diffuse glioma * WHO (histological) grade: II * Molecular parameters:   o *IDH1* R132H mutation  o *ATRX* mutation  o *TP53* mutation  o 1p/19q retention  **2016 WHO Classification of Tumours of the Central Nervous System1**   | **Entities** | **ICD-O code** | | --- | --- | | Diffuse astrocytic and oligodendroglial tumours |  | | Diffuse astrocytoma, IDH-mutant | 9400/3 | | Gemistocytic astrocytoma, IDH-mutant | 9411/3 | | Diffuse astrocytoma, IDH-wildtype | 9400/3 | | Diffuse astrocytoma, NOS | 9400/3 | | Anaplastic astrocytoma, IDH-mutant | 9401/3 | | Anaplastic astrocytoma, IDH-wildtype | 9401/3 | | Anaplastic astrocytoma, NOS | 9401/3 | | Glioblastoma, IDH-wildtype | 9440/3 | | Giant cell glioblastoma | 9441/3 | | Gliosarcoma | 9442/3 | | Epithelioid glioblastoma | 9440/3 | | Glioblastoma, IDH-mutant | 9445/3\* | | Glioblastoma, NOS | 9440/3 | | Diffuse midline glioma, H3 K27M–mutant | 9385/3\* | | Oligodendroglioma, IDH-mutant and 1p/19q-codeleted | 9450/3 | | Oligodendroglioma, NOS | 9450/3 | | Anaplastic oligodendroglioma, IDH-mutant and 1p/19q-codeleted | 9451/3 | | Anaplastic oligodendroglioma, NOS | 9451/3 | | Oligoastrocytoma, NOS | 9382/3 | | Anaplastic oligoastrocytoma, NOS | 9382/3 | | Other astrocytic tumours |  | | Pilocytic astrocytoma | 9421/1 | | Pilomyxoid astrocytoma | 9425/3 | | Subependymal giant cell astrocytoma | 9384/1 | | Pleomorphic xanthoastrocytoma | 9424/3 | | Anaplastic pleomorphic xanthoastrocytoma | 9424/3 | | Ependymal tumours |  | | Subependymoma | 9383/1 | | Myxopapillary ependymoma | 9394/1 | | Ependymoma | 9391/3 | | Papillary ependymoma | 9393/3 | | Clear cell ependymoma | 9391/3 | | Tanycytic ependymoma | 9391/3 | | Ependymoma, RELA fusion–positive | 9396/3\* | | Anaplastic ependymoma | 9392/3 | | Other gliomas |  | | Chordoid glioma of the third ventricle | 9444/1 | | Angiocentric glioma | 9431/1 | | Astroblastoma | 9430/3 | | Choroid plexus tumours |  | | Choroid plexus papilloma | 9390/0 | | Atypical choroid plexus papilloma | 9390/1 | | Choroid plexus carcinoma | 9390/3 | | Neuronal and mixed neuronal-glial tumours |  | | Dysembryoplastic neuroepithelial tumour | 9413/0 | | Gangliocytoma | 9492/0 | | Ganglioglioma | 9505/1 | | Anaplastic ganglioglioma | 9505/3 | | Dysplastic cerebellar gangliocytoma (Lhermitte–Duclos disease) | 9493/0 | | Desmoplastic infantile astrocytoma and ganglioglioma | 9412/1 | | Papillary glioneuronal tumour | 9509/1 | | Rosette-forming glioneuronal tumour | 9509/1 | | Diffuse leptomeningeal glioneuronal tumour |  | | Central neurocytoma | 9506/1 | | Extraventricular neurocytoma | 9506/1 | | Cerebellar liponeurocytoma | 9506/1 | | Paraganglioma | 8693/1 | | Tumours of the pineal region |  | | Pineocytoma | 9361/1 | | Pineal parenchymal tumour of intermediate differentiation | 9362/3 | | Pineoblastoma | 9362/3 | | Papillary tumour of the pineal region | 9395/3 | | Embryonal tumours |  | | Medulloblastomas, genetically defined |  | | Medulloblastoma, WNT-activated | 9475/3\* | | Medulloblastoma, SHH-activated and TP53-mutant | 9476/3\* | | Medulloblastoma, SHH-activated and TP53-wildtype | 9471/3 | | Medulloblastoma, non-WNT/non-SHH | 9477/3\* | | Medulloblastoma, group 3 |  | | Medulloblastoma, group 4 |  | | Medulloblastomas, histologically defined |  | | Medulloblastoma, classic | 9470/3 | | Medulloblastoma, desmoplastic/nodular | 9471/3 | | Medulloblastoma with extensive nodularity | 9471/3 | | Medulloblastoma, large cell / anaplastic | 9474/3 | | Medulloblastoma, NOS | 9470/3 | | Embryonal tumour with multilayered rosettes, C19MC-altered | 9478/3\* | | Embryonal tumour with multilayered rosettes, NOS | 9478/3 | | Medulloepithelioma | 9501/3 | | CNS neuroblastoma | 9500/3 | | CNS ganglioneuroblastoma | 9490/3 | | CNS embryonal tumour, NOS | 9473/3 | | Atypical teratoid/rhabdoid tumour | 9508/3 | | CNS embryonal tumour with rhabdoid features | 9508/3 | | Tumours of the cranial and paraspinal nerves |  | | Schwannoma | 9560/0 | | Cellular schwannoma | 9560/0 | | Plexiform schwannoma | 9560/0 | | Melanotic schwannoma | 9560/1 | | Neurofibroma | 9540/0 | | Atypical neurofibroma | 9540/0 | | Plexiform neurofibroma | 9550/0 | | Perineurioma | 9571/0 | | Hybrid nerve sheath tumours |  | | Malignant peripheral nerve sheath tumour | 9540/3 | | Epithelioid MPNST | 9540/3 | | MPNST with perineurial differentiation | 9540/3 | | Meningiomas |  | | Meningioma | 9530/0 | | Meningothelial meningioma | 9531/0 | | Fibrous meningioma | 9532/0 | | Transitional meningioma | 9537/0 | | Psammomatous meningioma | 9533/0 | | Angiomatous meningioma | 9534/0 | | Microcystic meningioma | 9530/0 | | Secretory meningioma | 9530/0 | | Lymphoplasmacyte-rich meningioma | 9530/0 | | Metaplastic meningioma | 9530/0 | | Chordoid meningioma | 9538/1 | | Clear cell meningioma | 9538/1 | | Atypical meningioma | 9539/1 | | Papillary meningioma | 9538/3 | | Rhabdoid meningioma | 9538/3 | | Anaplastic (malignant) meningioma | 9530/3 | | Mesenchymal, non-meningothelial tumours |  | | Solitary fibrous tumour / haemangiopericytoma\*\* |  | | Grade 1 | 8815/0 | | Grade 2 | 8815/1 | | Grade 3 | 8815/3 | | Haemangioblastoma | 9161/1 | | Haemangioma | 9120/0 | | Epithelioid haemangioendothelioma | 9133/3 | | Angiosarcoma | 9120/3 | | Kaposi sarcoma | 9140/3 | | Ewing sarcoma / PNET | 9364/3 | | Lipoma | 8850/0 | | Angiolipoma | 8861/0 | | Hibernoma | 8880/0 | | Liposarcoma | 8850/3 | | Desmoid-type fibromatosis | 8821/1 | | Myofibroblastoma | 8825/0 | | Inflammatory myofibroblastic tumour | 8825/1 | | Benign fibrous histiocytoma | 8830/0 | | Fibrosarcoma | 8810/3 | | Undifferentiated pleomorphic sarcoma / malignant fibrous histiocytoma | 8802/3 | | Leiomyoma | 8890/0 | | Leiomyosarcoma | 8890/3 | | Rhabdomyoma | 8900/0 | | Rhabdomyosarcoma | 8900/3 | | Chondroma | 9220/0 | | Chondrosarcoma | 9220/3 | | Osteoma | 9180/0 | | Osteochondroma | 9210/0 | | Osteosarcoma | 9180/3 | | Melanocytic tumours |  | | Meningeal melanocytosis | 8728/0 | | Meningeal melanocytoma | 8728/1 | | Meningeal melanoma | 8720/3 | | Meningeal melanomatosis | 8728/3 | | Lymphomas |  | | Diffuse large B-cell lymphoma of the CNS | 9680/3 | | Immunodeficiency-associated CNS lymphomas |  | | AIDS-related diffuse large B-cell lymphoma |  | | EBV-positive diffuse large B-cell lymphoma, NOS |  | | Lymphomatoid granulomatosis | 9766/1 | | Intravascular large B-cell lymphoma | 9712/3 | | Low-grade B-cell lymphomas of the CNS |  | | T-cell and NK/T-cell lymphomas of the CNS |  | | Anaplastic large cell lymphoma, ALK-positive | 9714/3 | | Anaplastic large cell lymphoma, ALK-negative | 9702/3 | | MALT lymphoma of the dura | 9699/3 | | Histiocytic tumours |  | | Langerhans cell histiocytosis | 9751/3 | | Erdheim–Chester disease | 9750/1 | | Rosai–Dorfman disease |  | | Juvenile xanthogranuloma |  | | Histiocytic sarcoma | 9755/3 | | Germ cell tumours |  | | Germinoma | 9064/3 | | Embryonal carcinoma | 9070/3 | | Yolk sac tumour | 9071/3 | | Choriocarcinoma | 9100/3 | | Teratoma | 9080/1 | | Mature teratoma | 9080/0 | | Immature teratoma | 9080/3 | | Teratoma with malignant transformation | 9084/3 | | Mixed germ cell tumour | 9085/3 | | Tumours of the sellar region |  | | Craniopharyngioma | 9350/1 | | Adamantinomatous craniopharyngioma | 9351/1 | | Papillary craniopharyngioma | 9352/1 | | Granular cell tumour of the sellar region | 9582/0 | | Pituicytoma | 9432/1 | | Spindle cell oncocytoma | 8290/0 | | Metastatic tumours |  |   The morphology codes are from the International Classification of Diseases for Oncology (ICD-O). Behaviour is coded /0 for benign tumours; /1 for unspecified, borderline, or uncertain behaviour; /2 for carcinoma in situ and grade III intraepithelial neoplasia; and /3 for malignant tumours.  The classification is modified from the previous WHO classification, taking into account changes in our understanding of these lesions.  \*These new codes were approved by the IARC/WHO Committee for ICD-O.  \*\*Grading similar to that of non-CNS solitary fibrous tumours as proposed in the 2013 WHO Classification of Tumours of Soft Tissue and Bone.3  References  1 Louis DN, Ohgaki H, Wiestler OD and Cavenee WK (eds) (2016). *WHO Classification of Tumours of the Central Nervous System, Revised. Fourth Edition*, IARC, Lyon.  2 Louis DN, Wesseling P, Paulus W, Giannini C, Batchelor TT, Cairncross JG, Capper D, Figarella-Branger D, Lopes MB, Wick W and van den Bent M (2018). cIMPACT-NOW update 1: Not Otherwise Specified (NOS) and Not Elsewhere Classified (NEC). *Acta Neuropathol.* 135(3):481-484.  3 Fletcher CDM, Bridge JA, Hogendoorn PCW and Mertens F (eds) (2013). *World Health Organization Classification of Tumours. Pathology and genetics of soft tissue and bone 4th Ed* IARC Press Lyon.  **Diagnosis not elsewhere classified**  In the event that *all diagnostic information is present* but the tumour still does not meet criteria for an entity defined by the 2016 WHO classification (e.g., a paediatric diffuse glioma that does not harbour IDH or H3 mutations), a “descriptive” or NEC (not elsewhere classified) diagnosis can be issued, which draws attention to the unusual nature of the lesion. Such designations are distinct from NOS diagnoses, which are included in the 2016 WHO classification and which are cases in which necessary diagnostic information is not available.1  References  1 Louis DN, Wesseling P, Paulus W, Giannini C, Batchelor TT, Cairncross JG, Capper D, Figarella-Branger D, Lopes MB, Wick W and van den Bent M (2018). cIMPACT-NOW update 1: Not Otherwise Specified (NOS) and Not Elsewhere Classified (NEC). *Acta Neuropathol.* 135(3):481-484. |  |
| Core | INTEGRATED DIAGNOSIS BASED ON | (Multi-select - select all that apply)  • Histology  • Molecular information |  |  |

**OVERVIEW OF SELECTED MOLECULAR MARKERS**

The table below summarizes selected molecular diagnostic markers for CNS tumours; the list of tests is not exhaustive and other assays may be helpful in some diagnostic circumstances. In addition, the tests listed are those related to ruling in the corresponding diagnoses; however, it should be realized that the assays may also be used in particular diagnostic situations to rule out other diagnoses. An example of this would be ATRX immunohistochemistry, which is commonly used to support a diagnosis of IDH-mutant diffuse astrocytoma, but which is also used to evaluate a possible diagnosis of oligodendroglioma, IDH-mutant and 1p/19q-codeleted. Some specific tests recommended in the commentaries below represent one of several validated and equivalent approaches to the evaluation of the described molecular variable; for those tests that have multiple testing modalities (e.g., sequencing and immunohistochemistry for BRAF V600E), it is assumed that only one of these testing modalities would be used per case unless one test yields equivocal results (e.g., a result of weak immunohistochemical positivity versus nonspecific background staining should be followed by gene sequencing). For some tests, relevance may be related to the age of the patient (e.g., EGFR gene amplification in adult high-grade gliomas rather than paediatric ones) and *the reader is referred to the commentaries under each molecular parameter for further information.*

**Summary of tests by tumour type**

Note: this is a summary and the reader is referred to the specific notes for details on use of each test.

**W** = component of the 2016 CNS WHO diagnostic criteria and 2017 WHO diagnostic criteria for pituitary adenomas

**D** = commonly used to support or refine the diagnosis, or provide important ancillary information in the corresponding tumour type

**D**\* = commonly used to rule out the diagnosis; see commentary for details

**(D)** = can be used to support or refine the diagnosis, or provide important ancillary information in specific tumour subtype(s); see commentary for details

DA = diffuse astrocytoma; AA = anaplastic astrocytoma; O = oligodendroglioma; AO = anaplastic oligodendroglioma; GBM = glioblastoma; PXA = pleomorphic xanthoastrocytoma; GG = ganglioglioma; AT/RT = atypical teratoid / rhabdoid tumour; ETMR = embryonal tumour with multilayered rosettes; SFT/HPC = solitary fibrous tumour / haemangiopericytoma; MPNST = malignant peripheral nerve sheath tumour

| **Test** | **Gliomas** | | | | | | | | **Embryonal tumours** | | | **Other** | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **DA, AA** | **O, AO** | **Diffuse midline glioma** | **GBM** | **Pilocytic astrocytoma** | **PXA, GG** | **Ependymoma - supratentorial** | **Ependymoma – posterior fossa** | **Medulloblastoma** | **AT/RT** | **ETMR** | **Extraventricular neurocytoma** | **Meningioma** | **SFT/HPC** | **Craniopharyngioma** | **MPNST** | **Pituitary tumours** |
| ***ATRX* mutation** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *ATRX* mutation | **D** |  |  | **D** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| ATRX loss of expression (immunohistochemistry) | **D** |  |  | **D** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| ***BRAF* alterations** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *BRAF* mutation | **(D)** |  |  | **(D)** | **D** | **D** |  |  |  |  |  |  |  |  | **D** |  |  |
| BRAF V600E expression (immunohistochemistry) | **(D)** |  |  | **(D)** | **D** | **D** |  |  |  |  |  |  |  |  | **D** |  |  |
| *BRAF* rearrangement/duplication |  |  |  |  | **D** |  |  |  |  |  |  |  |  |  |  |  |  |
| ***CDKN2A/B* homozygous deletion** | **(D)** |  |  |  |  | **(D)** |  |  |  |  |  |  |  |  |  |  |  |
| **C19MC alteration** |  |  |  |  |  |  |  |  |  |  | **W** |  |  |  |  |  |  |
| **Chromosomal arm 1p/19q codeletion** |  | **W** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Chromosome 7 gain combined with chromosome 10 loss (see below)** |  |  |  | **D** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Chromosome 10q23 (PTEN locus) deletion and *PTEN* mutation** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Chromosome 10q23 *(PTEN* locus)deletion or  monosomy 10 |  |  |  | **D** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *PTEN* mutation |  |  |  | **D** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| ***EGFR* amplification and EGFRvIII mutation** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *EGFR* amplification |  |  |  | **D** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| EGFRvIII mutation |  |  |  | **D** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Histone H3 mutation and H3 K27 trimethylation (me3)** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Histone H3 K27M mutation (sequencing) and expression (immunohistochemistry) | **(D)** |  | **W** | **D** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Histone H3 G34 mutation (sequencing) and expression (immunohistochemistry) | **(D)** |  |  | **D** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Histone H3 K27me3 expression (immunohistochemistry) |  |  | **D** |  |  |  |  | **D** |  |  |  |  |  |  |  | **D** |  |
| ***IDH1/IDH2* mutation** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *IDH1/IDH2* mutation | **W** | **W** | **D\*** | **W** | **D\*** | **D\*** |  |  |  |  |  | **D\*** |  |  |  |  |  |
| IDH1 R132Hexpression (immunohistochemistry) | **W** | **W** | **D\*** | **W** | **D\*** | **D\*** |  |  |  |  |  | **D\*** |  |  |  |  |  |
| **Ki-67 immunohistochemistry** |  | **D** |  |  |  |  |  |  |  |  |  |  | **D** |  |  |  | **D** |
| **L1CAM expression (immunohistochemistry)** |  |  |  |  |  |  | **D** |  |  |  |  |  |  |  |  |  |  |
| **LIN28A expression (immunohistochemistry)** |  |  |  |  |  |  |  |  |  |  | **D** |  |  |  |  |  |  |
| **Medulloblastoma immunohistochemistry** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| β-catenin nuclear expression (immunohistochemistry) |  |  |  |  |  |  |  |  | **D** |  |  |  |  |  | **D** |  |  |
| GAB1 expression (immunohistochemistry) |  |  |  |  |  |  |  |  | **D** |  |  |  |  |  |  |  |  |
| YAP1 expression (immunohistochemistry) |  |  |  |  |  |  |  |  | **D** |  |  |  |  |  |  |  |  |
| ***MGMT* promoter methylation** |  |  |  | **D** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Monosomy 6** |  |  |  |  |  |  |  |  | **D** |  |  |  |  |  |  |  |  |
| **MYC gene family amplification** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *MYC* amplification |  |  |  |  |  |  |  |  | **D** |  |  |  |  |  |  |  |  |
| *MYCN* amplification |  |  |  |  |  |  |  |  | **D** |  |  |  |  |  |  |  |  |
| ***NAB2-STAT6* fusion** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *NAB2-STAT6* fusion |  |  |  |  |  |  |  |  |  |  |  |  |  | **D** |  |  |  |
| STAT6 nuclear expression (immunohistochemistry) |  |  |  |  |  |  |  |  |  |  |  |  |  | **D** |  |  |  |
| **Pituitary hormones and transcription factors immunohistochemistry** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | **W** |
| ***RELA* fusion** |  |  |  |  |  |  | **W** |  |  |  |  |  |  |  |  |  |  |
| ***SMARCA4/BRG1* alteration** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *SMARCA4/BRG1* mutation |  |  |  |  |  |  |  |  | **D** | **W** |  |  |  |  |  |  |  |
| BRG1 loss of expression (immunohistochemistry) |  |  |  |  |  |  |  |  | **D** | **W** |  |  |  |  |  |  |  |
| ***SMARCB1/INI1/HNSF5* alteration** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *SMARCB1/INI1/HNSF5* mutation |  |  |  |  |  |  |  |  | **D** | **W** |  |  |  |  |  |  |  |
| INI1 (BAF47) loss of expression (immunohistochemistry) |  |  |  |  |  |  |  |  | **D\*** | **W** |  |  |  |  |  |  |  |
| ***TERT* promoter mutation** |  | **D** |  | **D** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| ***TP53* mutation** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *TP53* mutation | **D** |  |  |  |  |  |  |  | **W** |  |  |  |  |  |  |  |  |
| p53 expression (immunohistochemistry) | **D** |  |  |  |  |  |  |  | **W** |  |  |  |  |  |  |  |  |
| ***YAP1* fusion** |  |  |  |  |  |  | **D** |  |  |  |  |  |  |  |  |  |  |