COLLEGE of AMERICAN PATHOLOGISTS

Protocol for the Examination of Specimens from Patients with Tumors of the Central Nervous System*

Version: CNS 4.0.0.0 **Protocol Posting Date:** August 2018
This protocol is NOT required for accreditation purposes

*This protocol applies to primary neoplasms of the brain and spinal cord

The following tumor types should NOT be reported using this protocol:

Tumor type
Lymphoma (consider the Hodgkin or non-Hodgkin Lymphoma protocols)
Primary bone tumors (consider the Primary Bone Tumor protocol)
Metastatic tumors
Malignant peripheral nerve sheath tumor (consider the Soft Tissue Tumor protocol)
Mesenchymal tumors (consider the Soft Tissue Tumor protocol)

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With guidance from the CAP Cancer and CAP Pathology Electronic Reporting Committees.
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Accreditation Requirements
The use of this protocol is recommended for clinical care purposes, but is not required for accreditation purposes.

Important Note
There is no American Joint Committee on Cancer (AJCC) pTNM classification system for primary central nervous system (CNS) neoplasms. The World Health Organization (WHO) grading system is recommended.

CAP CNS Protocol Summary of Changes

Version 4.0.0.0

The following data elements were modified:
Histological Classification World Health Organization (WHO) 2016
Histologic Grade World Health Organization (WHO) 2016
Ancillary Studies

The following data elements were added:
Integrated Diagnosis
Biomarker Information

Surgical Pathology Cancer Case Summary

Protocol posting date: August 2018

CNS: Integrated Diagnosis

Note: This case summary is recommended for reporting the integrated diagnosis for CNS neoplasms, but is not required for accreditation purposes. If CNS Integrated Diagnosis section is not applicable, proceed to histological assessment summary.

Select a single response unless otherwise indicated.

Integrated Diagnosis (WHO 2016) (Note A)
___ (Specify): _____
___ Pending
___ Not applicable (proceed to Histological Assessment Case Summary)

Histologic Type (WHO 2016) (Note B)
___ (Specify): _____
___ Cannot be determined

Histologic Grade (WHO 2016) (Note C)
___ WHO grade I
___ WHO grade II
___ WHO grade III
___ WHO grade IV
___ Other (Specify): _____
___ Not applicable
___ Cannot be assessed

Biomarker Studies (Note D)
Note: For biomarker reporting the CAP CNS Biomarker Template should be used.
___ Testing performed (complete relevant findings in CNS Biomarker Template): _____
___ Not performed
___ Not applicable

Testing Performed on Block Number(s): _____

Comment(s)

Surgical Pathology Cancer Case Summary

Protocol posting date: August 2018

CNS: Histological Assessment

Note: This case summary is recommended for reporting the histologic assessment of CNS neoplasms, but is not required for accreditation purposes.

Select a single response unless otherwise indicated.

History of Prior Therapy for this Neoplasm (Note E)
___ Not administered
___ Not known
___ Administered (specify): _____

History of Previous Tumor and/or Familial Syndrome (not the current neoplasm) (Note E)
___ Not known
___ Known (specify): _____
___ Not specified

Neuroimaging Findings (Note F)
___ (specify):_____
___ Not available

Procedure (Note G)
___ Open biopsy
___ Resection
___ Stereotactic biopsy
___ Other (specify): _____
___ Not specified

Specimen Size, gross description (Note H)[#]
Greatest dimension (centimeters): ___ cm
___ Additional dimensions (centimeters): ___ x ___ cm
___ Cannot be determined (explain)
[#] *For fragmented tissue, an aggregate size may be given*

Tumor Site (select all that apply) (Note I)
___ Skull (specify precise location, if known): _____
___ Dura (specify precise location, if known): _____
___ Leptomeninges (specify precise location, if known): _____
___ Brain
___ Cerebral lobes (specify precise location, if known): _____
___ Deep grey matter (specify precise location, if known): _____
___ Ventricle (specify precise location, if known): _____
___ Cerebellum (specify precise location, if known): _____
___ Brain stem (specify precise location, if known): _____
___ Other (specify, if known): _____
___ Cerebellopontine angle
___ Sellar/Suprasellar/Pituitary
___ Pineal
___ Cranial nerve (specify I–XII, 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): _____
___ Other (specify): _____
___ Not specified

Tumor Laterality (Note I)
___ Right
___ Left
___ Midline
___ Bilateral
___ Not specified
___ Other (specify): _____

Tumor Focality (Note I)
___ Unifocal
___ Multifocal (specify number of lesions): _____
___ Cannot be determined

Histologic Type (WHO 2016) (Note B)
___ (Specify): _____
___ Cannot be determined

Histologic Grade (WHO 2016) (Note C)
___ WHO grade I
___ WHO grade II
___ WHO grade III
___ WHO grade IV
___ Other (Specify): _____
___ Not applicable
___ Cannot be assessed

Treatment Effect (Histological Evidence of Prior Therapy) (Note J)
___ Not identified
___ Present (specify type of response): _____
___ Cannot be determined

Additional Pathologic Findings
Specify: _____

Biomarker Studies (Note D)
Note: For biomarker reporting the CAP CNS Biomarker Template should be used.
___ Testing performed (complete relevant findings in CNS Biomarker Template)
___ Pending[#]
___ Not performed
___ Not applicable
[#] *Pending biomarker studies may be listed in the Comments section.*

Designate block for future studies: _____

Comment(s)

CNS Biomarker Reporting Template

Protocol posting date: August 2018

CNS Biomarker Reporting Template

Note: This case summary is recommended for reporting biomarkers for CNS neoplasms at the completion of testing, but is not required for accreditation purposes.

Select a single response unless otherwise indicated.

Testing Performed on Block Number(s): _____

Biomarker Studies (Note D)
Note: Pending biomarker studies may be listed in the Comments section of this report.

ATRX

***ATRX* mutation**
___ Absent
___ Present (specify): _____
___ Cannot be determined (explain): _____

ATRX expression (immunohistochemistry)
___ Intact nuclear expression
___ Loss of nuclear expression
___ Cannot be determined (explain): _____

***BRAF* alterations**

***BRAF* mutation**
___ Absent
___ *BRAF* V600E *(c.1799T>A)* mutation present
___ Other *BRAF* mutation present (specify): _____
___ Cannot be determined (explain): _____

***KIAA:BRAF* rearrangement/duplication**
___ Absent
___ Present
___ Cannot be determined (explain): _____

BRAF V600E expression (immunohistochemistry)
___ Negative
___ Positive
___ Cannot be determined (explain): _____

Beta-Catenin expression / *CTNNB1* mutation

Beta-catenin expression (immunohistochemistry)
___ Absence of nuclear expression
___ Positive nuclear expression
___ Cannot be determined (explain): _____

***CTNNB1* mutation**
___ Absent
___ Present

___ Cannot be determined (explain): _____

***C19MC* alteration**
___ Absent
___ Absent with low level gain
___ Present
___ Cannot be determined (explain): _____

Chromosomal arm 1p/19q codeletion
___ No deletion
___ 1p/19q codeletion
___ 1p only deleted
___ 19q only deleted
___ Polysomy (specify): _____
___ Monosomy (specify): _____
___ Relative deletion (specify): _____
___ Cannot be determined (explain): _____

Chromosomal 7 gain[#]
[#]typically identified by *EGFR* locus, often combined with chromosome 10 loss
___ Absent
___ Present
___ Cannot be determined (explain): _____

Chromosome 10q23 (*PTEN* locus) deletion and *PTEN* mutation

Chromosome 10q23 (*PTEN* locus) deletion
___ No deletion
___ Deletion identified
___ Polysomy (specify): _____
___ Monosomy (specify): _____
___ Cannot be determined (explain): _____

***PTEN* mutation**
___ Absent
___ Present (specify): _____
___ Cannot be determined (explain): _____

***EGFR* amplification and *EGFRvIII* mutation**

***EGFR* amplification**
___ Absent
___ Absent with low level gain
___ Present
___ Cannot be determined (explain): _____

***EGFRvIII* mutation**
___ Absent
___ Present
___ Cannot be determined (explain): _____

***FGFR1* mutation**
___ Absent
___ Present (specify): _____
___ Cannot be determined (explain): _____

GAB1 expression (immunohistochemistry)
___ Negative
___ Positive
___ Cannot be determined (explain): _____

Histone H3 mutation and K27me3

H3 gene family mutation
___ Negative
___ Positive
___ Cannot be determined (explain): _____

Histone H3 K27M expression (immunohistochemistry)
___ Negative
___ Positive
___ Cannot be determined (explain): _____

H3 K27me3 expression (immunohistochemistry)
___ Intact nuclear expression
___ Loss of nuclear expression
___ Cannot be determined (explain): _____

***IDH1/IDH2* mutation**

***IDH1/IDH2* mutation**
___ Absent
___ Present (specify): _____
___ Cannot be determined (explain): _____

***IDH1 R132H* expression (immunohistochemistry)**
___ Negative
___ Positive
___ Cannot be determined (explain): _____

Isochromosome 17q (i17q)
___ Absent
___ Present
___ Cannot be determined (explain): _____

Ki-67 expression (immunohistochemistry)
Hotspot percentage of positive tumor cell nuclei: ____ %

L1CAM expression (immunohistochemistry)
___ Negative
___ Positive
___ Cannot be determined (explain): _____

LIN28A expression (immunohistochemistry)
___ Negative
___ Positive
___ Cannot be determined (explain): _____

***MGMT* promoter methylation**
___ Absent
___ Present
If laboratory reports by level:
___ Low level
___ High level
___ Cannot be determined (explain): _____

Monosomy 6
___ Absent
___ Present
___ Cannot be determined (explain): _____

<u>*MYC* gene family amplification</u>

***MYC* amplification**
___ Absent
___ Present
___ Cannot be determined (explain): _____

***MYCN* amplification**
___ Absent
___ Present
___ Cannot be determined (explain): _____

<u>*NAB2-STAT6* fusion</u>

***NAB2-STAT6* fusion**
___ Negative
___ Positive
___ Cannot be determined (explain): _____

STAT6 expression (immunohistochemistry)
___ Absence of nuclear expression
___ Positive nuclear expression
___ Cannot be determined (explain): _____

<u>Pituitary hormones and transcription factors immunohistochemistry</u>

Tumor Cell(s) Reactivity (select all that apply)
___ Alpha subunit
___ Adrenocorticotrophic hormone (ACTH)
___ Follicular stimulating hormone (beta FSH)
___ Human growth hormone
___ Luteinizing hormone (beta LH)
___ Prolactin
___ PIT1
___ SF1
___ Thyroid stimulating hormone (beta TSH)
___ TPIT
___ Other (specify)

___ Cannot be determined (explain): _____

***RELA* fusion**
___ Negative
___ Positive
___ Cannot be determined (explain): _____

***SMARCA4/BRG1* alteration**

***SMARCA4/BRG1* mutation**
___ Absent
___ Present (specify): _____
___ Cannot be determined (explain): _____

BRG1 expression (immunohistochemistry)
___ Intact nuclear expression
___ Loss of nuclear expression
___ Cannot be determined (explain): _____

***SMARCB1/INI1/HSNF5* alteration**

***SMARCB1/INI1/HSNF5* mutation**
___ Absent
___ Present (specify): _____
___ Cannot be determined (explain): _____

INI1 (BAF47) expression (immunohistochemistry)
___ Intact nuclear expression
___ Loss of nuclear expression
___ Cannot be determined (explain): _____

***TERT* promoter mutation**
___ Absent
___ Hotspot mutation (C228T or C250T)
___ Other *TERT* mutation (specify): _____
___ Cannot be determined (explain): _____

***TP53* mutation**

***TP53* mutation**
___ Absent
___ Present (specify): _____
___ Cannot be determined (explain): _____

p53 expression (immunohistochemistry)
___ Negative or rare
___ Intermediate
___ Positive (diffuse and strong nuclear positivity)
___ Cannot be determined (explain): _____

YAP1

***YAP1* fusion**
___ Negative
___ Positive
___ Other (specify): _____

___ Cannot be determined (explain): _____

YAP1 expression (immunohistochemistry)
___ Negative
___ Positive
___ Cannot be determined (explain): _____

Other biomarker(s)

Point Mutations (specify): _____
Copy Number Alterations (specify): _____
Insertions (specify): _____
Deletions (specify): _____

Comment(s)

Explanatory Notes

A. Integrated Diagnosis

Historically, the diagnosis and classification of CNS tumors has been based exclusively on the histologic appearance of the tumor. In recent decades, however, our knowledge of the molecular basis of many of these tumors has increased significantly. In the updated 2016 WHO Classification of Tumours of the Central Nervous System[1], molecular information is now integrated into some of the tumor diagnostic entities. In such cases, including the diffuse gliomas and embryonal tumors, the final diagnosis should reflect the integration of both histologic and molecular information.

When applicable, it is suggested that all histologic and molecular information be presented in a "layered" report format as follows[2]:

Layer 1: Integrated diagnosis (incorporating all tissue-based information)
Layer 2: Histological classification
Layer 3: Histologic (WHO) grade
Layer 4: Biomarker studies

At centers where molecular testing is not available, an NOS (not otherwise specified) designation is available for some tumor entities. The NOS designation implies that insufficient information is available to provide a more specific integrated diagnosis, and may occasionally be used for tumors that do not precisely fit into one of the defined tumor categories.

References

1. Louis DN, Ohgaki H, Wiestler OD, et al. *World Health Organization Classification of Tumours of the Central Nervous System*. Lyon, France: IARC Press; 2016.
2. Louis DN, Perry A, Burger P, et al. International Society of Neuropathology-Haarlem Consensus Guidelines for Nervous System Tumor Classification and Grading. *Brain Pathol.* 2014;24:429-435.

B. Histologic Type

Classification should be made according to the WHO classification of tumors of the nervous system and the WHO classification of tumors of the endocrine organs whenever possible.[1,2] The list below contains WHO 2016 diagnostic entities for which the Central Nervous System (CNS) Cancer Protocol is recommended:

<u>Diffuse astrocytic and oligodendroglial tumors</u>
Diffuse astrocytoma, NOS
Diffuse astrocytoma, IDH-mutant
Diffuse astrocytoma, IDH-wildtype
Gemistocytic astrocytoma, IDH-mutant
Anaplastic astrocytoma, NOS
Anaplastic astrocytoma, IDH-mutant
Anaplastic astrocytoma, IDH-wildtype
Glioblastoma, NOS
Glioblastoma, IDH-mutant
Glioblastoma, IDH-wildtype
Epithelioid glioblastoma
Giant cell glioblastoma
Gliosarcoma
Diffuse midline glioma, H3 K27M-mutant
Oligodendroglioma, NOS
Oligodendroglioma, IDH-mutant and 1p/19q-codeleted
Anaplastic oligodendroglioma, NOS
Anaplastic oligodendroglioma, IDH-mutant and 1p/19q-codeleted
Oligoastrocytoma, NOS
Anaplastic oligoastrocytoma, NOS

Other astrocytic tumors
Pilocytic astrocytoma
Pilomyxoid astrocytoma
Subependymal giant cell astrocytoma
Pleomorphic xanthoastrocytoma
Anaplastic pleomorphic xanthoastrocytoma

Ependymal tumors
Subependymoma
Myxopapillary ependymoma
Ependymoma
Clear cell ependymoma
Papillary ependymoma
Tanycytic ependymoma
Ependymoma, RELA fusion-positive
Anaplastic ependymoma

Other gliomas
Chordoid glioma of the third ventricle
Angiocentric glioma
Astroblastoma

Choroid plexus tumors
Choroid plexus papilloma
Atypical choroid plexus papilloma
Choroid plexus carcinoma

Neuronal and mixed neuronal–glial tumors
Dysembryoplastic neuroepithelial tumor
Gangliocytoma
Ganglioglioma
Anaplastic ganglioglioma
Dysplastic cerebellar gangliocytoma (Lhermitte–Duclos disease)
Desmoplastic infantile astrocytoma and ganglioglioma
Papillary glioneuronal tumor
Rosette-forming glioneuronal tumor
Diffuse leptomeningeal glioneuronal tumor
Central neurocytoma
Extraventricular neurocytoma
Cerebellar liponeurocytoma
Paraganglioma

Tumors of the pineal region
Pineocytoma
Pineal parenchymal tumor of intermediate differentiation
Pineoblastoma
Papillary tumor of the pineal region

Embryonal tumors
Medulloblastomas, histologically defined
Medulloblastoma, NOS
Medulloblastoma, classic
Medulloblastoma, desmoplastic/nodular
Medulloblastoma with extensive nodularity
Medulloblastoma, large cell/anaplastic

Medulloblastomas, genetically defined
Medulloblastoma, NOS
Medulloblastoma, WNT-activated

Medulloblastoma, SHH activated
Medulloblastoma, SHH activated and TP53-mutant
Medulloblastoma, SHH activated and TP53-wildtype
Medulloblastoma, non-WNT/non-SHH
Medulloblastoma, non-WNT/non-SHH: Medulloblastoma, group 3
Medulloblastoma, non-WNT/non-SHH: Medulloblastoma, group 4

Atypical teratoid/rhabdoid tumor
Embryonal tumor with multilayered rosettes, NOS
Embryonal tumor with multilayered rosettes, C19MC-altered
Medulloepithelioma
CNS neuroblastoma
CNS ganglioneuroblastoma
CNS embryonal tumor, NOS
CNS embryonal tumor with rhabdoid features

Meningiomas
Meningioma
Angiomatous meningioma
Fibrous meningioma
Lymphoplasmacyte-rich meningioma
Meningothelial meningioma
Metaplastic meningioma
Microcystic meningioma
Psammomatous meningioma
Secretory meningioma
Transitional meningioma
Chordoid meningioma
Clear cell meningioma
Atypical meningioma
Papillary meningioma
Rhabdoid meningioma
Anaplastic (malignant) meningioma

Mesenchymal, non-meningothelial tumors
Solitary fibrous tumor/hemangiopericytoma, NOS
Solitary fibrous tumor/hemangiopericytoma, grade 1
Solitary fibrous tumor/hemangiopericytoma, grade 2
Solitary fibrous tumor/hemangiopericytoma, grade 3
Hemangioblastoma

Melanocytic tumors
Meningeal melanocytosis
Meningeal melanocytoma
Meningeal melanoma
Meningeal melanomatosis

Germ cell tumors
Germinoma
Embryonal carcinoma
Yolk sac tumor
Choriocarcinoma
Teratoma
Mature teratoma
Immature teratoma
Teratoma with malignant transformation
Mixed germ cell tumor

Tumors of the sellar region
Craniopharyngioma
Adamantinomatous craniopharyngioma
Papillary craniopharyngioma
Granular cell tumor of the sellar region
Pituicytoma
Spindle cell oncocytoma

Pituitary tumors
Pituitary adenomas
Pituitary adenoma
Corticotroph adenoma
Gonadotroph adenoma
Lactotroph adenoma
Somatotroph adenoma
Thyrotroph adenoma
Null cell adenoma
Plurihormonal and double adenomas
Pituitary carcinoma
Pituitary carcinoma

References

1. Louis DN, Ohgaki H, Wiestler OD, et al. *World Health Organization Classification of Tumours of the Central Nervous System*. Lyon, France: IARC Press; 2016.
2. Lloyd RV, Osamura RY, Klöppel G, et al. *WHO Classification of Tumours: Pathology & Genetics of Tumours of Endocrine Organs.* Lyon, France: IARC Press; 2017.

C. Histologic Grade

Below is a list of possible WHO grades for CNS tumors.[1] The WHO grading of some of the more common CNS tumors is shown in Table 1. There is no formal TNM-based classification and staging system for CNS tumors.

WHO Grades for CNS Tumors
WHO grade I
WHO grade II
WHO grade III
WHO grade IV
WHO grade not assigned

References

1. Louis DN, Ohgaki H, Wiestler OD, et al. *World Health Organization Classification of Tumours of the Central Nervous System*. Lyon, France: IARC Press; 2016.

D. Biomarker Studies

Immunohistochemical and molecular genetic studies are often performed to assist with diagnosis, prognosis, or to predict therapeutic response.[1] The most recent update of the World Health Organization's Classification of Tumours of the Central Nervous System has incorporated many of these biomarkers into this formal diagnostic classification system, thereby formally encouraging their use in the evaluation of these neoplasms. Currently, the 2016 WHO Classification of Tumours of the Central Nervous System and the 2017 (WHO) Pathology & Genetics of Tumours of Endocrine Organs incorporates molecular genetic studies into several entities while the diagnosis of the majority of CNS tumors remain largely morphologic.[1,2] It is expected that, as our understanding of the biology of CNS tumors improves, the list of entities requiring molecular genetic studies will continue to grow. For those defined entities, the use of the biomarker template is encouraged.

Additional common ancillary molecular testing in neurooncology includes *MGMT* promoter methylation studies; *ATRX* expression/mutations; *TP53* expression/mutations; copy number alterations in *EGFR* and *PTEN*; and

BRAF alterations and mutations.[3-5] For medulloblastoma, assessment of *MYC* or *MYCN* amplification and beta-catenin nuclear localization has prognostic significance.

In the absence of access to these biomarkers, the WHO has provided the “NOS” nomenclature appended to the end of the histologic diagnosis to indicate the absence of molecular testing on the individual case.

Embryonal neoplasms may benefit from ancillary studies for proper diagnostic categorization. Assigning medulloblastomas to appropriate genetic groups may be done by immunohistochemistry in most cases: WNT-activated (group 1) cases show nuclear beta-catenin and YAP1 expression; SHH-activated (group 2) cases express markers GAB1 and YAP1; groups 3 and 4 do not express neither GAB1 nor YAP1 and exhibit only nonnuclear beta-catenin immunostaining, if any.[6,7] Some copy number changes are useful for molecular grouping of medulloblastomas, but are not necessary to assess in most cases: monosomy 6 is present in the vast majority of WNT-activated cases; deletion of 9q (*PTCH* gene) is common in SHH-activated cases; loss of 17p and duplication of 17 (resulting in an “isochromosome 17q”) is limited to groups 3 and 4.[8] SHH-activated medulloblastomas can be diagnostically segregated by *TP53* mutation status; those medulloblastomas with a *TP53* mutation have a much worse prognosis.[9] Aberrant p53 immunostaining is an effective surrogate for the presence of a mutation, either as diffuse, strong nuclear reactivity or, less commonly, complete lack of nuclear expression in all tumor cells. Additional assessment for *MYC* or *MYCN* amplification for prognosis is indicated regardless of molecular group.

Any embryonal neoplasm with lumen-forming, multilayered rosettes can be tested for amplification of the C19MC region on chromosome 19.[10] The immunostain LIN28A, when strongly and diffusely positive, correlates highly with C19MC amplification, which confers a grim prognosis.[11] Medulloepitheliomas have multilayered rosettes, yet may not always exhibit C19MC amplification or LIN28 expression. Such cases should be specified as non-C19MC altered.

Embryonal tumors can be assessed for *SMARCB1/INI1* status to identify atypical teratoid/rhabdoid tumors (AT/RT), which have a significantly different treatment regimen from other CNS embryonal malignancies. This may be effectively done by demonstrating absence of SMARCB1/INI1 nuclear immunostaining in tumor cells (for example using the BAF47 antibody).[11] Morphologically rhabdoid embryonal malignancies with retained SMARCB1/INI1 nuclear expression can be assessed for loss of SMARCA4/BRG1, which is also diagnostic for AT/RT. The diagnosis "CNS embryonal tumor with rhabdoid features, NOS (WHO grade IV)" should be used when SMARCB1/INI1 or SMARCA4/BRG1 expression is retained or cannot be assessed in a malignant embryonal neoplasm with rhabdoid morphology.

Pediatric embryonal tumors in the supratentorial compartment can be tested for the H3F3A K27 or G34 mutations typically found in pediatric glioblastomas, which can display embryonal, neuroblastic morphology and immunophenotype.[13] Antibodies are available for immunohistochemical detection of both the H3K27M and the mutant proteins.[14] H3 G34-mutant glioblastomas have high rates of ATRX loss and TP53 mutations, immunostaining for which can help distinguish them from the embryonal tumors.

Supratentorial ependymomas can be tested for fusion rearrangements of the *RELA* gene, which are associated with a poor prognosis and constitute a separate diagnostic category in the WHO 2016 classification.[15] Immunostaining for L1CAM is a surrogate marker for *RELA* fusion in ependymomas, although it may also be seen in other tumor types. Gain of 1q implies worse prognosis in posterior fossa ependymomas. In posterior fossa tumors, loss of H3 K27me3 staining reliably identifies the PF-A ependymomas, which have a much worse prognosis than PF-B.[15]

The advent of DNA next generation sequencing (NGS) techniques has led to the evaluation of many more biomarkers than can be performed one at a time in most FISH or immunohistochemical laboratories. NGS also allows the evaluation of biomarkers that are too large for routine sequencing methods such as *NF1*. The capture of these data may lead to the identification of less common genetic alterations that the oncologists may identify as clinically relevant, targetable pathways, particularly in the less common tumors of childhood and young adulthood.[16] In such cases in which NGS analyses are obtained, we have left room at the end of the section to record the deviations found in these biomarkers. Similarly, research in brain tumor biomarkers is ongoing, making

the updating of this protocol a dynamic process. Such new discoveries can be added also in the additional spaces provided.

Additional biomarker information and references developed by the International Collaboration on Cancer Reporting (ICCR) may be found at http://www.iccr-cancer.org/datasets/published-datasets/central-nervous-system.[17]

The ICCR Central Nervous System Molecular Notes includes an overview of selected molecular diagnostic markers for CNS tumors:

Overview of selected molecular diagnostic markers for CNS tumours

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).

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.[17]

Test	Gliomas								Embryonal tumours			Other					
	DA, AA	O, AO	Diffuse midline glioma	Glioblastoma	Pilocytic astrocytoma	PXA, GG	Ependymoma - supratentorial	Ependymoma – posterior fossa	Medulloblastoma	AT/RT	ETMR	Extraventricular neurocytoma	Meningioma	SFT/HPC	Craniopharyngioma	MPNST	Pituitary adenomas
***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												
C19MC alteration											W						
Chromosomal arm 1p/19q codeletion		W															
Chromosome 7 gain combined with chromosome 10 loss				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		W								D*					
IDH1 R132H expression (immunohistochemistry)	W	W		W								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**										

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; 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

References
1. Louis DN, Ohgaki H, Wiestler OD, et al. *World Health Organization Classification of Tumours of the Central Nervous System*. Lyon, France: IARC Press; 2016.
2. Lloyd RV, Osamura RY, Klöppel G, et al. WHO Classification of Tumours: Pathology & Genetics of Tumours of Endocrine Organs. Lyon, France: IARC Press; 2017.
3. Nikiforova MN, Hamilton RL. Molecular diagnostics of gliomas. *Arch Pathol Lab Med.* 2011;135:558-568.
4. Taylor MD, Northcott PA, Korshunov A, et al. Molecular subgroups of medulloblastoma: the current consensus. *Acta Neuropathol.* 2012 ;123:465-472.
5. Nageswara Rao AA, Packer RJ. Impact of molecular biology studies on the understanding of brain tumors in childhood. *Curr Oncol Rep.* 2012;14:206-212.
6. Kaur K, Kakkar A, Kumar A, et al. Integrating Molecular Subclassification of Medulloblastomas into Routine Clinical Practice: A Simplified Approach. *Brain Pathol*. 2016;26:334-343.
7. Ellison DW, Dalton J, Kocak M, et al. Medulloblastoma: clinicopathological correlates of SHH, WNT, and non-SHH/WNT molecular subgroups. *Acta Neuropathol.* 2011;121:381-396.
8. Kool M, Korshunov A, Remke M, et al. Molecular subgroups of medulloblastoma: an international meta-analysis of transcriptome, genetic aberrations, and clinical data of WNT, SHH, Group 3, and Group 4 medulloblastomas. *Acta Neuropathol.* 2012;123:473-484.
9. Zhukova N, Ramaswamy V, Remke M, et al. Subgroup-specific prognostic implications of TP53 mutation in medulloblastoma. *J Clin Oncol.* 2013;31:2927-2935.
10. Korshunov A, Sturm D, Ryzhova M, et al. Embryonal tumor with abundant neuropil and true rosettes (ETANTR), ependymoblastoma, and medulloepithelioma share molecular similarity and comprise a single clinicopathological entity. *Acta Neuropathol.* 2014;128:279-289.
11. Korshunov A, Ryzhova M, Jones DT, et al. LIN28A immunoreactivity is a potent diagnostic marker of embryonal tumor with multilayered rosettes (ETMR). *Acta Neuropathol.* 2012;124:875-881.
12. Judkins AR, Mauger J, Ht A, Rorke LB, Biegel JA. Immunohistochemical analysis of hSNF5/INI1 in pediatric CNS neoplasms. *Am J Surg Pathol.* 2004;28:644-650.
13. Korshunov A, Capper D, Reuss D, et al. Histologically distinct neuroepithelial tumors with histone 3 G34 mutation are molecularly similar and comprise a single nosologic entity. *Acta Neuropathol.* 2016;131:137-46.
14. Haque F, Varlet P, Puntonet J, et al. Evaluation of a novel antibody to define histone 3.3 G34R mutant brain tumours. *Acta Neuropathol Commun*. 2017:5:45.
15. Pajtler KW, Witt H, Sill M, et al. Molecular Classification of Ependymal Tumors across All CNS Compartments, Histopathological Grades, and Age Groups. *Cancer Cell.* 2015;27:728-743.
16. Cole BL, Pritchard CC, Anderson M, et al. Targeted sequencing of malignant supratentorial pediatric brain tumors demonstrates a high frequency of clinically relevant mutations. *Pediatr Dev Pathol.* 2017 Jan 1:1093526617743905. doi: 10.1177/1093526617743905.
17. http://www.iccr-cancer.org/datasets/published-datasets/central-nervous-system.

E. Relevant History

Previous Diagnoses or CNS Biopsies

Knowledge of the presence or absence of previous intracranial or extracranial disease (eg, immunosuppression, previous CNS or other primary neoplasm) is essential for specimen interpretation. If a previous tumor is included in the differential diagnosis, it is useful to have microscopic slides of the lesion available for review and comparison.[1,2]

Family History of Cancer or Primary CNS Tumors

Several genetic conditions/syndromes are associated with an increased predisposition to the development of specific forms of CNS neoplasms (eg, neurofibromatosis types 1 and 2, Turcot/Lynch, tuberous sclerosis, von Hippel-Lindau, Cowden, Li-Fraumeni, and Gorlin syndromes).[3,4]

References
1. Burger PC, Scheithauer BW, Vogel FS. *Surgical Pathology of the Nervous System and Its Coverings.* 4th ed. New York: Churchill Livingstone; 2002.
2. Perry A, Brat DJ. Practical Surgical Pathology: A Diagnostic Approach. Philadelphia: Elsevier; 2010.

3. McLendon RE, Rosenblum MK, Bigner DD, eds. *Russell and Rubinstein's Pathology of Tumors of the Nervous System*. 7th ed. New York: Hodder Arnold; 2006.
4. Burger PC, Scheithauer BW. *Atlas of Tumor Pathology, Third Series. Tumors of the Central Nervous System.* Washington, DC: Armed Forces Institute of Pathology; 2003.

F. Neuroimaging Findings

Knowledge of neuroimaging features is extremely helpful in specimen interpretation.[1] A differential diagnosis may be generated based on patient age, tumor location, and neuroimaging features. Neuroimaging also can be helpful in providing correlation with or highlighting discrepancy with pathologic diagnosis (e.g., contrast enhancement with hypocellularity). A close collaboration with the neuroradiologist and neurosurgeon is essential.

References

1. Vincentelli C, Hwang SN, Holder CA, Brat DJ. The use of neuroimaging to guide the histologic diagnosis of central nervous system lesions. *Adv Anat Pathol.* 2012;19:97-107.

G. Procedure

It is useful to know if the specimen was procured by open craniotomy or stereotactic biopsy. Since tumors may be heterogeneous, adequate sampling is an issue. The reliability of the prognostic information derived from such specimens may vary depending on how the specimen was obtained.

Specimen Handling, Triage, and Special Procedures

(While the reporting of specimen handling is not required in this protocol, the following information may be helpful.)
It may be necessary to divide biopsy/resection tissue into portions for the following procedures:

- Squash/smear/touch preparations
- Frozen sections
- Unfrozen, routine, permanent paraffin sections (essential to avoid artifacts of freezing tissue)
- Electron microscopy (retain a small portion in glutaraldehyde, or "embed and hold" for electron microscopy, if necessary)
- Frozen tissue, for possible molecular diagnostic studies (freeze fresh tissue as soon as possible and store)
- Other (microbiology, flow cytometry, cytogenetics, molecular diagnostics)

Since cytologic details are essential for interpreting CNS neoplasms, previously frozen tissue with its inherent artifacts is suboptimal, especially for subclassifying and grading gliomas. Recommendations for optimal freezing and frozen sections from CNS tissue have been published.[1] It is imperative to retain tissue that has not been previously frozen for permanent sections. Avoid using sponges in cassettes because they produce angular defects that resemble vascular/luminal spaces in the final sections. It is more appropriate to wrap small biopsies in lens paper or into tissue sacs prior to submitting in cassettes. If frozen and permanent sections are nondiagnostic, tissue that was retained in glutaraldehyde may be submitted for additional paraffin sections.

In touch, smear, and squash preparations, the presence of cells with long delicate processes is suggestive of a primary CNS cell type. The identification of macrophages is important since a macrophage-rich lesion is more likely a subacute infarct or demyelination, rather than a neoplasm.

If an infectious etiology is suspected, the neurosurgeon should be alerted to submit a fresh sample to microbiology to be processed for bacterial, fungal, and/or viral cultures.

If a lymphoproliferative disorder is suspected and sufficient tissue is available, a portion of fresh tissue should be set aside for appropriate workup.

References

1. Burger PC, Nelson JS. Stereotactic brain biopsies: specimen preparation and evaluation. *Arch Pathol Lab Med.* 1997;121:477-480.

H. Specimen Size

For most CNS tumors, specimen size is not used for staging or grading. However, in heterogeneous lesions, tissue sampling may become important, and the size of the biopsy relative to the overall size of the lesion provides useful information concerning whether the sample is representative of the overall lesion. The total specimen size may not correspond to the tumor size within the specimen, and this discrepancy should be noted. The protocol may not be applicable to biopsy specimen if the tissue sample is limited.

Table 1. WHO Grading System for Some of the More Common Tumors of the CNS[1,2]

Tumor Group	Tumor Type	Grade			
		I	II	III	IV
Diffuse astrocytic and oligodendroglial tumors	Diffuse astrocytoma, IDH-mutant		X		
	Anaplastic astrocytoma, IDH-mutant			X	
	Glioblastoma, IDH-wildtype				X
	Glioblastoma, IDH-mutant				X
	Oligodendroglioma, IDH-mutant and 1p/19q-codeleted		X		
	Anaplastic oligodendroglioma, IDH-mutant and 1p/19q-codeleted			X	
Other astrocytic tumors	Pilocytic astrocytoma	X			
	Subependymal giant cell astrocytoma	X			
	Pleomorphic xanthoastrocytoma		X		
	Anaplastic pleomorphic xanthoastrocytoma			X	
Ependymal tumors	Subependymoma	X			
	Myxopapillary ependymoma	X			
	Ependymoma		X		
	Ependymoma, RELA fusion-positive		X	X	
	Anaplastic ependymoma			X	
Other gliomas	Angiocentric glioma	X			
	Chordoid glioma of the third ventricle		X		
Choroid plexus tumors	Choroid plexus papilloma	X			
	Atypical choroid plexus papilloma		X		
	Choroid plexus carcinoma			X	
Neuronal and mixed neuronal–glial tumors	Dysembryoplastic neuroepithelial tumor	X			
	Gangliocytoma	X			
	Ganglioglioma	X			
	Anaplastic ganglioglioma			X	
	Central neurocytoma		X		
	Extraventricular neurocytoma		X		
	Cerebellar liponeurocytoma		X		
Tumors of the pineal region	Pineocytoma	X			
	Pineal parenchymal tumor of intermediate		X	X	
	Pinealoblastoma				X
	Papillary tumor of the pineal region		X	X	
Embryonal tumors	Medulloblastoma (all subtypes)				X
	Embryonal tumor with multilayered rosettes				X
	Medulloepithelioma				X
	CNS embryonal tumor, NOS				X
	Atypical teratoid/rhabdoid tumor				X
	CNS embryonal tumor with rhabdoid features				X
Meningiomas	Meningioma	X			
	Atypical meningioma		X		
	Anaplastic (malignant) meningioma			X	
Mesenchymal, non-	Solitary fibrous tumor/hemangiopericytoma	X	X	X	

meningothelial tumors	Hemangioblastoma	X			
Tumors of the sellar region	Craniopharyngioma	X			
	Granular cell tumor of the sellar region	X			
	Pituicytoma	X			
	Spindle cell oncocytoma	X			

Tumor histology and grade are strong predictors of clinical behavior for astrocytomas and meningiomas. Tables 2 and 3 list the grading criteria for these common CNS tumor types.[1]

Table 2. 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 endothelial proliferation and/or necrosis

Table 3. WHO Grading of Meningiomas

WHO grade I Benign meningioma
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

References

1. Louis DN, Ohgaki H, Wiestler OD, et al. *World Health Organization Classification of Tumours of the Central Nervous System*. Lyon, France: IARC Press; 2016.
2. Lloyd RV, Osamura RY, Klöppel G, et al. *WHO Classification of Tumours: Pathology & Genetics of Tumours of Endocrine Organs.* Lyon, France: IARC Press; 2017.

I. Primary Tumor Site, Laterality, and Focality

Since the anatomic site of a neoplasm may correlate with tumor type and prognosis, it should be recorded, if known.

- For skull location, specify bone involved, such as frontal, parietal, temporal, occipital, etc, if known. The College of American Pathologists (CAP) cancer protocol for bone should be used for primary tumors of bone.[1]
- For dural location, indicate cerebral convexity/lobe, falx, tentorium, posterior fossa, sphenoid wing, skull base, spinal, or other, if known.
- For leptomeningeal location, indicate cerebral convexity/lobe, posterior fossa, spinal, or other, if known.
- For cerebral lobe location, indicate frontal, temporal, parietal, or occipital lobe, if known. For a deep gray matter location, indicate basal ganglia, thalamus, or hypothalamus.
- For an intraventricular location, indicate lateral, third, fourth, or cerebral aqueduct, if known.
- For a brain stem location, indicate midbrain, pons, or medulla, if known.
- For spine (vertebral bone), spinal cord, spinal root or spinal ganglion, indicate level (eg, C5, T2, L3), if known. The CAP cancer protocol for bone should be used for primary tumors of bone.[1]

The laterality of a neoplasm should be indicated as involving the left or right side of the CNS structure. In some instances, such as tumors arising in the pineal, pituitary, third ventricular, and other locations, the tumor will be situated in the midline. A tumor would be considered bilateral if it involved both sides of the brain, such as glioblastoma extending through the corpus callosum to involve the left and right hemispheres. The focality of a lesion should be indicated, if possible. Multifocality implies that multiple, noncontiguous lesions are noted on neuroimaging, such as might be seen in primary CNS lymphoma. A solitary lesion would be considered unifocal.

Margins

Resection margins provide no prognostic information and generally are not required for most CNS neoplasms.

References

1. Laurini JA, Antonescu CR, Cooper K, et al. Protocol for the examination of specimens from patients with tumors of bone. 2017. Available at www.cap.org/cancerprotocols.

J. Preoperative Treatment and Treatment Effect

Knowledge of preoperative treatment, including radiation therapy, chemotherapy, corticosteroid therapy, embolization, and other therapy, is helpful for specimen interpretation.[1-3] In particular, prior radiation therapy or radiosurgery may alter the interpretation of specimens in which there are increased cellular atypia, decreased proliferative activity, or large areas of radiation-induced change (e.g., coagulative [nonpalisading] necrosis, vascular hyalinization, and gliosis). The addition of chemotherapy to radiation may further alter histomorphological appearance. For patients with malignant gliomas, the presence and degree of radiation necrosis appear to be of prognostic significance. Tumors that show evidence of radiation necrosis are associated with a longer survival, and the degree of necrosis appears to be prognostically significant.[4] Corticosteroid treatment can alter the pathologic features of some CNS diseases. In particular, the treatment of primary CNS lymphoma with corticosteroids can be associated with widespread tumor necrosis or infiltration by macrophages, which may limit or misguide interpretation. Embolization of certain tumor types, especially meningiomas, may introduce histologic changes in the neoplasm.

References

1. Burger PC, Scheithauer BW, Vogel FS. *Surgical Pathology of the Nervous System and Its Coverings.* 4th ed. New York: Churchill Livingstone; 2002.
2. Perry A, Brat DJ. Practical Surgical Pathology: A Diagnostic Approach. Philadelphia: Elsevier; 2010.
3. McLendon RE, Rosenblum MK, Bigner DD, eds. *Russell and Rubinstein's Pathology of Tumors of the Nervous System*. 7th ed. New York: Hodder Arnold; 2006.
4. Forsyth PA, Kelly PJ, Cascino TL, et al. Radiation necrosis or glioma recurrence: is computer-assisted stereotactic biopsy useful? *J Neurosurg.* 1995;82:436-444.