

Sarra M. Belakhoua, MD*[‡]

Fausto J. Rodriguez, MD   

*Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA; [‡]School of Medicine, University of Tunis El Manar, Tunis, Tunisia; [§]Department of Ophthalmology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA; [¶]Sydney Kimmel Cancer Center, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Correspondence:

Fausto J. Rodriguez, MD,
Johns Hopkins University School of
Medicine,
Sheikh Zayed Tower, Room M2101,
1800 Orleans St,
Baltimore, MD 21231, USA.
Email: frodrig4@jhmi.edu

Received, May 15, 2021.

Accepted, September 9, 2021.

Published Online, February 16, 2021.

© Congress of Neurological Surgeons
2021. All rights reserved.

For permissions, please e-mail:
journals.permissions@oup.com

Diagnostic Pathology of Tumors of Peripheral Nerve

Neoplasms of the peripheral nervous system represent a heterogeneous group with a wide spectrum of morphological features and biological potential. They range from benign and curable by complete excision (schwannoma and soft tissue perineurioma) to benign but potentially aggressive at the local level (plexiform neurofibroma) to the highly malignant (malignant peripheral nerve sheath tumors [MPNST]). In this review, we discuss the diagnostic and pathologic features of common peripheral nerve sheath tumors, particularly those that may be encountered in the intracranial compartment or in the spine and paraspinal region. The discussion will cover schwannoma, neurofibroma, atypical neurofibromatous neoplasms of uncertain biological potential, intraneural and soft tissue perineurioma, hybrid nerve sheath tumors, MPNST, and the recently renamed enigmatic tumor, malignant melanotic nerve sheath tumor, formerly referred to as melanotic schwannoma. We also discuss the diagnostic relevance of these neoplasms to specific genetic and familial syndromes of nerve, including neurofibromatosis 1, neurofibromatosis 2, and schwannomatosis. In addition, we discuss updates in our understanding of the molecular alterations that represent key drivers of these neoplasms, including neurofibromatosis type 1 and type 2, *SMARCB1*, *LZTR1*, and *PRKAR1A* loss, as well as the acquisition of *CDKN2A/B* mutations and alterations in the polycomb repressor complex members (*SUZ12* and *EED*) in the malignant progression to MPNST. In summary, this review covers practical aspects of pathologic diagnosis with updates relevant to neurosurgical practice.

KEY WORDS: Neurofibroma, Schwannoma, MPNST, Neurofibromatosis, Schwannomatosis, Schwann cell, *NF1*, *NF2*, *SMARCB1*, *LZTR1*, *PRKAR1A*

Neurosurgery 88:443–456, 2021

DOI:10.1093/neuros/nyab021

www.neurosurgery-online.com

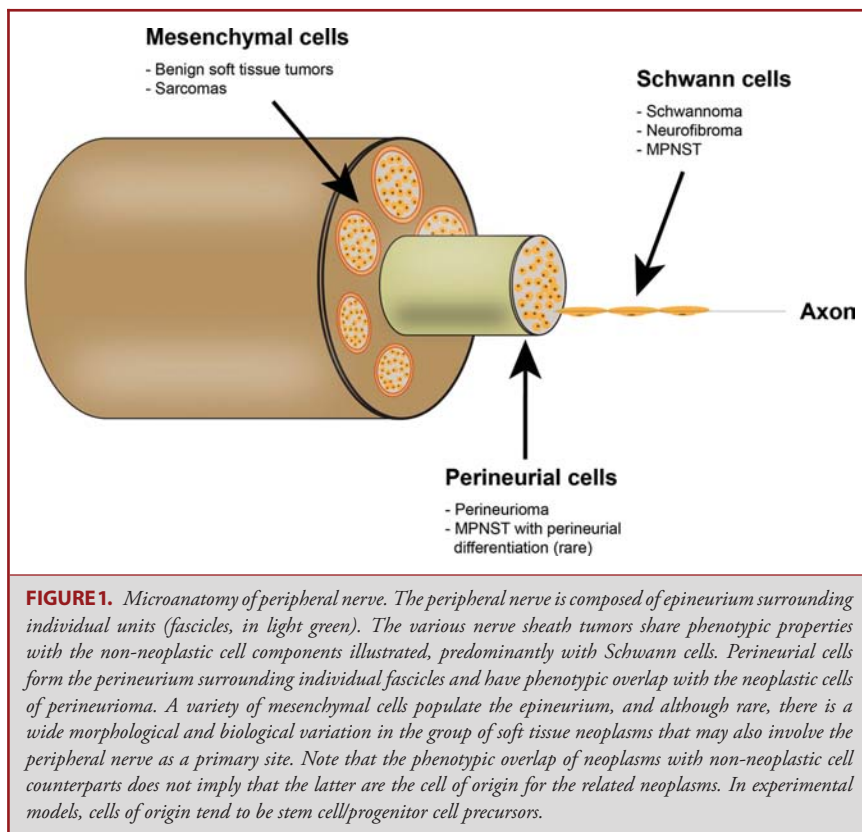
The peripheral nervous system is composed of peripheral nerves and ganglia, which arise from the brain stem or the spinal cord and course throughout the body to reach its various targets. Its function is to provide somatic and autonomic innervation. The peripheral nerves are formed by bundles of axons and are defined at the microscopic level by myelination mediated by Schwann cells. Peripheral nerves are composed of organized specialized tissue forming 3 layers: the endoneurium, the perineurium, and the epineurium (Figure 1). The anatomical distribution and histological complexity of the

peripheral nervous system is reflected in the morphological and biological heterogeneity of the tumors that arise or differentiate toward its various cell components. Tumors of peripheral nerve range from benign to highly malignant.

Comprehensive molecular genetic profiling using high resolution platforms is currently transforming the approach to pathologic diagnosis of disease and cancer, including peripheral nerve sheath tumors (PNSTs). Well-defined morphological criteria still allow for classification in most cases, but global methylation profiling¹ in tumor and next-generation sequencing platforms in tumor and blood^{2,3} may provide additional diagnostic and prognostic information. Although PNSTs usually develop sporadically in patients lacking a familial history or genetic predisposition, they are important components of a variety of genetic syndromes and pathologic characterization constitute important diagnostic criteria with consequences for clinical follow-up and genetic counseling. These syndromes mainly include

ABBREVIATIONS: ANNUBP, atypical neurofibromatous neoplasms of uncertain biological potential; EBV, Epstein-Barr virus; EMA, epithelial membrane antigen; GTP, guanosine triphosphate; HPFs, high power fields; MMNST, malignant melanotic nerve sheath tumor; NFP, neurofilament protein; PRC, polycomb repressive complex

CNS Spotlight available at cns.org/spotlight.



neurofibromatosis type 1 (NF1), neurofibromatosis type 2 (NF2), schwannomatosis, and Carney complex⁴ (Figure 2).

In this review, we focus on the major categories of PNST that are likely to be encountered in neurosurgical practice. We focus on those that develop predominantly intracranially and in spinal nerve roots (Table). Key histologic and molecular genetic features that are useful for diagnosis are particularly emphasized.

SCHWANNOMA

Schwannomas are benign nerve sheath tumors composed of neoplastic Schwann cells. They develop at multiple body sites but intracranially they favor the vestibular branch of the VIII cranial nerve. On imaging, they present as masses of the cerebellopontine angle (Figure 3). As discussed below, bilateral vestibular schwannomas are diagnostic of NF2.⁵ Schwannomas may also involve other cranial nerves and in rare occasions be entirely intraparenchymal within the brain.⁶ Grossly, they are usually solitary, and the cut surfaces display a light-tan appearance surrounded by a fibrous capsule. A yellow color is frequent because of lipid content or lipid-laden macrophages. Variably sized cysts and hemorrhagic changes may be present. Histologically, in their classic form, schwannomas contain compact patterns (Antoni A), alternating with loose patterns (Antoni B). The Antoni A zones are characterized by increased cellularity and spindle

nuclei. These zones are studded with clumps of cellular aggregates, where palisading patterns called Verocay bodies may be encountered, which are almost diagnostic at the histologic level (Figure 3). Paradoxically, Verocay bodies are frequently absent in vestibular schwannomas. The Antoni B zones are disorganized arrangements that are hypocellular and feature a variable macrophage infiltrate. The surrounding capsule is formed by multiple layers of collagen fibers. Other common histologic findings in schwannomas include hyalinized vessels with perivascular hemosiderin deposition, cystic spaces, and degenerative atypia (“ancient change”). Malignant transformation in schwannomas is exquisitely rare but usually takes the form of epithelioid malignant peripheral nerve sheath tumor (MPNST), a round cell malignancy or angiosarcoma.^{7,8}

On immunohistochemistry, schwannomas are diffusely positive for mature Schwann cell markers, including S100 and SOX10 protein (Figure 3). Collagen IV and reticulin special stains are positive around individual cells. Epithelial membrane antigen (EMA) outlines perineurial cells peripherally but is negative in neoplastic cells. Neurofilament protein (NFP) highlights rare entrapped axons, but these are usually encountered in the periphery. Neoplastic cells in schwannoma also express vascular endothelial growth factor and antiangiogenic therapy with bevacizumab is often used for progressive vestibular schwannomas in NF2 patients.⁹

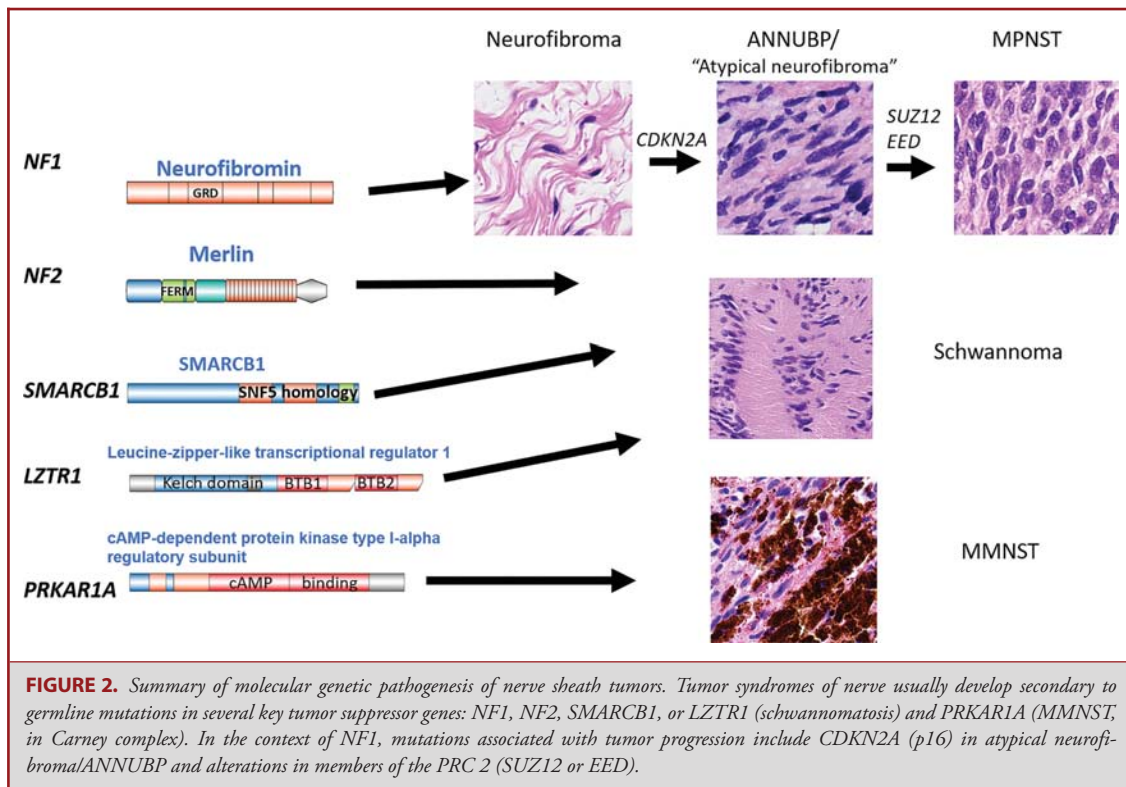


TABLE. Peripheral Nerve Tumors Relevant to Intracranial and Paraspinal Locations

Schwannoma
Neurofibroma
Perineurioma
Hybrid nerve sheath tumor
Schwannoma/perineurioma
Neurofibroma/schwannoma
Neurofibroma/perineurioma
ANNUBP
MMNST
MPNST
Miscellaneous
Lipoma of VIII cranial nerve
Glomus tumor of nerve
Sarcomas
Primary central nervous system tumors presenting in nerve root (myxopapillary ependymoma, hemangioblastoma)
Lymphoma
Metastatic carcinoma

Cellular Schwannoma

This type of schwannoma is composed almost entirely of Antoni A patterns lacking Verocay bodies (Figure 3). Although benign, it often displays local aggressiveness (eg, bone erosion). Dense cellularity and mitotic activity make this schwannoma

variant particularly prone to be mistaken for malignancy.¹⁰ Important typical features that help in avoiding this pitfall include the presence of a continuous peritumoral capsule, a rich macrophagic infiltrate, and the diffuse expression of Schwann cell markers (S100 and SOX10)¹¹ and, in contrast to most MPNST, retain H3K27 trimethylation (or H3K27me3).¹² A diffuse lymphocytic cuff under the capsule may be present, occasionally containing even well-formed lymphoid follicles. It must be highlighted that malignant transformation of schwannoma into MPNST is exquisitely rare and takes the form of a morphological change (eg, epithelioid, small cell, and angiosarcoma). Therefore, once the neoplasm is identified as a schwannoma, a simple increase in cellularity and/or mitotic activity, in the context of the features listed above, should not suggest malignant change. Cellular schwannomas may be encountered in a variety of body sites, including paravertebral areas. Involvement of the intracranial compartment is rare, but we have recently reported our experience with cellular schwannomas in this anatomic location.¹³ The intracranial cellular schwannomas mostly developed in association with cranial nerve VIII or V. Local recurrences developed in a subset of patients, but no metastases or deaths were documented.

Plexiform Schwannoma

This schwannoma variant has a predilection for superficial sites, growing mostly in cutaneous or subcutaneous tissues, but

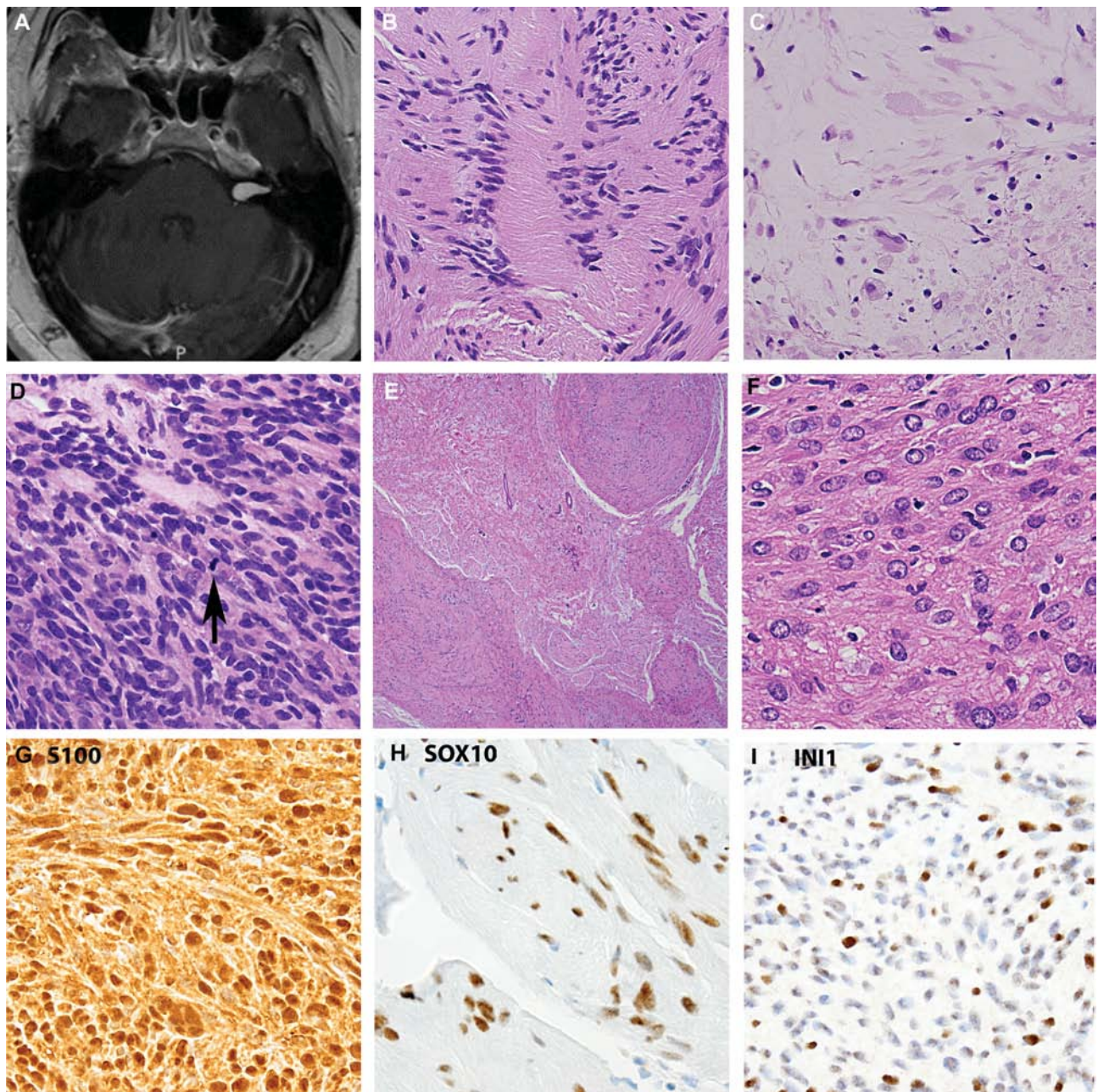


FIGURE 3. Schwannoma. **A**, The vestibular branch of cranial nerve VIII is the main intracranial site of origin of schwannoma. **B**, Antoni A areas are compact and may contain characteristic palisades known as Verocay bodies. **C**, Antoni B patterns are composed of loose tissue with variable histiocytic infiltrates. Cellular schwannoma is a specific variant that is particularly prone to be mistaken for malignancy. **D**, Mitotic figures may be encountered (arrow). **E**, Plexiform neurofibromas are composed of multinodular aggregates of predominantly Antoni A tissue. **F**, Epithelioid schwannomas are rare, composed of plump cells with prominent nuclei and nucleoli. Strong expression of mature Schwann cell markers is typical of all schwannomas, including S100 **G** and SOX10 **H**. **I**, Patchy loss or a “mosaic” pattern of INI1/SMARCB1 immunostaining is typical of syndrome-associated (NF2 and schwannomatosis) schwannomas.

may also develop in major peripheral nerves and plexi.¹⁴ Their distinctive feature is a plexiform or nodular growth pattern (Figure 3). A capsule is usually absent. Plexiform schwannomas may be associated with local recurrences, but malignant transformation is exceedingly rare. Most occur sporadically, but a small subset (~5%) develops in genetic syndromes (NF2 or schwannomatosis). Other rare schwannoma variants include epithelioid,¹⁵ neuroblastoma-like,¹⁶ and reticular.¹⁷

Molecular Genetics

One important aspect of the diagnostic pathology of schwannomas is that when multiple, they are an important component of NF2 and schwannomatosis, and they represent important diagnostic criteria for these syndromes.^{5,18} The hallmark of NF2 is the presence of bilateral vestibular schwannomas. Additionally, these patients develop other tumor types, particularly meningiomas and ependymomas. Approximately half of NF2 patients have a familial history. Schwannomatosis is sporadic in most instances (75%-85%), with approximately 15% to 25% being inherited. These patients almost exclusively develop schwannomas, but in rare instances, they also develop meningiomas.^{19,20} Although schwannomas developing in genetic syndromes and sporadically are indistinguishable in most instances, features overrepresented in syndrome-associated cases, include whorling patterns, myxoid change, nerve edema/infiltration, and a mosaic pattern of SMARCB1/INI1 loss by immunohistochemistry.²¹ The exception is NF2-associated vestibular schwannomas, where SMARCB1/INI1 positivity remains intact.²²

A loss of function mutation in the *NF2* gene is the most common genetic feature of schwannomas in sporadic and familial forms. The *NF2* gene is located at the 22q12.2 chromosomal locus and encodes Merlin. Merlin is known to interact with cell junctional complexes and plays a role in contact-dependent inhibition. It has multiple additional cellular functions, including activation of oncogenic signaling pathways. Recent genetic profiling studies of schwannomas have also found recurrent mutations in *ARID1A*, *ARID1B*, and *DDR1* as well as a novel *SH3PXD2A-HTRA1* fusion in subsets of schwannoma.²³ Germline *NF2* mutations are diagnostic of NF2 syndrome. The germline alterations responsible for schwannomatosis are unknown in most instances, but *SMARCB1* (Chr 22q11.23 locus)²⁴ or *LZTR1* (Chr 22q11.21 locus)²⁵ germline mutations occur in subsets. SMARCB1 is a component of the switch/sucrose nonfermentable (SWI/SNF) chromatin remodeling multiunit complexes that are Adenosine triphosphate dependent and mediate changes in chromatin architecture and gene expression. *LZTR1* encodes the *leucine zipper-like transcription regulator 1*, a member of the BTB-kelch superfamily. *LZTR1* is a Golgi complex protein and interacts with the CUL3-based ubiquitin ligase complex. In addition to schwannomatosis, this gene has also been found to be altered in Noonan syndrome and glioblastoma, and may participate in the regulation of RAS/MAPK signaling.²⁶

Schwannomas developing in the context of SMARCB1-associated schwannomatosis represent an illustrative model on the cooperation of tumor suppressor loss in human neoplasia. In this context, the sequence involves a “four-hit” event in which sequential inactivation of both copies of *SMARCB1* and *NF2* occur in the individual tumors.²⁴ This event is probably facilitated by the localization of both genes in the chromosome 22q arm.

NEUROFIBROMA

Neurofibroma is another benign tumor composed of neoplastic Schwann cells, but unlike schwannoma, it also contains additional non-neoplastic components, including fibroblasts, mast cells, perineurial-like cells, and residual axons. Sporadic cutaneous neurofibroma is the most common subtype. However, multiple neurofibromas are a key feature of NF1, in which there may be involvement of multiple cutaneous sites, peripheral nerves, and spinal roots (Figure 4). Gross examination of neurofibromas involving peripheral nerves demonstrate fusiform enlargement of the parent nerve fascicle. Cut surfaces are variably yellow and translucent depending on the extent of myxoid stroma. The histologic features of neurofibroma are similar independent of specific subtype, and include a predominance of Schwann cells with thin wavy nuclei embedded in a myxoid matrix with variable amounts of collagen arranged in a haphazard fashion (Figure 4). Other cell components that are present in variable amounts include mast cells, lymphocytes, fibroblasts, and EMA-positive perineurial cells that all contribute to the tumor microenvironment. When involving nerve roots in the spine, neurofibromas may entrap ganglion cells through infiltration of sensory ganglia. In contrast to schwannoma, entrapped axons are frequent in the substance of the neoplasm because it is more infiltrative and, therefore, more challenging to resect without affecting the parent fascicle. The immunophenotype is characterized by uniform S100 and SOX10 expression in the neoplastic Schwann cells, whereas EMA labels perineurial cells and CD34 stromal cells. NFP typically outlines numerous entrapped axons.

Diffuse Neurofibroma

Diffuse neurofibromas typically form plaque-like enlargements of the skin and underlying subcutis. Approximately 10% of cases develop in NF1 patients. They lack a capsule and are poorly demarcated. These tumors may entrap adnexal and underlying soft tissue structures and frequently contain pseudomeissnerian corpuscles (spherical arrangements of S100-positive cell processes) (Figure 4). Transformation into MPNST in diffuse neurofibromas is rare, compared to plexiform and intraneural neurofibromas, but well documented.²⁷

Plexiform Neurofibroma

Plexiform neurofibroma is a neurofibroma variant defined at the architectural level as involving multiple peripheral nerve fascicles, and therefore, it is frequently associated with larger

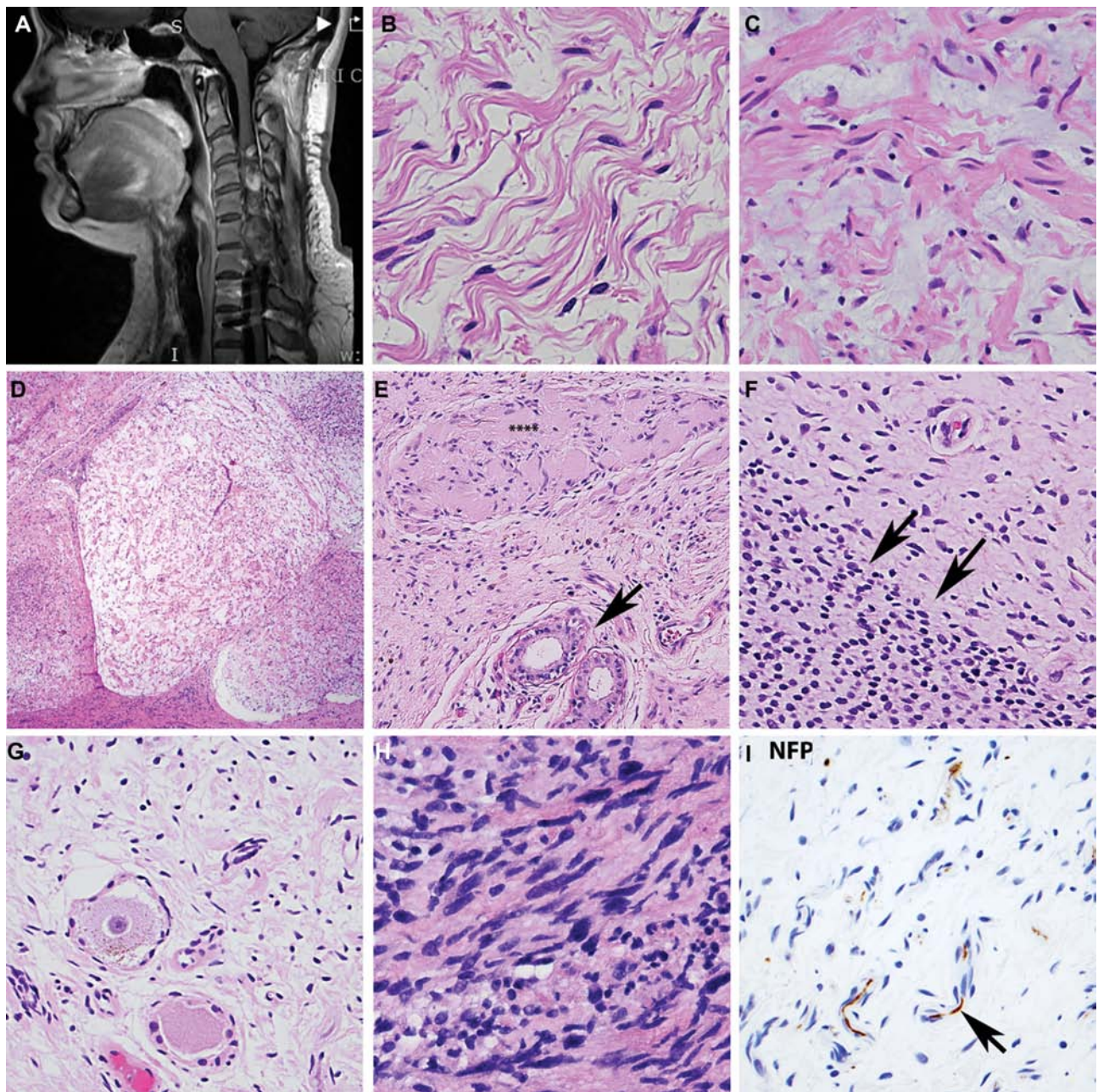


FIGURE 4. **A**, Neurofibroma. Multiple neurofibromas involving cervical nerve roots in a patient with NF1. Neurofibromas are composed of wavy nuclei, somewhat smaller than those of schwannoma, with associated delicate collagen **B** and variable myxoid stroma **C**. **D**, Plexiform neurofibromas are typical NF1-associated tumors and form multiple tortuous nodules, reflecting multifascicular involvement best appreciated at low power. **E**, Diffuse neurofibromas may be infiltrating, frequently entrapping adnexal structures (arrow) and containing characteristic pseudomeissnerian corpuscles (asterisks). Massive soft tissue neurofibromas are typically enormous neoplasms, developing exclusively in NF1 patients. **F**, A cellular, but benign, round cell component is frequent (arrows). **G**, Neurofibromas frequently involve the dorsal roots in NF1 patients. Infiltration of dorsal root ganglia should not be mistaken for ganglion cell differentiation. The designation ANNUBP is usually reserved for the identification of some worrisome histologic features in small biopsies from NF1 patients but not meeting criteria for malignancy. **H**, This example demonstrates hypercellularity and nuclear enlargement. **I**, NFP immunostain is useful in highlighting underlying axons in the substance of neurofibromas (arrow).

nerves and plexi. This subtype frequently affects (20%-50%) patients with NF1,²⁸ representing important clinical criteria for the diagnosis, and in these patients, it is an important precursor for MPNST.²⁹ In fact, plexiform neurofibromas involving large nerves and body regions are almost limited to patients satisfying clinical criteria for NF1. The rare cases lacking other clinical criteria for NF1 are usually classified as localized or “segmental” NF1. Malignant degeneration of plexiform neurofibromas occurs in approximately 5% of cases. In plexiform neurofibroma, the proliferation of Schwann cells involves multiple fascicles, englobing long nerve segments (Figure 4). Their distinctive feature is a multinodular enlargement, giving them the appearance of “bag of worms” or “string of onions.” The histologic features are similar to neurofibromas occurring at other sites; therefore, the imaging, gross appearance, and low power histologic features are essential for diagnosis. Plexiform and diffuse components can co-exist in individual tumors.

Massive Soft Tissue Neurofibroma

This rare neurofibroma variant occurs exclusively in NF1 patients. These tumors are remarkable for their often-colossal size (which may span an entire limb) and the exquisite infiltration of local soft tissues, including skeletal muscle and even bone. They are characterized by slow progression, often beginning in childhood. They arise in subcutaneous tissue and may be covered by hyperpigmented skin.³⁰ Histologically, the tumors are characterized by extensive infiltration of underlying soft tissues, including skeletal muscle. Pseudomeisnerian corpuscles and cellular areas are frequently present (Figure 4).

Molecular Genetics

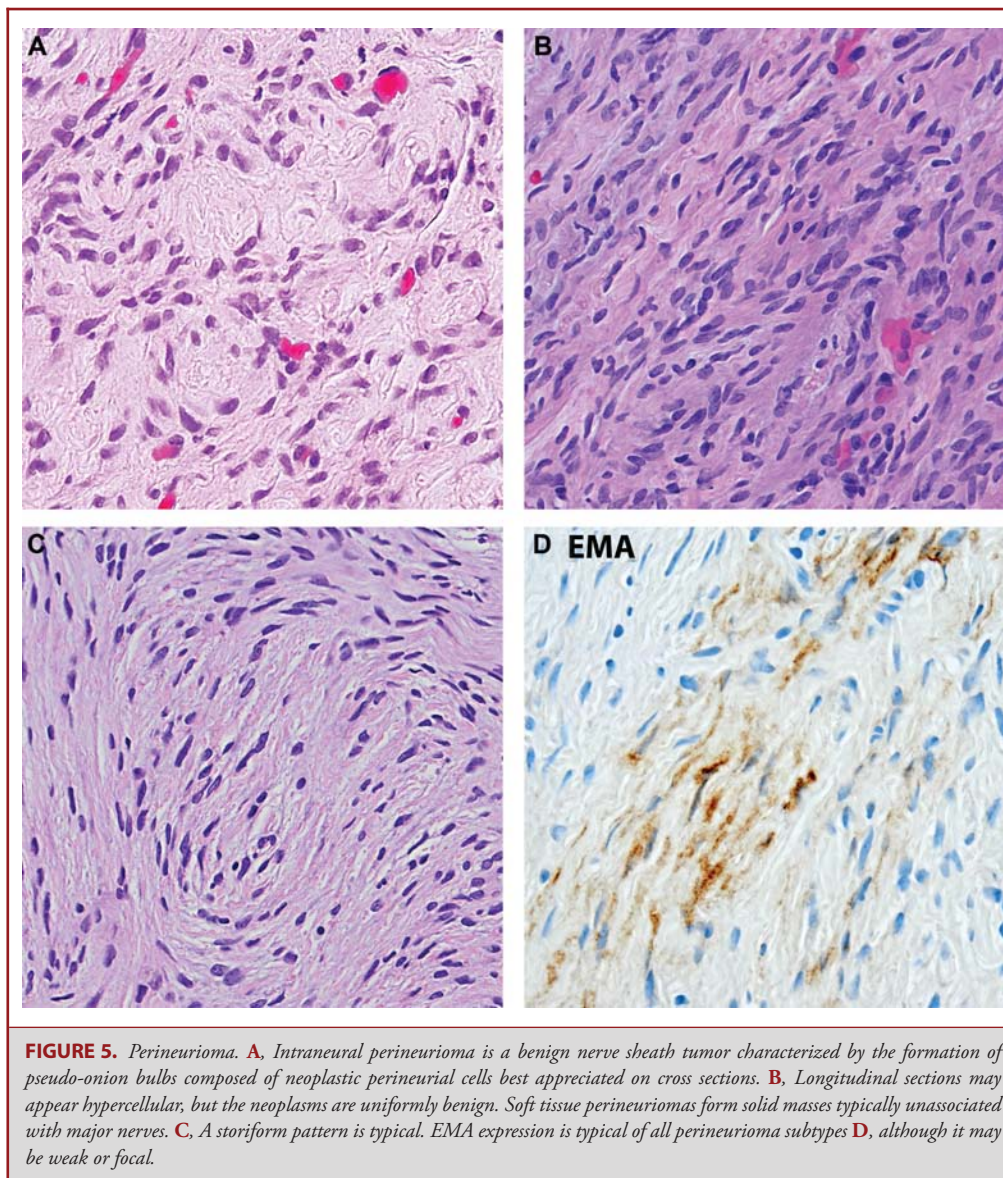
The defining genetic event for all neurofibroma subtypes is inactivation of the *NF1* gene (chromosome locus 17q11.2), encoding neurofibromin, a GTPase-activating protein that is expressed in many cell types in the central and peripheral nervous system. It regulates RAS signaling by mediating the conversion of Guanosine triphosphate (GTP)-bound RAS (active) to its Guanosine diphosphate (GDP)-bound state (inactive).³¹⁻³³ GTP-bound RAS results in activation of MAPK, extracellular signal-regulated kinase 1 and 2 (ERK1 and ERK2), and increased PI3K-mTOR signaling. The end result is increased transcription of target genes, cell growth, survival, and proliferation.³⁴⁻³⁹ *NF1* inactivation also results in altered cAMP levels through regulation of adenylate cyclase, which affects non-neoplastic (eg, cognitive) as well as neoplastic manifestations of the disease. *NF1* gene inactivation is the sole recurring abnormality in neurofibromas,⁴⁰ with additional alterations acquired as part of atypical and malignant progression. Of therapeutic relevance, MEK inhibitors (eg, selumetinib) are currently feasible and may provide therapeutic benefit for some patients with plexiform neurofibroma.⁴¹

ATYPICAL NEUROFIBROMATOUS NEOPLASM OF UNCERTAIN BIOLOGICAL POTENTIAL

An atypical neurofibroma category has been controversial given the nosologic confusion that it creates and applied in various ways to denote a range of changes ranging from totally benign and clinically inconsequential (eg, degenerative atypia or “ancient change”) to low-grade malignant change. Clear-cut diagnostic criteria for these important changes in the management of neurofibromas, particularly in the context of NF1, are lacking. A recent published consensus⁴² proposed the designation of *atypical neurofibromatous neoplasms of uncertain biological potential (ANNUBP)* for a group of tumors with some worrisome histologic features, but not clearly deserving a diagnosis of malignancy (Figure 4). Criteria for the designation of ANNUBP include neurofibromas with at least 2 of the following 4 features: histologic atypia, loss of the typical neurofibroma architecture, hypercellularity, and increased mitotic activity ($>1/50$ but <3 mitotic figures per 10 High power fields (HPFs) or <1.5 mitoses/mm²). This diagnosis should prompt additional evaluation (eg, tissue sampling) and close clinical follow-up. The sequence of molecular alterations responsible for malignant change in NF1-associated tumors is also beginning to be characterized, with premalignant change in neurofibroma demonstrating *NF1* inactivation, a low mutation burden, and *CDKN2A/B* loss.⁴³

PERINEURIOMA

Perineuriomas are benign tumors composed of neoplastic perineurial cells. Two types of perineuriomas can be distinguished: intraneural and soft tissue perineuriomas, depending on their association to a major nerve or lack thereof, respectively. Historically, most cases of intraneural perineurioma were interpreted as localized hypertrophic neuropathy, which suggested a reactive process involving nerve and overlapping with a variety of reactive neuropathies. Immunohistochemical, ultrastructural, and molecular genetic studies have clarified intraneural perineurioma to be a neoplastic proliferation of perineurial cells rather than a reactive process. Macroscopically, intraneural perineuriomas appear as segmental enlargements of nerve. Histologic examination demonstrates multiple concentric cytoplasmic layers forming whorls of variable size surrounding central axons (“pseudoonion bulbs”)⁴⁴ (Figure 5). Despite the increased cellularity, mitotic activity is low to absent. Immunohistochemical features include membranous staining with EMA, GLUT-1, and Claudin-1 positivity, whereas S100 is negative, which differentiates neoplastic cells from underlying resident Schwann cells. Residual non-neoplastic Schwann cells and central axons in the pseudo-onion bulbs are identifiable with S100 and NFP immunoreactivity, respectively. At the molecular genetic level, most intraneural perineuriomas have *TRAF7* alterations, and a minority has NF2/chromosome 22 alterations.⁴⁵ Soft tissue perineuriomas share the same immunophenotype with



intraneural perineuriomas, but they form well-circumscribed masses unassociated with major nerves.⁴⁶ Molecular genetic alterations reported include deletions involving the *NF1* or *NF2* locus in a mutually exclusive fashion.⁴⁷ A few cases of intracranial perineuriomas have been reported, related to dura⁴⁸ or inside the ventricles.⁴⁹

HYBRID NERVE SHEATH TUMORS

It has been increasingly recognized that a subset of benign nerve sheath tumors remains difficult to classify and place in one category because they have features of more than one recognized subtype (Figure 6). The most frequent variety is

schwannoma/perineurioma, which more commonly occurs in the extremities in the form of subcutaneous/dermal masses. The neoplastic cells show a schwannian morphology, but the overall architecture resembles that of soft tissue perineuriomas. On immunohistochemistry, all tumors are positive for S100 and EMA.⁵⁰

Another hybrid combination is neurofibroma/schwannoma, which may involve larger peripheral nerves, and appears to be overrepresented in patients with genetic syndromes (schwannomatosis, NF1, and NF2).⁵¹ Monosomy 22 is frequent in these tumors.⁵² Recently, recurrent *ERBB2* mutations were identified in a subset of hybrid neurofibroma/schwannomas developing in the context of schwannomatosis.⁵³

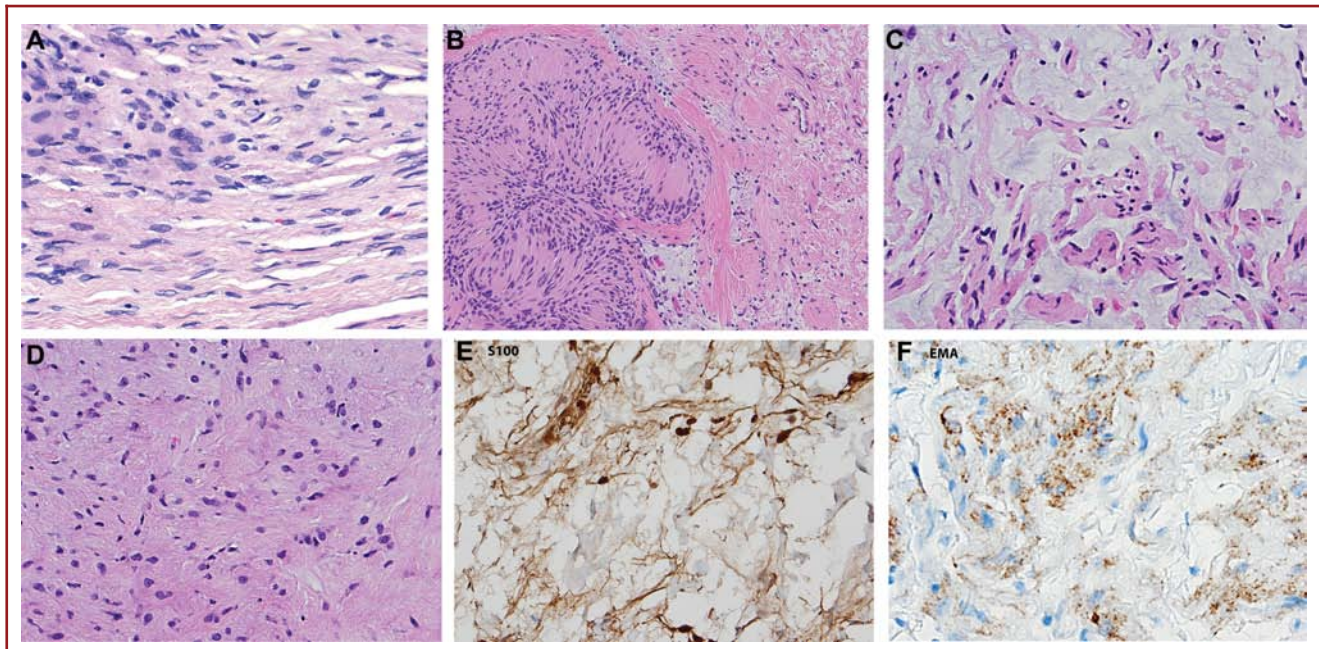


FIGURE 6. Hybrid nerve sheath tumors. It is increasingly recognized that a subset of benign nerve sheath tumors may contain components of more than 1 tumor type. **A**, Hybrid schwannoma-perineurioma is the most frequent hybrid nerve sheath tumor. **B**, Schwannoma-neurofibroma usually takes the form of schwannian nodules (asterisk) in a plexiform neurofibroma background. Hybrid neurofibroma-perineurioma with neurofibroma **C** and perineurioma **D** components highlighted by S100 **E** and EMA immunostains **F**, respectively.

The least common variation is neurofibroma/perineurioma, the recognition of which is often done through immunohistochemistry, which highlights EMA-positive areas within a plexiform neurofibroma.⁵⁴

MALIGNANT MELANOTIC NERVE SHEATH TUMOR

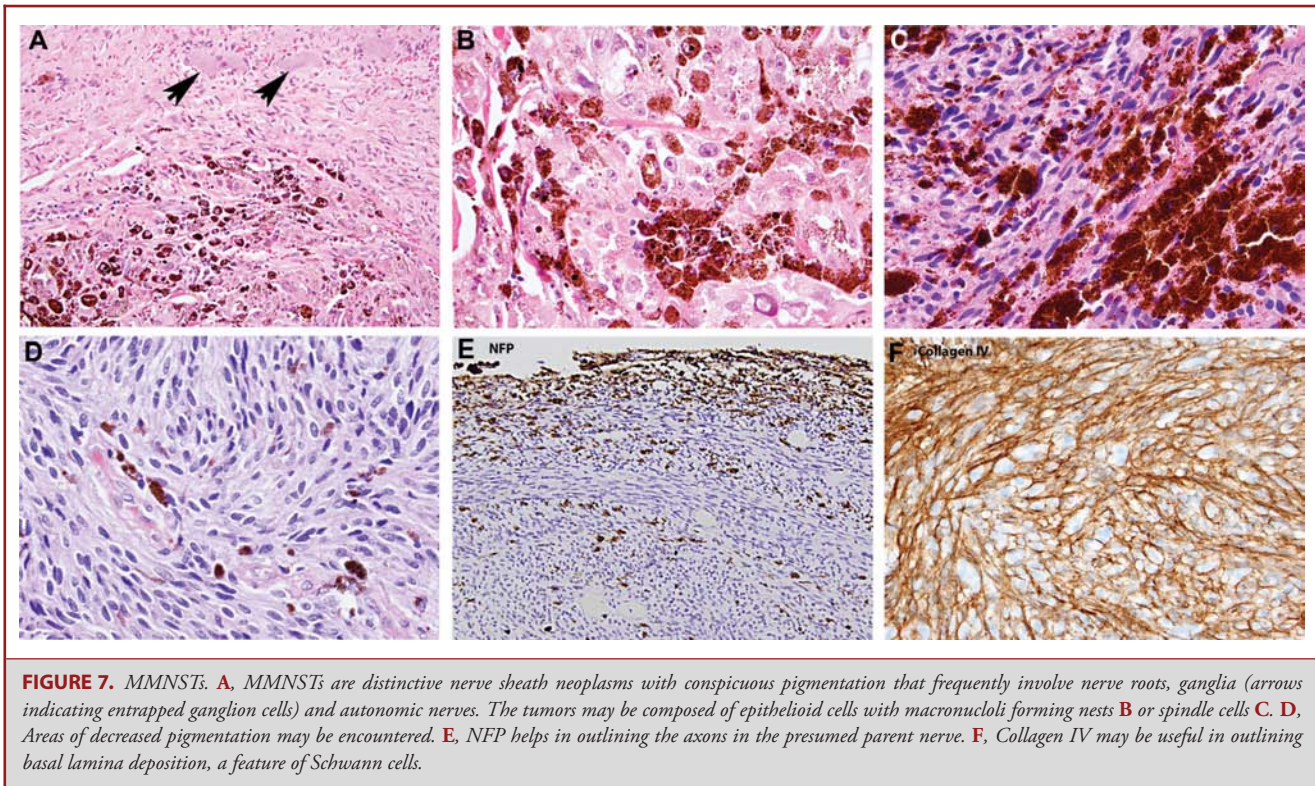
Malignant melanotic nerve sheath tumors (MMNSTs) are PNSTs composed of neoplastic Schwann cells with melanocytic differentiation. They present as solitary outgrowths bulging from spinal nerve roots and autonomic ganglia, and a subset is associated with Carney complex. On gross examination, MMNSTs form well-circumscribed, heavily pigmented tumors, with central necrosis and hemorrhage variably present. Histopathology shows a mix of spindled and epithelioid neoplastic cells arranged in fascicular patterns (Figure 7). Each neoplastic cell contains multiple intracytoplasmic brown inclusions corresponding to melanosomes. The nuclei are round and uniform with small nucleoli. A subset contains psammoma bodies, half of which have been associated with Carney complex in historic series,⁵⁵ but contemporary series have not found clinical differences between psammomatous and nonpsammomatous subtypes.⁵⁶ This syndrome is also characterized by endocrine hyperactivity, patchy skin hyperpigmentation, and a variety of tumor types, including cardiac myxomas.

On immunohistochemistry, MMNSTs are positive for both Schwann cell and melanocytic markers, including S100, SOX10, melan-A, and HMB45. Collagen IV tends to outline tumor lobules. Molecular genetic studies of these tumors (sporadic and syndrome associated) have demonstrated frequent loss of function mutations in *PRKARIA*⁵⁷ located on the chromosome locus 17p22-24. There is an additional, less-well-characterized locus on 2p16 that is responsible for a minority of Carney complex cases. Loss of the protein product of *PRKARIA* can be detected through immunohistochemistry, which has diagnostic value. The mutations take the form of single base pair substitutions, deletions and insertions, or rearrangements.⁵⁸ *PRKARIA* is one of the main regulatory subunits that form the Protein kinase A holoenzyme, and loss results in an increase in cAMP signaling.

The clinical course of MMNSTs historically has been hard to predict, and the tumors were formerly designated melanotic schwannoma, given the usual well-developed mature Schwann cell phenotype. However, Torres-Mora et al⁵⁶ reported high recurrence and metastatic rates in these tumors, justifying their reinterpretation as malignant neoplasms.

MALIGNANT PERIPHERAL NERVE SHEATH TUMORS

MPNST are usually high-grade malignant spindle cell neoplasms arising in association with nerves, in pre-existing



Schwann cell neoplasms (most frequently a plexiform neurofibroma) and/or demonstrating a variable Schwann cell phenotype (Figure 8). They are aggressive tumors characterized by a tendency to invade surrounding soft tissue and potential for hematogenous metastatic spread. MPNST can develop in association with NF1, after radiation exposure, or arise sporadically. Macroscopically, MPNST present as adherent, exophytic masses. They have a large variability in terms of size and consistency, ranging from microscopic change in a pre-existing neoplasm to massive tumors. Areas of necrosis and hemorrhage are common. Microscopically, they are usually cellular neoplasms composed of spindle-shaped cells with tapered nuclei. They frequently have alternating hyper- and hypocellular areas, which impart a marbled appearance at low power. Brisk mitotic activity and necrosis are frequent. Perivascular aggregates with herniation inside the vascular lumen are a characteristic feature. A small subset of MPNST is considered low grade, a designation usually applicable to areas of malignant change within plexiform neurofibromas in NF1.

Immunohistochemical stains demonstrate variable S100 and/or SOX10 expression, which is decreased in comparison with schwannomas, and maybe altogether negative.¹¹ MPNST also demonstrate loss of neurofibromin expression and H3K27 trimethylation. The latter is a key epigenetic marker that is lost in MPNST and has relatively high specificity for the diagnosis.¹² However, close mimickers such as melanoma may also demonstrate loss.⁵⁹

As mentioned above, most MPNST are high grade and easily recognized as malignant at the histologic level. The diagnosis of low grade MPNST is usually made in the context of NF1-associated neurofibromas with areas of ANNUPB transitioning to low grade MPNST. Although difficult to define, proposed criteria include the presence of changes typical of ANNUPB but, in addition, greater increases in mitotic activity (1.5-4.5 mitoses/mm²) or 3 to 9 mitotic figures per 10 HPFs and no necrosis.⁶⁰ High-grade MPNST are characterized by increased mitotic activity, and necrosis is frequent.

MPNST typically develop in association with larger nerves and plexiform neurofibromas. Rarely they may develop in association with cranial nerves and/or in the intracranial compartment.⁶¹ In a series of 17 cases, the vestibular nerves were most commonly involved, and the tumors were aggressive as those occurring in other sites. Careful consideration must be given to exclusion of desmoplastic/spindle cell melanoma, a more common diagnosis in head and neck sites.

The morphological spectrum of MPNST is remarkable. Heterologous differentiation may present with a variety of morphologies, including cartilage, bone, skeletal, and smooth muscle. Glandular differentiation is a rare but well documented phenomenon. Other MPNST variants include epithelioid MPNST and MPNST with perineurial differentiation.

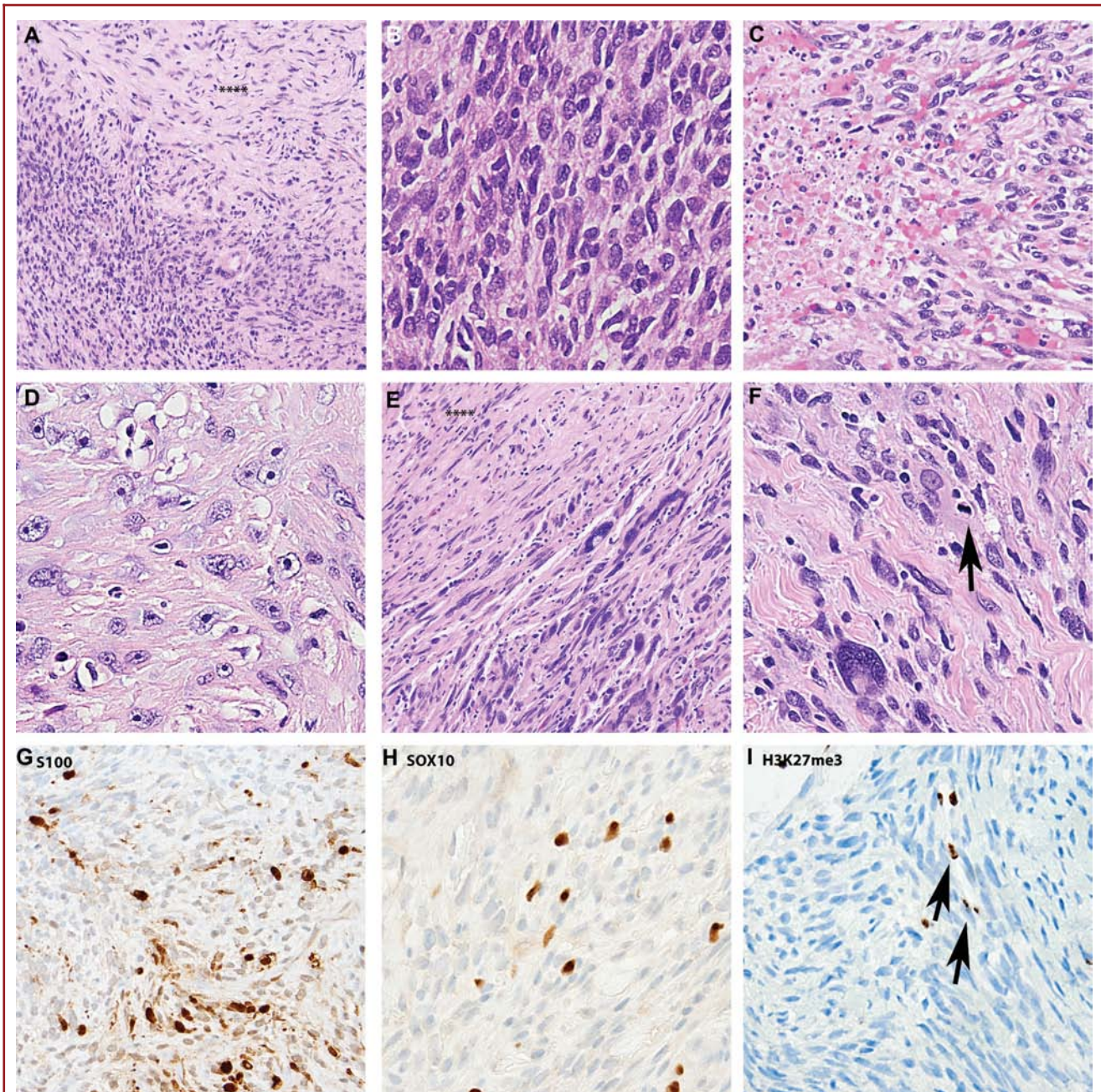


FIGURE 8. MPNSTs. MPNST are usually high-grade neoplasms that may arise in a neurofibroma precursor (asterisks) **A** and are characterized by hypercellularity/nuclear atypia **B** and frequent necrosis **C**. **D**, Epithelioid MPNST is a distinctive subset characterized by neoplastic cells with large nuclei with macronucleoli. MPNST developing in a pre-existing neurofibroma (asterisks) **E** and displaying low-grade histology, including lesser cell density and rare mitotic figures (arrow) **F**. MPNSTs typically have decreased expression of mature Schwann cell markers, including S100 **G** and SOX10 **H**, and in fact may be completely negative. Loss of H3K27 trimethylation (H3K27me3) is a relatively specific feature. **I**, Retained positivity in underlying endothelial cells is a useful internal positive control (arrows).

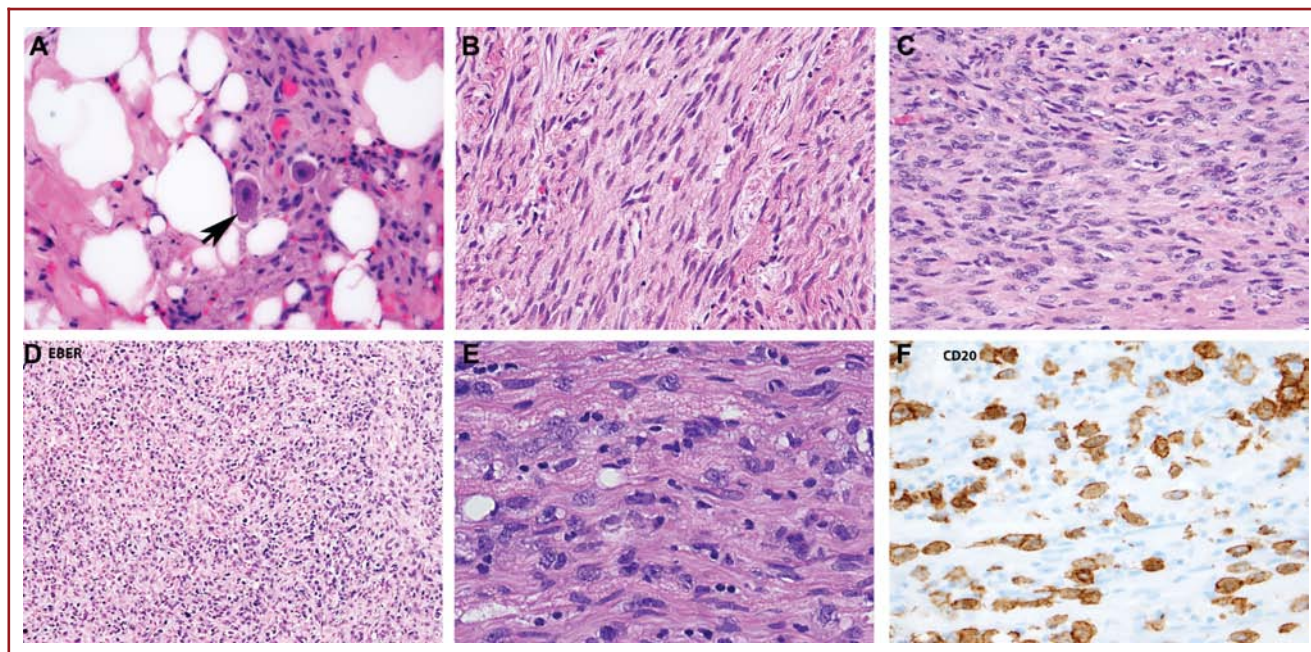


FIGURE 9. Miscellaneous tumors of peripheral nerve. A variety of soft tissue neoplasms may develop in association with a major nerve or nerve root. **A**, Lipoma of cranial nerve VIII with associated entrapped ganglion cells (arrow). Synovial sarcoma is a malignant difficult mimic of nerve sheath tumors when arising primarily in nerve. **B**, The distinction may require molecular testing. **C**, Smooth muscle tumors may also involve peripheral nerve occasionally. **D**, This example developed in an immunosuppressed patient and was EBV associated (in Situ hybridization study for EBV encoded ribonucleic acid). Diffuse large B-cell lymphoma may present as a peripheral nerve infiltrate overriding axons **E**, and is typically outlined by CD20 immunostain **F**.

Molecular Genetics

In MPNST germline or acquired *NF1*, inactivation is considered an important initiating event, which is followed by a cascade of other mutations that contribute to the malignant phenotype.

CDKN2A inactivation and somatic mutations in members of the polycomb repressive complex (PRC) *SUZ12* and/or *EED* are additional important events.^{62,63} The mutations in PRC components lead to loss of H3K27 trimethylation, which is more typical of high-grade tumors.

MISCELLANEOUS

A variety of benign and malignant neoplasms may involve peripheral nerves, both intracranially and at spinal/perispinal sites. They vary from rare (lipoma) to relatively more frequent (metastatic carcinoma/melanoma).

Lipoma of the 8th cranial nerve is a unique benign lesion that differs from lipomas arising at other anatomic locations. Magnetic resonance imaging studies are excellent at preoperative diagnosis given the fatty content, which results in hypersensitivity on postcontrast T1-weighted images, disappearing on fat suppression. These lesions are composed predominantly of fat (Figure 9), but other mesenchymal components may be encountered such as smooth or skeletal muscle.

Myxopapillary ependymoma, hemangioblastoma, and paraganglioma are usually considered tumors of the central nervous system, but they can present as purely involving the spinal nerve roots. A variety of sarcomas may involve the paraspinal region, nerve roots, and major nerves and are important tumors in the differential diagnosis of MPNST. Leiomyosarcoma may have morphological overlap with MPNST and may also express S100. However, immunohistochemical markers of smooth muscle are consistently present, such as smooth muscle actin and desmin. A group of smooth muscle tumors that may also involve the intracranial compartment and spinal nerve roots in immunosuppressed patients are the Epstein Barr Virus (EBV)-related smooth muscle tumors (Figure 9). Monophasic synovial sarcoma is a more troublesome mimic of MPNST and may present as a purely intraneural mass⁶⁴ (Figure 9). Cytokeratin and EMA are typically positive, but they may also express S100 and SOX10. Neurofibromin and H3K27 trimethylation immunoreactivity are usually retained.^{12,65-68} The identification of SSX1/SSX2 gene fusions are diagnostic.⁶⁹

Melanoma, particularly the desmoplastic variant, may be difficult to distinguish from nerve sheath tumors, and it may be a mimic of both neurofibroma and MPNST. Prominent neural infiltration (“neurotropic melanoma”) is a well-described phenomenon, and may in fact be more frequent in head and neck sites than MPNST. Lymphoma, usually the diffuse large

B-cell type, can present with prominent peripheral nerve or nerve root infiltration (Figure 9). Carcinoma from a primary site may also involve cranial or spinal nerves by direct spread from head and neck sites (eg, squamous cell carcinoma and adenoid cystic carcinoma) or pelvis (eg, prostatic adenocarcinoma) all the way up to their point of origin, in addition to involving nerve as metastatic deposits.

CONCLUSION

The spectrum of neoplasms that may involve the peripheral nervous system is wide, but most primary tumors have a Schwann cell phenotype, at least in part. They range from the most benign to the most malignant tumors encountered in surgical pathology. They also illustrate the value of diagnostic precision in their pathologic evaluation, even when benign, given their solid place in the diagnostic criteria of importance to the management of patients with genetic syndromes.

Funding

This work was supported in part by NIH grant P30 CA006973 to the Sidney Kimmel Comprehensive Cancer Center (PI: W. Nelson).

Disclosures

The authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article.

REFERENCES

- Rohrich M, Koelsche C, Schrimpf D, et al. Methylation-based classification of benign and malignant peripheral nerve sheath tumors. *Acta Neuropathol.* 2016;131(6):877-887.
- Louvrier C, Pasmant E, Briand-Suleau A, et al. Targeted next-generation sequencing for differential diagnosis of neurofibromatosis type 2, schwannomatosis, and meningiomatosis. *Neuro Oncol.* 2018;20(7):917-929.
- Carlson ML, Smadbeck JB, Link MJ, Klee EW, Vasmatzis G, Schimmenti LA. Next generation sequencing of sporadic vestibular schwannoma: necessity of biallelic NF2 inactivation and implications of accessory non-NF2 variants. *Otol Neurotol.* 2018;39(9):e860-e871.
- Rodriguez FJ, Stratakis CA, Evans DG. Genetic predisposition to peripheral nerve neoplasia: diagnostic criteria and pathogenesis of neurofibromatosis, Carney complex, and related syndromes. *Acta Neuropathol.* 2012;123(3):349-367.
- Smith MJ, Bowers NL, Bulman M, et al. Revisiting neurofibromatosis type 2 diagnostic criteria to exclude LZTR1-related schwannomatosis. *Neurology.* 2017;88(1):87-92.
- Casadei GP, Komori T, Scheithauer BW, Miller GM, Parisi JE, Kelly PJ. Intracranial parenchymal schwannoma. A clinicopathological and neuroimaging study of nine cases. *J Neurosurg.* 1993;79(2):217-222.
- Woodruff JM, Selig AM, Crowley K, Allen PW. Schwannoma (neurilemoma) with malignant transformation. A rare, distinctive peripheral nerve tumor. *Am J Surg Pathol.* 1994;18(9):882-895.
- McMenamin ME, Fletcher CD. Expanding the spectrum of malignant change in schwannomas: epithelioid malignant change, epithelioid malignant peripheral nerve sheath tumor, and epithelioid angiosarcoma: a study of 17 cases. *Am J Surg Pathol.* 2001;25(1):13-25.
- Plotkin SR, Duda DG, Muzikansky A, et al. Multicenter, prospective, phase II and biomarker study of high-dose bevacizumab as induction therapy in patients with neurofibromatosis type 2 and progressive vestibular schwannoma. *J Clin Oncol.* 2019;37(35):3446-3454.
- Woodruff JM, Godwin TA, Erlandson RA, Susin M, Martini N. Cellular schwannoma: a variety of schwannoma sometimes mistaken for a malignant tumor. *Am J Surg Pathol.* 1981;5(8):733-744.
- Pekmezci M, Reuss DE, Hirbe AC, et al. Morphologic and immunohistochemical features of malignant peripheral nerve sheath tumors and cellular schwannomas. *Mod Pathol.* 2015;28(2):187-200.
- Schaefer IM, Fletcher CD, Hornick JL. Loss of H3K27 trimethylation distinguishes malignant peripheral nerve sheath tumors from histologic mimics. *Mod Pathol.* 2016;29(1):4-13.
- D'Almeida Costa F, Dias TM, Lombardo KA, et al. Intracranial cellular schwannomas: a clinicopathological study of 20 cases. *Histopathology.* 2020;76(2):275-282.
- Hebert-Blouin MN, Amrami KK, Scheithauer BW, Spinner RJ. Multinodular/plexiform (multifascicular) schwannomas of major peripheral nerves: an underrecognized part of the spectrum of schwannomas. *J Neurosurg.* 2010;112(2):372-382.
- Hart J, Gardner JM, Edgar M, Weiss SW. Epithelioid schwannomas: an analysis of 58 cases including atypical variants. *Am J Surg Pathol.* 2016;40(5):704-713.
- Goldblum JR, Beals TF, Weiss SW. Neuroblastoma-like neurilemoma. *Am J Surg Pathol.* 1994;18(3):266-273.
- Liegl B, Bennett MW, Fletcher CD. Microcystic/reticular schwannoma: a distinct variant with predilection for visceral locations. *Am J Surg Pathol.* 2008;32(7):1080-1087.
- Evans DG, King AT, Bowers NL, et al. Identifying the deficiencies of current diagnostic criteria for neurofibromatosis 2 using databases of 2777 individuals with molecular testing. *Genet Med.* 2019;21(7):1525-1533.
- Melean G, Velasco A, Hernandez-Imaz E, et al. RNA-based analysis of two SMARCB1 mutations associated with familial schwannomatosis with meningiomas. *Neurogenetics.* 2012;13(3):267-274.
- Bacci C, Sestini R, Provenzano A, et al. Schwannomatosis associated with multiple meningiomas due to a familial SMARCB1 mutation. *Neurogenetics.* 2010;11(1):73-80.
- Patil S, Perry A, Maccollin M, et al. Immunohistochemical analysis supports a role for INI1/SMARCB1 in hereditary forms of schwannomas, but not in solitary, sporadic schwannomas. *Brain Pathol.* 2008;18(4):517-519.
- Caltabiano R, Magro G, Polizzi A, et al. 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.* 2017;33(6):933-940.
- Agnihotri S, Jalali S, Wilson MR, et al. The genomic landscape of schwannoma. *Nat Genet.* 2016;48(11):1339-1348.
- Sestini R, Bacci C, Provenzano A, Genuardi M, Papi L. Evidence of a four-hit mechanism involving SMARCB1 and NF2 in schwannomatosis-associated schwannomas. *Hum Mutat.* 2008;29(2):227-231.
- Piotrowski A, Xie J, Liu YF, et al. Germline loss-of-function mutations in LZTR1 predispose to an inherited disorder of multiple schwannomas. *Nat Genet.* 2014;46(2):182-187.
- Abe T, Umeki I, Kanno SI, Inoue SI, Niihori T, Aoki Y. LZTR1 facilitates polyubiquitination and degradation of RAS-GTPases. *Cell Death Differ.* 2020;27(3):1023-1035.
- Schaefer IM, Fletcher CD. Malignant peripheral nerve sheath tumor (MPNST) arising in diffuse-type neurofibroma: clinicopathologic characterization in a series of 9 cases. *Am J Surg Pathol.* 2015;39(9):1234-1241.
- Dombi E, Baldwin A, Marcus LJ, et al. Activity of selumetinib in neurofibromatosis type 1-related plexiform neurofibromas. *N Engl J Med.* 2016;375(26):2550-2560.
- McCarron KF, Goldblum JR. Plexiform neurofibroma with and without associated malignant peripheral nerve sheath tumor: a clinicopathologic and immunohistochemical analysis of 54 cases. *Mod Pathol.* 1998;11(7):612-617.
- Bongiorno MR, Cefalu AB, Arico M, Averna M. Clinical, pathologic, and genetic features of massive soft tissue neurofibromas in a Sicilian patient. *Dermatol Ther.* 2008;21(Suppl 3):S21-S25.
- Gutmann DH, Ferner RE, Listernick RH, Korf BR, Wolters PL, Johnson KJ. Neurofibromatosis type 1. *Nat Rev Dis Primers.* 2017;3(1):17004.
- Dasgupta B, Yi Y, Chen DY, Weber JD, Gutmann DH. Proteomic analysis reveals hyperactivation of the mammalian target of rapamycin pathway in neurofibromatosis 1-associated human and mouse brain tumors. *Cancer Res.* 2005;65(7):2755-2760.

33. Tsiipi M, Poulou M, Fylaktou E, et al. Phenotypic expression of a spectrum of neurofibromatosis type 1 (NF1) mutations identified through NGS and MLPA. *J Neurol Sci*. 2018;395:95-105.
34. Gutmann DH. Review article: neurofibromin in the brain. *J Child Neurol*. 2002;17(8):592-601.
35. Jones DTW, Gronych J, Lichter P, Witt O, Pfister SM. MAPK pathway activation in pilocytic astrocytoma. *Cell Mol Life Sci*. 2012;69(11):1799-1811.
36. DeClue JE, Papageorge AG, Fletcher JA, et al. Abnormal regulation of mammalian p21ras contributes to malignant tumor growth in von Recklinghausen (type 1) neurofibromatosis. *Cell*. 1992;69(2):265-273.
37. Donovan S, Shannon KM, Bollag G. GTPase activating proteins: critical regulators of intracellular signaling. *Biochim Biophys Acta*. 2002;1602(1):23-45.
38. Downward J. Targeting RAS signalling pathways in cancer therapy. *Nat Rev Cancer*. 2003;3(1):11-22.
39. Mendoza MC, Er EE, Blenis J. The Ras-ERK and PI3K-mTOR pathways: cross-talk and compensation. *Trends Biochem Sci*. 2011;36(6):320-328.
40. Pemov A, Li H, Patidar R, et al. The primacy of NF1 loss as the driver of tumorigenesis in neurofibromatosis type 1-associated plexiform neurofibromas. *Oncogene*. 2017;36(22):3168-3177.
41. Gross AM, Wolters PL, Dombi E, et al. Selumetinib in children with inoperable plexiform neurofibromas. *N Engl J Med*. 2020;382(15):1430-1442.
42. Miettinen MM, Antonescu CR, Fletcher CDM, et al. Histopathologic evaluation of atypical neurofibromatous tumors and their transformation into malignant peripheral nerve sheath tumor in patients with neurofibromatosis 1—a consensus overview. *Hum Pathol*. 2017;67:1-10.
43. Pemov A, Hansen NF, Sindiri S, et al. Low mutation burden and frequent loss of CDKN2A/B and SMARCA2, but not PRC2, define pre-malignant neurofibromatosis type 1-associated atypical neurofibromas. *Neuro Oncol*. 2019;21(8):981-992.
44. Mauermann ML, Amrami KK, Kuntz NL, et al. Longitudinal study of intraneural perineurioma—a benign, focal hypertrophic neuropathy of youth. *Brain*. 2009;132(8):2265-2276.
45. Klein CJ, Wu Y, Jentoft ME, et al. Genomic analysis reveals frequent TRAF7 mutations in intraneural perineuriomas. *Ann Neurol*. 2017;81(2):316-321.
46. Hornick JL, Fletcher CD. Soft tissue perineurioma: clinicopathologic analysis of 81 cases including those with atypical histologic features. *Am J Surg Pathol*. 2005;29(7):845-858.
47. Carter JM, Wu Y, Blessing MM, et al. Recurrent genomic alterations in soft tissue perineuriomas. *Am J Surg Pathol*. 2018;42(12):1708-1714.
48. Vajtai I, Hewer E, Andres R, Neuenschwander M, Kappeler A, Gugger M. Meningeal perineurioma: a benign peripheral nerve sheath tumor in a previously unrecognized central nervous system location, mimicking meningioma. *Pathol Res Pract*. 2011;207(9):592-596.
49. Giannini C, Scheithauer BW, Steinberg J, Cosgrove TJ. Intraventricular perineurioma: case report. *Neurosurgery*. 1998;43(6):1478-1481; discussion 1481-1472.
50. Hornick JL, Bundock EA, Fletcher CD. Hybrid schwannoma/perineurioma: clinicopathologic analysis of 42 distinctive benign nerve sheath tumors. *Am J Surg Pathol*. 2009;33(10):1554-1561.
51. Harder A, Wesemann M, Hagel C, et al. Hybrid neurofibroma/schwannoma is overrepresented among schwannomatosis and neurofibromatosis patients. *Am J Surg Pathol*. 2012;36(5):702-709.
52. Stahn V, Nagel I, Fischer-Huchzermeyer S, et al. Molecular analysis of hybrid neurofibroma/schwannoma identifies common monosomy 22 and α -T-Catenin/CTNNA3 as a novel candidate tumor suppressor. *Am J Pathol*. 2016;186(12):3285-3296.
53. Ronellenfitsch MW, Harter PN, Kirchner M, et al. Targetable ERBB2 mutations identified in neurofibroma/schwannoma hybrid nerve sheath tumors. *J Clin Invest*. 2020;130(5):2488-2495.
54. Agaimy A. Microscopic intraneural perineural cell proliferations in patients with neurofibromatosis type 1. *Ann Diagn Pathol*. 2014;18(2):95-98.
55. Carney JA. Psammomatous melanotic schwannoma. A distinctive, heritable tumor with special associations, including cardiac myxoma and the Cushing syndrome. *Am J Surg Pathol*. 1990;14(3):206-222.
56. Torres-Mora J, Dry S, Li X, Binder S, Amin M, Folpe AL. Malignant melanotic schwannian tumor: a clinicopathologic, immunohistochemical, and gene expression profiling study of 40 cases, with a proposal for the reclassification of “melanotic schwannoma”. *Am J Surg Pathol*. 2014;38(1):94-105.
57. Wang L, Zehir A, Sadowska J, et al. Consistent copy number changes and recurrent PRKARIA mutations distinguish melanotic schwannomas from melanomas: SNP-array and next generation sequencing analysis. *Genes Chromosomes Cancer*. 2015;54(8):463-471.
58. Horvath A, Bossis I, Giatzakis C, et al. Large deletions of the PRKARIA gene in Carney complex. *Clin Cancer Res*. 2008;14(2):388-395.
59. Le Guellec S, Macagno N, Velasco V, et al. Loss of H3K27 trimethylation is not suitable for distinguishing malignant peripheral nerve sheath tumor from melanoma: a study of 387 cases including mimicking lesions. *Mod Pathol*. 2017;30(12):1677-1687.
60. Perry A, Rodriguez FJ, Reuss DE. Neurofibroma. In: *WHO Classification of Tumours of Soft Tissue and Bone*. Lyon, France: IARC Press; 2019.
61. Scheithauer BW, Erdogan S, Rodriguez FJ, et al. Malignant peripheral nerve sheath tumors of cranial nerves and intracranial contents: a clinicopathologic study of 17 cases. *Am J Surg Pathol*. 2009;33(3):325-338.
62. Lee W, Teckie S, Wiesner T, et al. PRC2 is recurrently inactivated through EED or SUZ12 loss in malignant peripheral nerve sheath tumors. *Nat Genet*. 2014;46(11):1227-1232.
63. Zhang M, Wang Y, Jones S, et al. Somatic mutations of SUZ12 in malignant peripheral nerve sheath tumors. *Nat Genet*. 2014;46(11):1170-1172.
64. Scheithauer BW, Amrami KK, Folpe AL, et al. Synovial sarcoma of nerve. *Hum Pathol*. 2011;42(4):568-577.
65. Reuss DE, Habel A, Hagenlocher C, et al. Neurofibromin specific antibody differentiates malignant peripheral nerve sheath tumors (MPNST) from other spindle cell neoplasms. *Acta Neuropathol*. 2014;127(4):565-572.
66. Rekhil B, Vogel U. Utility of characteristic ‘weak to absent’ INI1/SMARCB1/BAF47 expression in diagnosis of synovial sarcomas. *APMIS*. 2015;123(7):618-628.
67. Prieto-Granada CN, Wiesner T, Messina JL, Jungbluth AA, Chi P, Antonescu CR. Loss of H3K27me3 expression is a highly sensitive marker for sporadic and radiation-induced MPNST. *Am J Surg Pathol*. 2016;40(4):479-489.
68. Cleven AH, Sanna GA, Briaire-de Bruijn I, et al. Loss of H3K27 tri-methylation is a diagnostic marker for malignant peripheral nerve sheath tumors and an indicator for an inferior survival. *Mod Pathol*. 2016;29(6):582-590.
69. Tamborini E, Agus V, Perrone F, et al. Lack of SYT-SSX fusion transcripts in malignant peripheral nerve sheath tumors on RT-PCR analysis of 34 archival cases. *Lab Invest*. 2002;82(5):609-618.

CNS Spotlight available at cns.org/spotlight.

Acknowledgments

The authors also thank Rick Tracey from Path Photo Graphic Arts Laboratory, Department of Pathology, Johns Hopkins Hospital, for assistance with illustrations.