COLLEGE of AMERICAN PATHOLOGISTS

Template for Reporting Results of Biomarker Testing of Specimens From Patients With Tumors of the Central Nervous System

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CAP CNS Biomarker Template Revision History

Version Code
The definition of the version code can be found at www.cap.org/cancerprotocols.

Version: CNS_Biomarkers 1.0.0.0

Summary of Changes
This is a new template.

CNS Biomarker Reporting Template

Template web posting date: December 2014

Completion of the template is the responsibility of the laboratory performing the biomarker testing and/or providing the interpretation. When both testing and interpretation are performed elsewhere (eg, a reference laboratory), synoptic reporting of the results by the laboratory submitting the tissue for testing is also encouraged to ensure that all information is included in the patient's medical record and thus readily available to the treating clinical team.

CENTRAL NERVOUS SYSTEM (CNS)

Select a single response unless otherwise indicated.

Note: *Use of this template is optional.*

+ RESULTS

+ GLIOMAS

+ *IDH1/2* Mutation
+ ___ Present (specify): ______________________
+ ___ Absent
+ ___ Cannot be determined (explain): ______________________

+ IDH1 R132H Immunohistochemistry
+ ___ Positive
+ ___ Negative
+ ___ Cannot be determined (explain): ______________________

+ 1p/19q Deletion
+ ___ 1p/19q co-deletion
+ ___ 1p only deleted
+ ___ 19q only deleted
+ ___ Polysomy (specify): ______________________
+ ___ Monosomy (specify): ______________________
+ ___ None detected
+ ___ Cannot be determined (explain): ______________________

+ *TP53* Mutation
+ ___ Present (specify): ______________________
+ ___ Absent
+ ___ Cannot be determined (explain): ______________________

+ *ATRX* Mutation
+ ___ Present (specify): ______________________
+ ___ Absent
+ ___ Cannot be determined (explain): ______________________

+ ATRX Immunohistochemistry
+ ___ Loss of nuclear expression
+ ___ Intact nuclear expression
+ ___ Cannot be determined (explain): ______________________

+ *EGFR* Amplification
+ ___ Present
+ ___ Absent
+ ___ Cannot be determined (explain): ______________________

+ 10q23 *(PTEN* Locus) Deletion
+ ___ Deletion identified
+ ___ Polysomy (specify): ______________________
+ ___ Monosomy (specify): ______________________
+ ___ None detected
+ ___ Cannot be determined (explain): ______________________

+ *PTEN* Mutation
+ ___ Present (specify): ______________________
+ ___ Absent
+ ___ Cannot be determined (explain): ______________________

+ *MGMT* Promoter Methylation
+ ___ Present
If laboratory reports by level:
    + ___ Low level
    + ___ High level
+ ___ Absent
+ ___ Cannot be determined (explain): ______________________

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

+ BRAF V600E Immunohistochemistry
+ ___ Positive
+ ___ Negative
+ ___ Cannot be determined (explain): ______________________

+ *BRAF* Rearrangement
+ ___ Present
+ ___ Absent
+ ___ Cannot be determined (explain): ______________________

+ Ki-67
+ Percentage of positive nuclei: ____ %

+ <u>**EMBRYONAL TUMORS**</u>

+ Nuclear Beta-Catenin Immunohistochemistry
+ ___ Positive (nuclear staining in at least 50% of tumor cells)
+ ___ Negative (no staining or nuclear staining in <50% of tumor cells)
+ ___ Cannot be determined (explain): ______________________

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

+ GAB1 Immunohistochemistry
+ ___ Positive
+ ___ Negative
+ ___ Cannot be determined (explain): ______________________

+ *MYC* Amplification
+ ___ Present
+ ___ Absent
+ ___ Cannot be determined (explain): ______________________

+ *MYCN* Amplification
+ ___ Present
+ ___ Absent
+ ___ Cannot be determined (explain): ______________________

+ Isochromosome 17 (i17q)
+ ___ Present
+ ___ Absent
+ ___ Cannot be determined (explain): ______________________

+ INI1 (BAF47) Immunohistochemistry
+ ___ Loss of nuclear expression
+ ___ Intact nuclear expression
+ ___ Cannot be determined (explain): ______________________

+ *SMARCB1/INI1/HNSF5* Mutation
+ ___ Present (specify): ______________________
+ ___ Absent (*SMARCB1/INI1/HNSF5*)
+ ___ Cannot be determined (explain): ______________________

+ METHODS

+ <u>**GLIOMAS**</u>

+ *IDH1/2* Mutational Analysis
+ <u>Testing Method</u> (select all that apply)
+ ___ Direct Sanger sequencing
+ ___ Pyrosequencing
+ ___ Polymerase chain reaction (PCR), allele-specific hybridization

+ Data elements preceded by this symbol are not required.

+ ___ Real-time PCR
+ ___ High-throughput next-generation sequencing
+ ___ Other (specify): ____________________

+ Immunohistochemistry for IDH1 R132H
+ Primary Antibody
+ ___ H09
+ ___ Other (specify): ______________________

+ 1p/19q Deletion Analysis
+ Testing Method (select all that apply)
+ ___ In situ hybridization
+ ___ Cytogenomic microarray (CMA)
+ ___ Loss of heterozygosity
+ ___ Other (specify): ________________________

+ *TP53* Mutational Analysis
+ Testing Method (select all that apply)
+ ___ Direct Sanger sequencing
+ ___ Pyrosequencing
+ ___ PCR, allele-specific hybridization
+ ___ Real-time PCR
+ ___ High-throughput next-generation sequencing
+ ___ Other (specify): ______________________

+ *ATRX* Mutational Analysis
+ Testing Method (select all that apply)
+ ___ Direct Sanger sequencing
+ ___ Pyrosequencing
+ ___ PCR, allele-specific hybridization
+ ___ Real-time PCR
+ ___ High-throughput next-generation sequencing
+ ___ Other (specify): ______________________

+ Immunohistochemistry for ATRX
+ Primary Antibody
+ Specify: ______________________

+ *EGFR* Amplification Analysis
+ Testing Method (select all that apply)
+ ___ In situ hybridization
+ ___ Cytogenomic microarray (CMA)
+ ___ Other (specify): ______________________

+ Chromosome 10q23 (*PTEN* Locus) Deletion Analysis
+ Testing Method (select all that apply)
+ ___ In situ hybridization
+ ___ Cytogenomic microarray (CMA)
+ ___ Loss of heterozygosity
+ ___ Other (specify): ______________________

+ *PTEN* Mutational Analysis
+ Testing Method (select all that apply)
+ ___ Direct Sanger sequencing
+ ___ Pyrosequencing
+ ___ PCR, allele-specific hybridization
+ ___ Real-time PCR
+ ___ High-throughput next-generation sequencing
+ ___ Other (specify): ______________________

+ *MGMT* Promoter Methylation
+ Testing Method (select all that apply)
+ ___ Methylation-specific PCR
+ ___ Other (specify): ______________________

+ *BRAF* V600E Mutational Analysis
+ Mutations Assessed (select all that apply)
+ ___ V600E
+ ___ Any mutation in exon 15
+ ___ Other (specify): ______________________

+ Testing Method (select all that apply)
+ ___ Direct Sanger sequencing
+ ___ Pyrosequencing
+ ___ PCR, allele-specific hybridization
+ ___ Real-time PCR
+ ___ High-throughput next-generation sequencing
+ ___ Other (specify): ______________________

+ Immunohistochemistry for BRAF V600E
+ Primary Antibody
+ ___ VE1
+ ___ Other (specify): ______________________

+ *BRAF* Rearrangement Analysis
+ Testing Method (select all that apply)
+ ___ In situ hybridization
+ ___ Cytogenomic microarray (CMA)
+ ___ Real-time PCR
+ ___ Other (specify): ______________________

+ Immunohistochemistry for Ki-67
+ Primary Antibody
+ ___ MIB1
+ ___ SP6
+ ___ Other (specify): ______________________

+ EMBRYONAL TUMORS

+ Immunohistochemistry for Beta-Catenin
+ Primary Antibody
+ ___ E-5
+ ___ 14

+ ___ Beta-catenin-1
+ ___ Other (specify): ________________________

+ Monosomy 6 Analysis
+ <u>Testing Method</u> (select all that apply)
+ ___ In situ hybridization
+ ___ Cytogenomic microarray (CMA)
+ ___ Other (specify): ________________________

+ Immunohistochemistry for GAB1
+ <u>Primary Antibody</u>
+ Specify: ________________________

+ *MYC* Amplification Analysis
+ <u>Testing Method</u> (select all that apply)
+ ___ In situ hybridization
+ ___ Cytogenomic microarray (CMA)
+ ___ Other (specify): ________________________

+ *MYCN* Amplification Analysis
+ <u>Testing Method</u> (select all that apply)
+ ___ In situ hybridization
+ ___ Cytogenomic microarray (CMA)
+ ___ Other (specify): ________________________

+ Isochromosome 17 (i17q) Analysis
+ <u>Testing Method</u> (select all that apply)
+ ___ In situ hybridization
+ ___ Cytogenomic microarray (CMA)
+ ___ Other (specify): ________________________

+ Immunohistochemistry for INI1 (BAF47)
+ <u>Primary Antibody</u>
+ ___ MRQ-27
+ ___ 25/BAF47
+ ___ Other (specify): ________________________

+ *SMARCB1/INI1/HNSF5* Mutational Analysis
+ <u>Testing Method</u> (select all that apply)
+ ___ Direct Sanger sequencing
+ ___ Pyrosequencing
+ ___ PCR, allele-specific hybridization
+ ___ Real-time PCR
+ ___ High-throughput next-generation sequencing
+ ___ Other (specify): ______________________

+ Comments:

____
____

Explanatory Notes

The diagnosis of central nervous system (CNS) tumors increasingly relies on molecular genetic applications to aid in classification, offer prognostic value, and predict response to therapy.[1-6] These applications may assess genetic losses, amplifications, translocations, mutations, or the expression levels of specific gene transcripts or proteins. Molecular diagnostics is quickly transitioning from testing for one biomarker at a time to a panel-based approach and whole genome analysis. Frequently employed methods for genetic testing are gene sequencing, fluorescence in situ hybridization (FISH), and cytogenomic microarray (CMA). In some cases, immunohistochemistry can be used as a surrogate for genetic analysis when the marker gene is consistently overexpressed or underexpressed**.** This template for reporting results of biomarker testing for CNS tumors represents a common framework for the reporting of molecular findings relevant to these diseases and does not advocate their specific application.

GLIOMAS

Isocitrate Dehydrogenase (IDH)

Isocitrate dehydrogenase (IDH) is an enzyme that exists in 5 isoforms, each of which catalyzes the reaction of isocitrate to α-ketoglutarate.[7] The finding of mutations in *IDH1* and *IDH2* in diffuse gliomas has dramatically changed the practice of neuropathology and neurooncology. Mutations in *IDH1* are frequent (70%-80%) in World Health Organization (WHO) grade II and III astrocytomas, oligodendrogliomas, and oligoastrocytomas, as well as glioblastomas (GBMs; WHO grade IV) that have progressed from these lower grade neoplasms (secondary GBMs).[8] Mutations in *IDH2* have also been detected in these same tumor types, but much less frequently. *IDH* mutations are infrequent in de novo GBMs. 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.[9] Inhibition of the family of histone demethylases and the TET family of 5-methylcytosine hydroxylases has profound effect on the epigenetic status of mutated cells and leads directly to a hypermethylator phenotype that has been referred to as the CpG island methylator phenotype (G-CIMP).[10] The finding of *IDH* mutations in an infiltrating glioma is associated with substantially improved prognosis, grade for grade. Indeed, *IDH* mutant GBMs, WHO grade IV, are associated with longer survivals than *IDH* wild-type anaplastic astrocytomas, WHO grade III. Over 90% of *IDH1* mutations in diffuse gliomas occur at a specific site and are characterized by a base exchange of guanine to adenine within codon 132, resulting in an amino acid change from arginine to histidine (R132H). Because of this consistent protein alteration, a monoclonal antibody has been developed to the mutant protein, allowing its use in paraffin-embedded specimens (mIDH1R132H).[11] The ability of the antibody to detect a small number of cells as mutant may make this method more sensitive than sequencing for identifying *R132H* mutant gliomas. However, mutations in *IDH2* and other mutations in *IDH1* will not be detected using immunohistochemistry with this antibody.

1p/19q

One of the best studied relationships between genetic alterations and glioma histology is the strong association of allelic losses on chromosomes 1p and 19q and the oligodendroglioma phenotype.[12,13] Approximately 60% to 80% of oligodendroglial neoplasms demonstrate combined 1p and 19q losses, and those oligodendrogliomas that are morphologically classic have even higher frequencies.[14] Most studies have indicated that combined losses of 1p and 19q are specific to oligodendrogliomas, with only few astrocytomas and a small subset of oligoastrocytomas harboring these alterations. Those oligodendrogliomas with 1p/19q loss show enhanced response to chemotherapy and are associated with prolonged survival. Co-deletion of 1p/19q occurs by an unbalanced translocation after which only one copy of the short arm of chromosome 1 and one copy of the long arm of chromosome 19 remain and der(1;19) (q10;p10) is produced.[15] Solitary losses of 1p or 19q are also occasionally noted within an infiltrating glioma, but are not as strongly linked to the oligodendroglioma histology and are not predictive of enhanced response to therapy or prolonged survival.[13] Polysomy of 1p, 19q or both is also noted in a subset of oligodendrogliomas and has been associated with a poor prognosis, independent of deletion status.[16, 17] Co-deletion of 1p/19q is highly associated with the *IDH1* mutation, with over 80% of 1p/19q co-deleted oligodendrogliomas also carrying the *IDH1* mutation.[18] Oligodendrogliomas of grades II and III that have 1p/19q co-deletion also have a high frequency of *TERT* promoter mutations, *CIC* mutations on the remaining chromosome 1p allele and *FUBP1* mutation on the remaining 19q allele.[18,19]

TP53

Mutations of *TP53* are found in over 60% to 80% of infiltrative astrocytomas, anaplastic astrocytomas and secondary GBMs, yet are rare in oligodendrogliomas.[8,20,21] The vast majority of diffuse astrocytomas that have *IDH* mutations also harbor a *TP53* mutation.[22] In one study, 80% of anaplastic astrocytomas and GBMs that had an *IDH1* or *IDH2* mutation also carried a *TP53* mutation. Conversely, *TP53* mutations were identified in only 18% of high-grade astrocytomas that lacked an *IDH1* or *IDH2* mutation.[8] Thus, there is a strong association between *IDH1* mutation and *TP53* mutation in diffuse astrocytomas, and this combination of mutations is helpful in distinguishing astrocytomas from oligodendroglimas. Immunohistochemical reactivity for the p53 protein is often used as a marker for astrocytic differentiation in diffuse gliomas, since the mutant protein is degraded more slowly and accumulates in the nucleus of tumor cells. This immunostain reacts with both the normal and mutant forms of p53 and therefore is not entirely specific for *TP53* mutations.[23]

ATRX

IDH1 mutation and *TP53* mutation in infiltrating gliomas are strongly associated with inactivating alterations in *Alpha Thalassemia/Mental Retardation Syndrome X-linked* (*ATRX*), a gene that encodes a protein involved in chromatin remodeling.[22,24] *ATRX* mutations are a marker of astrocytic lineage among the *IDH* mutant gliomas and are mutually exclusive with 1p/19q codeletion. Mutations are most frequent in grade II (67%) and grade III (73%) astrocytomas and secondary GBMs (57%), while they are uncommon in primary GBMs and oligodendrogliomas. Nearly all diffuse gliomas with *IDH* and *ATRX* mutations also harbor *TP53* mutation and are associated with the alternative lengthening of telomeres (ALT) phenotype.[24] Immunohistochemistry for ATRX demonstrates a loss of protein expression in neoplastic cells that harbor inactivating mutations, while expression is retained in nonneoplastic cells within the sample (eg, endothelial cells).[25,26]

EGFR

Epidermal growth factor receptor (EGFR) is a transmembrane receptor tyrosine kinase, whose ligands include EGF and TGF-α. *EGFR* is the most frequently amplified oncogene in astrocytic tumors, being amplified in over 40% of all GBMs and less frequently in anaplastic astrocytomas (5%-10%).[27] *EGFR* amplification is much more frequent in de novo (primary) GBMs than in secondary GBMs.[28] Approximately one-half of those GBMs with *EGFR* amplification also have specific *EGFR* mutations (the *vIII* mutant), which produce a truncated transmembrane receptor with constitutive activity. Both *EGFR* amplification and the *EGFRvIII* mutant are mutually exclusive with *IDH* mutations. *EGFR* amplification is specific to those gliomas that are astrocytic in differentiation and of higher grade, such as anaplastic astrocytoma, WHO grade III, and GBM, WHO grade IV.[29] This molecular finding can be useful in distinguishing the morphologically similar small cell GBM, which harbor the amplification, from anaplastic oligodendrogliomas, which does not.[30]

PTEN and LOH Chromosome 10

Loss of the entire chromosome (monosomy), deletions, and copy neutral loss of heterozygosity (LOH) of chromosome 10 occurs in 60% to 95% of GBMs and less frequently in grade II or III diffuse astrocytomas.[28] Loss of large regions at 10p, 10q23 and 10q25-26 loci, or loss of an entire copy of chromosome 10 are the most frequent genetic alterations in GBMs.[1] Loss of the long arm, which occurs more frequently than the short arm in GBMs, occurs equally in primary and secondary GBMs. The *PTEN* gene at 10q23.3 has been most strongly implicated as a glioma-related tumor suppressor on chromosome 10q, with *PTEN* mutations identified in about 25% of GBMs and less frequently in anaplastic astrocytomas, WHO grade III.[29] *PTEN* mutations are more common in primary GBMs than secondary GBMs. Losses on chromosome 10 and mutations in *PTEN* are considered to be specific for astrocytic differentiation and are rare in oligodendrogliomas. They are also markers of high-grade progression and aggressive clinical behavior in astrocytomas.[4,31] The clinical significance of polysomy involving chromosome 10 is not fully understood.

MGMT

The current standard therapy for GBM includes radiation and chemotherapy with temozolomide, which acts by crosslinking DNA by alkylating multiple sites including the O^6 position of guanine.[32] DNA crosslinking at the O^6 position of guanine is reversed by the DNA repair enzyme MGMT (O^6-methylguanine-DNA methyltransferase). Thus, low levels of MGMT expression by GBM cells would be expected to be associated with an enhanced response to alkylating agents. The expression level of MGMT is determined in large part by the methylation status of the gene's promoter. This "epigenetic silencing" of *MGMT* occurs in 40% to 50% of GBMs and can be assessed by its promoter methylation status on PCR-based tests of genomic DNA. Some laboratories report the promoter methylation status as "low level" and "high level," or indicate that "partial methylation" is present, yet the clinical implications of this distinction are not fully understood. Most investigations have shown that epigenetic gene silencing of *MGMT* is a strong predictor of prolonged survival, independent of other clinical factors or treatment.[33] It has also been demonstrated that *MGMT* promoter methylation is associated with prolonged progression-free and overall survival in patients with GBM treated with chemotherapy and radiation therapy. [33, 34]

BRAF

Genomic alterations involving *BRAF* are common in sporadic cases of pilocytic astrocytoma and result in the downstream activation of the ERK/MAPK pathway.[2] *BRAF* activation in pilocytic astrocytoma occurs most commonly through a gene fusion between *KIAA1549* and *BRAF*, producing a fusion protein that lacks the BRAF regulatory domain and demonstrates constitutive activity.[35] This fusion is seen in the majority of cerebellar and midline pilocytic astrocytomas, but is present at lower frequency in cerebral tumors.[36] Cerebral hemispheric pilocytic astrocytomas are more likely to harbor activating *BRAF* V600E point mutations. Other genomic alterations in pilocytic astrocytomas include other *BRAF* gene fusions, *RAF1* rearrangements, and *RAS* mutations, but these are less common. Given the role of neurofibromatosis 1 (NF1) deficiency in activating the ERK/MAPK pathway, *BRAF* genomic alterations are uncommon in pilocytic astrocytoma associated with NF1. *BRAF* point mutations (V600E) are also observed in other low-grade gliomas and glioneuronal neoplasms, including approximately two-thirds of pleomorphic xanthoastrocytomas (PXAs) and lower percentages of ganglioglioma, desmoplastic infantile ganglioglioma (DIG), and dysembryoplastic neuroepithelial tumor (DNT).[37] While these tumor types are most frequently encountered in children, they are also occasionally seen in adults and have similar *BRAF* mutations. Although less common, diffusely infiltrative gliomas including GBM, particularly the epithelioid variant, may also demonstrate the V600E mutation.[27,38-40] More recently, *BRAF* mutations have been identified in papillary craniopharyngiomas.[41]

Ki-67

The most reliable and technically feasible marker of proliferation for gliomas is Ki-67, a nuclear antigen expressed in cells actively engaged in the cell cycle but not expressed in the resting phase, G0.[5] Results are expressed as a percentage of positive staining tumor cell nuclei (Ki-67 labeling index). Numerous investigations have demonstrated a positive correlation between Ki-67 indices and histologic grade for astrocytomas, oligodendrogliomas, and oligoastrocytomas.[42,43] Among grade II and III diffuse gliomas, the Ki-67 index provides prognostic value, as there is a strong inverse relation with survival on multivariate analysis.[42] In contrast, investigations of Ki-67 proliferation on patient outcome for GBM, WHO grade IV, have consistently concluded that it does not provide prognostic value in this set of tumors.[44] One potential shortcoming of Ki-67 as a marker is the high degree of variability in tissue processing, immunohistochemical staining, and quantization techniques between laboratories, making it difficult to standardize proliferation indices.[45] Large variations in proliferation rates within a single tumor may also be noted. Nonetheless, when interpreted uniformly within a given laboratory, the Ki-67 proliferation index provides prognostic value to clinicians and can be helpful in histologically borderline cases, such as those that are at the grade II to III and III to IV border. A high labeling index in this setting may indicate a more aggressive neoplasm.

EMBRYONAL TUMORS

Medulloblastoma Markers

Medulloblastomas are primitive embryonal neoplasms of the cerebellum, generally arising in childhood, whose molecular genetic alterations have now been well defined. Four subgroups have been described based on gene expression profiles: wingless (WNT), sonic hedgehog (SHH), "group 3," and "group 4."[3, 46] WNT medulloblastomas display monosomy 6 and most also show nuclear accumulation of the WNT pathway protein beta-catenin, the latter serving as a useful immunohistochemical screen for this group.[47] Medulloblastomas with >50% nuclear staining for beta-catenin have been shown to have WNT pathway activation, *CTNNB1* mutations, and monosomy 6, whereas those with only focal nuclear staining do not.[48] The overall survivals for WNT pathway medulloblastomas are dramatically longer than those of the other subtypes, and clinical practices are changing in light of this.[49] SHH medulloblastomas often show a nodular/desmoplastic histology and are associated with a better prognosis in younger children and infants. 9q deletion is characteristic of the SHH group, and *MYCN* amplifications are occasionally noted. GAB1 is expressed in the cytoplasm of nearly all SHH medulloblastomas but not in other groups and can be detected immunohistochemically, making it a valuable SHH-group marker.[47] Targeted therapies directed at this subgroup have been established and are entering clinical practice.[50,51] Group 3 has the worst overall prognosis and contains the vast majority of *MYC* amplified tumors. *MYC* and *MYCN* amplification are strong negative prognostic factors, although they occur in only a small percentage of cases.[49] Approximately 30% to 40% of all medulloblastomas have i(17q), making it the most common genetic defect. Those tumors with i(17q) have a worse prognosis than those that don't. Among the genetic markers for medulloblastoma, monosomy 6 (or nuclear beta-catenin immunoreactivity), GAB1 expression, *MYC* or *MYCN* amplification, and i(17q) appear to be the most reliable and carry the strongest prognostic and therapeutic implications.

INI1

The atypical teratoid/rhabdoid tumor (AT/RT) is a clinically aggressive embryonal tumor of infancy that occurs in the posterior fossa and cerebral hemispheres.[6] The tumor is characterized by deletions and mutations of *SMARCB1/INI1* (*HSNF5*) (22q11.2).[52,53] Immunohistochemical evaluation of AT/RT for the INI1 protein (using the BAF47 antibody) shows a loss of labeling in tumor cell nuclei, but retention of nuclear labeling in nonneoplastic cells, such as endothelial cells. The recognition of AT/RT is important for clinical management, since AT/RTs have morphologic overlap with medulloblastoma, CNS primitive neuroectodermal tumor (PNET), choroid plexus carcinoma, GBM, and other malignant tumors of childhood.[54] The diagnosis of AT/RT and the finding of *SMARCB1/INI1* loss or mutation also carry potential implications for inheritance. These tumors are often a component of the rhabdoid tumor predisposition syndrome (RTPS), characterized by germline mutations of *SMARCB1/INI1* and manifested by a marked predisposition to the development of malignant rhabdoid tumors of infancy and early childhood.[52,55] Up to one-third of AT/RTs arise in the setting of RTPS, and the majority of these occur within the first year of life.[56] The diagnosis of RTPS is established with certainty by sequencing of *SMARCB1/INI1* on tissue representing the patient's germline. Because of the risk associated with the RTPS, the germline status of *SMARCB1/INI1* is typically assessed for each new case of AT/RT.

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