

## Comparative Pathology of Nerve Sheath Tumors in Mouse Models and Humans

Anat O. Stemmer-Rachamimov,<sup>1</sup> David N. Louis,<sup>1,14</sup> Gunnlaugur P. Nielsen,<sup>1</sup> Cristina R. Antonescu,<sup>3</sup> Alexander D. Borowsky,<sup>4</sup> Roderick T. Bronson,<sup>5</sup> Dennis K. Burns,<sup>6</sup> Pascale Cervera,<sup>8</sup> Margaret E. McLaughlin,<sup>9</sup> Guido Reifenberger,<sup>10</sup> Michael C. Schmale,<sup>11</sup> Mia MacCollin,<sup>2</sup> Richard C. Chao,<sup>12</sup> Karen Cichowski,<sup>13</sup> Michel Kalamarides,<sup>8</sup> Shanta M. Messerli,<sup>2</sup> Andrea I. McClatchey,<sup>14</sup> Michiko Niwa-Kawakita,<sup>8</sup> Nancy Ratner,<sup>15</sup> Karlyne M. Reilly,<sup>16</sup> Yuan Zhu,<sup>7,17</sup> and Marco Giovannini<sup>8</sup>

Departments of <sup>1</sup>Pathology and <sup>2</sup>Neurology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts; <sup>3</sup>Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, New York; <sup>4</sup>Medical Pathology Center for Comparative Medicine, University of California at Davis, Davis, California; <sup>5</sup>Department of Pathology, Harvard Medical School, Boston, Massachusetts; <sup>6</sup>Department of Pathology and <sup>7</sup>Center for Developmental Biology, University of Texas Southwestern Medical Center, Dallas, Texas; <sup>8</sup>INSERM U434, Fondation Jean Dausset-CEPH, Paris, France; <sup>9</sup>Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts; <sup>10</sup>Department of Neuropathology, Heinrich-Heine-University, Duesseldorf, Germany; <sup>11</sup>Division of Marine Biology and Fisheries, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, Florida; <sup>12</sup>Department of Medicine, Veterans' Affairs Medical Center and University of California at San Francisco, San Francisco, California; <sup>13</sup>Genetics Division, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts; <sup>14</sup>Massachusetts General Hospital Cancer Center, Harvard Medical School Department of Pathology, Charlestown, Massachusetts; <sup>15</sup>Department of Cell Biology, Neurobiology, and Anatomy, University of Cincinnati College of Medicine, Cincinnati, Ohio; <sup>16</sup>Genetic Modifiers of Tumorigenesis Section, Mouse Cancer Genetics Program, National Cancer Institute at Frederick, Frederick, Maryland; and <sup>17</sup>Division of Molecular Medicine and Genetics, University of Michigan Medical School, Ann Arbor, Michigan

### Abstract

Despite the progress made in our understanding of the biology of neurofibromatosis (NF), the long-term clinical outcome for affected patients has not changed significantly in the past decades, and both NF1 and NF2 are still associated with a significant morbidity and a decreased life span. A number of NF1 and NF2 murine models have been generated to aid in the study of NF tumor biology and in the development of targeted therapies for NF patients. A single, universal pathological classification of the lesions generated in these murine models is essential for the validation of the models, for their analysis and comparison with other models, and for their future effective use in preclinical treatment trials. For the formulation of a pathological classification of these lesions, the WHO classification of human tumors was used as a reference. However, it was not adopted for the classification of the GEM lesions because of some important differences between the human and murine lesions. A novel classification scheme for peripheral nerve sheath tumors in murine models was therefore devised.

### Introduction

The neurofibromatoses (NFs) NF1 and NF2 are autosomal dominant tumor suppressor gene syndromes in which affected individuals are predisposed to develop multiple tumors and hamartomas. NF1 and NF2 are clinically and genetically distinct (1).

NF1 affects approximately 1 in 3000 births. The hallmark of NF1 is the development of multiple neurofibromas and plexiform neurofibromas. Plexiform neurofibromas may undergo malignant transformation to malignant peripheral nerve sheath tumors (MPNSTs). Other neoplasms associated with NF1 include pilocytic astrocytomas, diffuse astrocytomas, leukemias, and pheochromocytomas. Nonneoplastic manifestations

include café au lait macules, skin fold freckling, Lisch nodules, learning disabilities, skeletal defects, and hypertension (1).

NF2 affects approximately 1 in 35,000 individuals, and its hallmark is the development of bilateral vestibular schwannomas. Patients often suffer from multiple schwannomas, meningiomas, and ependymomas as well as a variety of clinically asymptomatic lesions, including Schwann cell tumorlets, glial microhamartomas, and meningioangiomas (1, 2).

Despite the progress made in our understanding of the biology of NF1 and NF2 in recent years, the mainstay of treatment remains local tumor control by repeated surgeries, and the long-term clinical outcome of these patients has not changed significantly (1, 3–5).

The development of several NF1 and NF2 murine models provides a unique opportunity to gain insight into tumor initiation and progression in these syndromes as well as to develop and test different prognostic and therapeutic tools. Furthermore, NF murine models may aid in development of therapies for sporadic tumors because the *NF1* and *NF2* genes are involved in the pathogenesis of some sporadic MPNSTs and plexiform neurofibromas (6), meningiomas, schwannomas, ependymomas, and mesotheliomas (7).

Biallelic inactivation of *Nf1* (*Nf1*<sup>-/-</sup>) or *Nf2* (*Nf2*<sup>-/-</sup>) in the mouse is embryonic lethal (8–12), and although hemizygosity for one of the murine *Nf* genes (*Nf1*<sup>-/+</sup> or *Nf2*<sup>-/+</sup>) is associated with increased incidence of tumors, these are not peripheral nerve sheath tumors [PNSTs (10, 13–18)]. Therefore, different strategies had to be used to promote development of PNSTs, including the generation of chimeric mice composed in part of <sup>-/-</sup> cells for the relevant *Nf* gene (19) or the generation of conditional knockout models with disruption of the respective *Nf* gene only in specifically targeted cells, using tissue-specific promoters and *Cre-LoxP* technology (14, 20–22). In addition, models have been generated to identify functional domains of the *Nf1* and *Nf2* proteins [including exon-specific knockout mice (23), models with overexpression of the mutant gene in a selective cell population (24), and models in which *Nf* mutants were crossed with other knockout mice, enabling the evaluation of cooperation among *Nf1*, *Nf2*, and other tumor-associated genes (such as *p53* or *EGFR*) in Schwann cells (19, 25, 26)].

### Materials and Methods

A panel of 10 pathologists with different areas of expertise reviewed 56 lesions from 8 laboratories representing most published and unpublished NF mouse models (Appendix A) and compared their histological features with the corresponding human tumors. The aim

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**Requests for reprints:** Marco Giovannini, INSERM U434, Fondation Jean Dausset-CEPH, 27 rue Juliette Dodu, 75010 Paris, France. E-mail:marco.giovannini@cephb.fr.

Table 1 GEM classification of PNSTs<sup>a</sup>

GEM neurofibroma, grade I
GEM schwannoma
Grade I
Grade II
GEM perineurioma, grade I
GEM PNST (NOS)
Grade II
Grade III
Grade III with divergent differentiation
GEM peripheral nerve hyperplasia
Diffuse, "global"
Schwann cell hyperplasia

<sup>a</sup> PNST, peripheral nerve sheath tumor; NOS, not otherwise specified.

of this workshop was to review all available PNSTs from the Nf1 and Nf2 murine models generated to date and develop a pathological classification to characterize these lesions in a uniform and consistent manner. The meeting and pathological classification follow a format successfully adopted by similar pathology workshops for murine models of other organ systems (27, 28).

## Results

**A Separate GEM Classification Proposed.** In formulating the GEM classification of PNSTs, the panel referred to criteria and terminology of the WHO classification of human nervous system tumors (29). However, although the WHO classification served as a useful point of reference, it was not adopted for the classification of the GEM lesions because there were some important differences between the human and murine lesions. The WHO classification of human PNSTs is based on both histological and clinical characteristics (and describes clinicopathological entities), whereas the GEM classification must necessarily be based on histological features alone because further work is needed to determine the clinical features of murine PNSTs. Furthermore, although many of the histological features displayed by GEM PNSTs were similar to those observed in human tumors, some GEM lesions presented unique and distinct morphology and did not fit into the human classification by histological criteria.

For these reasons, a separate classification scheme for GEM is

proposed (Table 1), which is based on morphological criteria alone. Specifically, grading in the GEM classification refers merely to the presence or absence of histological features such as high cellularity, necrosis, nuclear pleomorphism, and brisk mitotic activity. As opposed to the WHO classification, where grade is an indicator of biological behavior, grading in the GEM classification is applied as a histological descriptor only, and it has no predictable bearing on the biological behavior of the tumors in the animals.

The WHO classification of PNSTs includes clinicopathological entities that are benign (WHO grade I) and defined by their cell composition as schwannomas, neurofibromas, and perineuriomas, and one malignant entity (WHO grade III or IV), the MPNST (Appendix B). In the GEM classification, grade I tumors are similarly defined by their cell composition as GEM neurofibromas, GEM schwannomas, and GEM perineuriomas. For the histologically malignant tumors (GEM grade III), the term "GEM PNST" is preferred because the tumors are composed of immature cells with some differentiation, and the distinction among the three above-mentioned entities becomes difficult (Table 2).

### Comparative Pathology of Human and Murine Neurofibromas.

Human neurofibromas (WHO grade I) are defined as benign nerve sheath tumors composed of a mixture of mature Schwann cells, perineurial cells, and fibroblasts. Clinically, neurofibromas may be dermal, nodular, or plexiform. Dermal and nodular neurofibromas are superficial or deep lesions, respectively, that arise in small nerves and may be well circumscribed or diffusely infiltrative. Plexiform neurofibromas arise almost exclusively in the context of NF1, causing diffuse enlargement of an affected plexus of large nerve and may undergo malignant transformation. Histologically, human neurofibromas are often hypocellular, composed of a mixed population of cells in a myxoid background (Fig. 1A). The mixed cell composition is important for diagnosis and can be confirmed by immunohistochemistry, which shows only a subset of S-100 protein-positive cells (Schwann cells), or by electron microscopy (EM). The lesions are often transversed by nerve axons and may contain varying amounts of collagen fibers and mast cells.

GEM neurofibromas (grade I) are similarly defined as tumors composed of a mixture of Schwann cells, perineurial cells, and fibro-

Table 2 GEM nerve sheath tumor classification: diagnostic criteria and definitions

#### GEM nerve sheath tumor diagnostic criteria

A spindled cell tumor arising in a mouse model can be designated as a nerve sheath tumor when at least one of the following criteria is met:

The lesion is clearly originating from a nerve.

In case of a high-grade lesion (see below), the lesion is clearly originating in a definite low-grade nerve sheath tumor.

The tumor contains S-100 immunopositive cells and/or there is evidence on EM<sup>a</sup> of Schwannian and/or perineurial differentiation.

Tumors are described using 3 different parameters: cellular composition, grade, and pattern of growth and spread.

**1. Cellular composition:** as defined by histology and immunohistochemistry (S-100-positive cells are present) and/or EM.

GEM schwannoma: a tumor composed of mature Schwann cells, as shown by immunohistochemistry or EM.

GEM neurofibroma: a tumor composed of a mixed cell composition: mature Schwann cells, fibroblasts, and perineurial cells, as shown by immunohistochemistry or EM.

GEM perineurioma: a tumor composed of mature perineurial cells as shown by EM.

GEM PNST (not otherwise specified): a tumor arising from a peripheral nerve, with some evidence of Schwannian differentiation, by immunohistochemistry or EM, that does not fit any of the above categories.

Divergent differentiation: differentiation along mesenchymal, epithelial, or neuroendocrine lines in a PNST.

#### 2. Grade

Grade evaluation depends on morphological features only. These include cellularity, pleomorphism, mitotic activity, and necrosis.

Grade I: GEM tumor with low cellularity. Tumor cells have bland/uniform nuclear cytology. Mitoses are absent, and there is no necrosis.

Grade II: GEM tumor with increased cellularity, nuclear atypia, and some mitotic activity. These lesions have histological similarities to low-grade MPNSTs in humans but may also include GEM cellular schwannomas (GEM schwannoma, grade II).

Grade III: GEM tumor with marked cellularity, nuclear atypia, and high rate of mitotic activity. May contain areas of necrosis or hemorrhage. These lesions histologically correspond to MPNSTs in the WHO classification.

#### 3. Stage (pattern of growth and spread)

In addition to the histological features of the tumors, the panel recommends describing the following features:

A) *Interface* of the tumor with adjacent normal tissues (circumscribed vs. infiltrative).

When infiltrative, the pattern of infiltration should be noted (destructive vs. infiltration with preservation of tissue).

B) *Tumor size*, measured as per greatest diameter.

C) *Metastasis*, evidence of metastasis in liver or lung. However, the panel recognizes that in some of the GEM models, the distinction between multiple primary tumors arising in targeted cells and metastasis may be impossible.

<sup>a</sup> EM, electron microscopy; PNST, peripheral nerve sheath tumor; MPNST, malignant PNST.

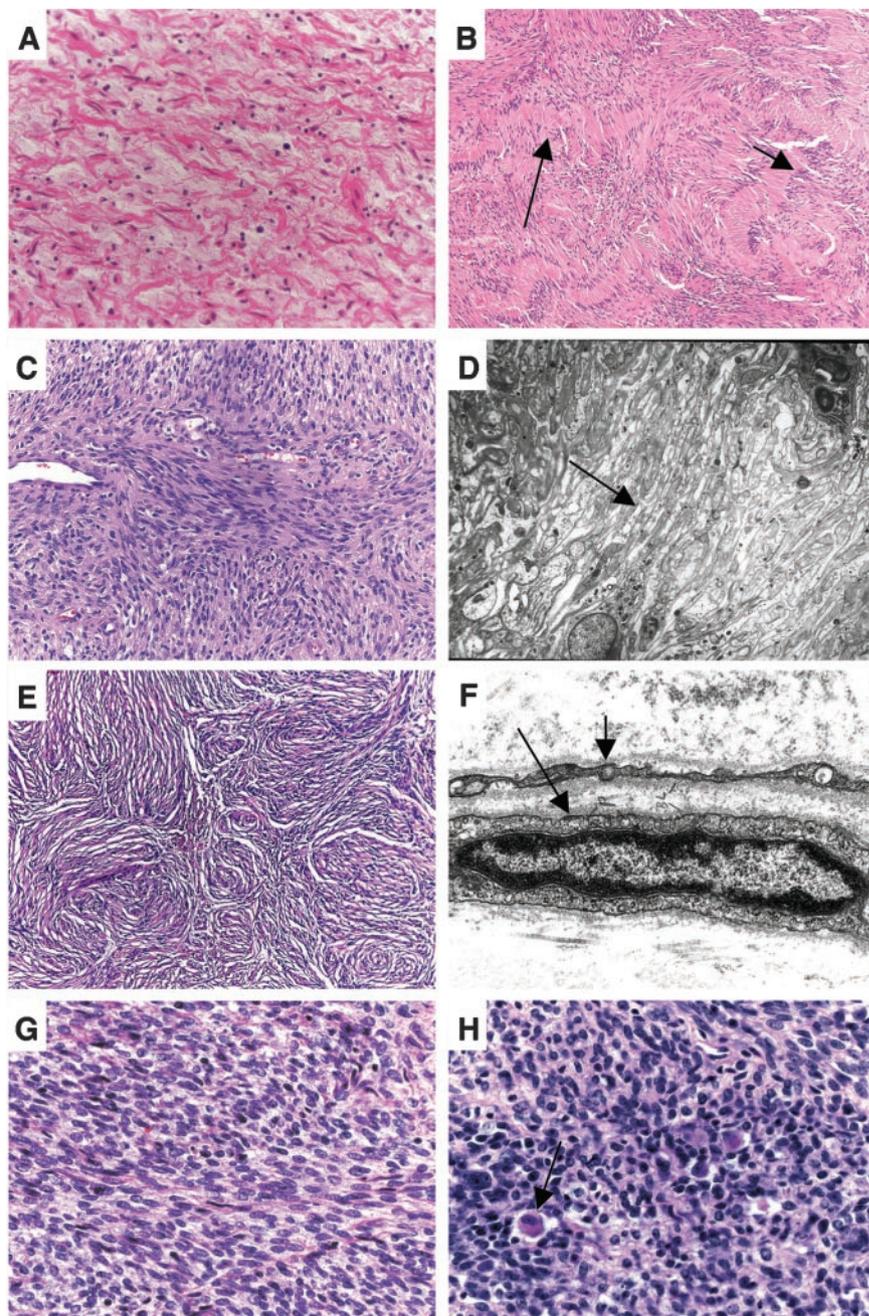


Fig. 1. Pathology of human peripheral nerve sheath tumors. *A*, neurofibroma composed of spindled and round cells and collagen bundles in a loose, myxoid background. *B*, Schwannoma with prominent, characteristic formation of Verocay bodies (arrows). *C*, cellular schwannoma, displaying moderate cellularity and nuclear pleomorphism and the absence of Verocay bodies. *D*, ultrastructural features of a schwannoma showing a Verocay body composed of intertwining of cytoplasmic processes surrounded by basal lamina. *E*, whorl formation and fascicles of spindle-shaped cells in a soft tissue perineurioma. *F*, diagnostic ultrastructural features of a perineurial cell include elongated cytoplasmic processes, a basal lamina (long arrow), and prominent pinocytotic vesicles (short arrow). *G*, malignant peripheral nerve sheath tumor composed of atypical spindle-shaped cells arranged in fascicles. *H*, large cells with abundant eosinophilic cytoplasm (arrow) represent skeletal muscle differentiation in a malignant peripheral nerve sheath tumor.

blasts, as shown by immunohistochemistry and/or ultrastructural analysis. Histologically, grade I GEM neurofibromas are closely similar to human neurofibromas. The tumors display low cellularity, minimal nuclear atypia, and rare mitoses. They are composed of elongated, thin nuclei in a loose, myxoid background (Fig. 2A) and contain collagen bundles and mast cells. A definite case of plexiform neurofibroma was not observed by the panel in the reviewed GEM lesions.

#### Comparative Pathology of Human and Murine Schwannomas.

The WHO defines human schwannomas as benign nerve sheath tumors (WHO grade I) composed of mature Schwann cells. Schwannomas are often encapsulated and do not infiltrate adjacent soft tissue. Histologically, classic features include a biphasic growth pattern with dense and loose areas (Antoni A and Antoni B, respectively) and the presence of Verocay bodies (rows of palisaded Schwann cell nuclei separated by a nuclear space; Fig. 1B). As opposed to neurofibromas, the presence of necrosis, mitotic activity, or nuclear pleomorphism in

schwannomas does not necessarily suggest malignant transformation. This is especially true in cellular schwannomas, a histological variant with high cellularity, moderate nuclear pleomorphism, and scattered mitotic figures (Fig. 1C). Despite all these “aggressive” histological features, cellular schwannomas are clinically benign tumors and thus have a different clinical behavior from neurofibromas displaying the same features (which would be classified as MPNSTs, WHO grade III). Confirmation, by S-100 protein immunohistochemistry or EM, of a pure Schwann cell population is essential for diagnosis (Fig. 1D).

GEM schwannomas (grade I) are similarly defined as murine tumors composed of mature Schwann cells, as shown by immunohistochemistry (diffuse S-100 staining) and/or ultrastructural analysis. Histologically, GEM schwannomas primarily display a dense pattern of growth (Antoni A) composed of intersecting fascicles or storiform designs. Verocay bodies are not common, but a similar pattern may be identified in some tumors (Fig. 2B). As opposed to human schwan-

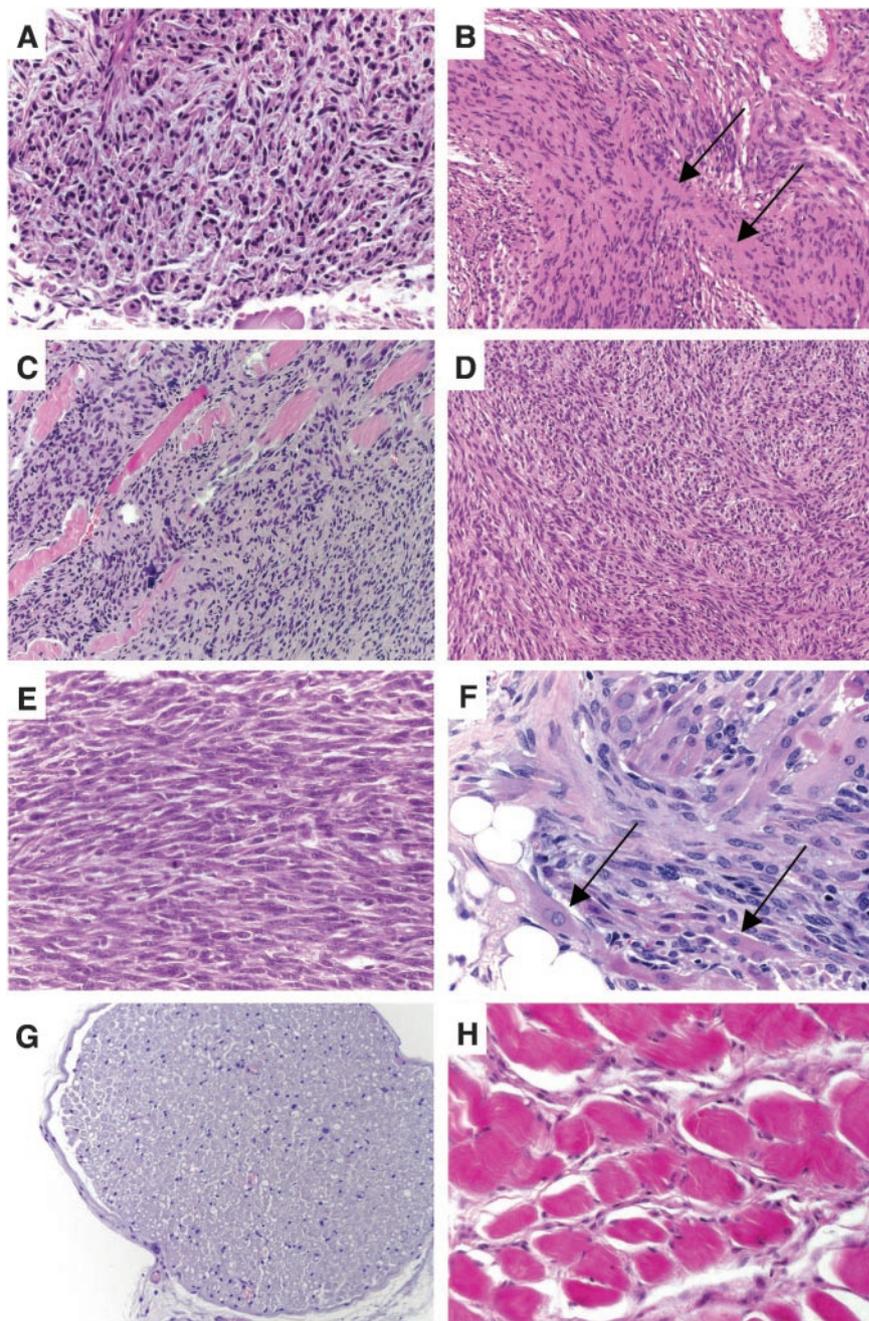


Fig. 2. Pathology of murine peripheral nerve sheath tumors. *A*, GEM neurofibromas, grade I, composed of small stellate cells in a loose, myxoid background. *B*, GEM schwannoma, grade I, with vague formation of Verocay bodies. *C*, GEM schwannoma widely infiltrating skeletal muscle. *D*, GEM schwannoma, grade II, a moderately cellular tumor with rare mitotic figures. *E*, GEM peripheral nerve sheath tumor, grade III, a cellular tumor composed of spindle-shaped cells with marked nuclear atypia arranged in fascicles. *F*, large cells with abundant cytoplasm (arrows) represent skeletal muscle differentiation in a GEM peripheral nerve sheath tumor, grade III. *G*, GEM global nerve hyperplasia. *H*, GEM diffuse Schwann cell hyperplasia.

nomas, GEM schwannomas are not encapsulated and often infiltrate into adjacent soft tissues, in a nondestructive fashion, entrapping fat and muscle (Fig. 2*C*). Other features commonly observed in human schwannomas such as hyalinized vessels, vascular malformations, thrombosed vessels, hemosiderin-laden macrophages, or hemorrhage, were not observed in GEM schwannomas.

Grade II GEM schwannomas display moderate nuclear pleomorphism, increased cellularity, and scattered mitotic figures (Fig. 2*D*). It has not yet been determined whether these lesions correspond in their behavior to human cellular schwannomas or to human MPNSTs (WHO grade III).

**Comparative Pathology of Human and Murine Perineuriomas.** Human perineurioma is a benign PNST composed of mature perineurial cells. There is an intraneural variant arising from a nerve, in which the cells form whorls around nerve fibers in a pseudo-onion bulb pattern, and a soft tissue variant, unassociated with nerve, that may

histologically mimic a meningioma or fibroma (Fig. 1*E*). Tumor cells are epithelial membrane antigen immunopositive and S-100 protein negative and have ultrastructural features of normal perineurial cells (Fig. 1*F*).

GEM perineuriomas (grade I) are similarly defined as tumors composed of perineurial cells. Unfortunately, epithelial membrane antigen immunohistochemistry has been problematic in mouse tissues. As a result, ultrastructural confirmation is essential for diagnosis because the pseudo-onion bulb pattern is not specific and can be seen in other tumor types. A definite case of GEM perineurioma was not observed by the panel in this meeting. A GEM tumor composed of thin elongated cells with tight whorls was reviewed, but electron microscopic data were not available on this case.

The GEM classification includes two other histological entities, which are not part of the WHO scheme: the GEM nerve sheath tumor not otherwise specified; and the GEM nerve sheath tumor with diver-

Table 3 Summary of peripheral nerve sheath tumors in NF mouse models reviewed at the workshop

Tumor (GEM)	Genotype	Source/reference
GEM schwannomas		
I	<i>Nf2</i> <sup>+/-</sup> : <i>Nf1</i> <sup>+/-</sup> P0Cre; <i>Nf2</i> <sup>flox/flox</sup> ; <i>Nf1</i> <sup>+/-</sup> P0-Sch-Δ(39-121)	I. Saotome, C-H. Liu, M. Niwa-Kawakita, M. Giovannini, A. I. McClatchey, unpublished results M. Niwa-Kawakita, M. Giovannini, unpublished results 24
II	<i>Nf2</i> <sup>+/-</sup> : <i>Nf1</i> <sup>+/-</sup> P0Cre; <i>Nf2</i> <sup>flox/flox</sup> ; <i>Nf1</i> <sup>+/-</sup> <i>Nf2</i> <sup>+/-</sup> : <i>p53</i> <sup>+/-</sup> cis	I. Saotome, C-H. Liu, M. Niwa-Kawakita, M. Giovannini, A. I. McClatchey, unpublished results M. Niwa-Kawakita, M. Giovannini, unpublished results 13
GEM neurofibromas		
I	<i>Nf1</i> <sup>flox/KO</sup> ;Krox20-cre+ <i>Nf1</i> <sup>-/-</sup> chimera	21 19
II	<i>Nf1</i> <sup>+/-</sup> : <i>p53</i> <sup>+/-</sup> cis	K. M. Reilly, D. A. Loisel, J. Ledger, R. T. Bronson, K. Cichowski, S. Shih, T. Jacks, unpublished results
GEM PNST <sup>a</sup>		
III	<i>Nf1</i> <sup>+/-</sup> : <i>p53</i> <sup>+/-</sup> P0-Sch-Δ(39-121) <i>p53</i> <sup>KO/+</sup> , <i>Nf1</i> <sup>KO/flox</sup> ;Krox20-cre+	19 24 L. F. Parada, F. Guignard, Y. Zhu, unpublished results

<sup>a</sup> PNST, peripheral nerve sheath tumor.

Table 4 WHO Classification for PNSTs<sup>a</sup>

Schwannoma (WHO grade I)
Cellular
Plexiform
Melanotic
Neurofibroma (WHO grade I)
Plexiform
Perineurioma
Intra-neural perineurioma
Soft tissue perineurioma
MPNST (WHO grade III or IV)
Epithelioid
MPNST with divergent mesenchymal differentiation and/or epithelial differentiation
Melanotic
Melanotic psammomatous

<sup>a</sup> PNST, peripheral nerve sheath tumor; MPNST, malignant PNST.

gent differentiation. The GEM nerve sheath tumor not otherwise specified is defined as any tumor clearly arising from a nerve or displaying Schwannian differentiation that does not fit into any of the above-mentioned categories. The GEM nerve sheath tumor with divergent differentiation is any GEM nerve sheath tumor in which there is clear evidence of divergent differentiation by histology, immunohistochemical staining, or EM (for example muscle, bone, cartilage, or epithelium). Divergent differentiation was seen in grade III GEM nerve sheath tumors.

**Comparative Pathology of Human and Murine MPNSTs.** In the WHO classification, MPNSTs are defined as malignant tumors arising from peripheral nerve sheath cells (WHO grade III or IV). Histologically, low-grade MPNSTs are cellular lesions with occasional enlarged hyperchromatic nuclei and scattered mitotic figures, whereas high-grade MPNSTs exhibit high cellularity with marked nuclear pleomorphism and brisk mitotic activity (Fig. 1G) and may display areas of necrosis. In some of the tumors, mesenchymal differentiation (including rhabdomyoblasts, cartilage, and bone) or epithelial and neuroendocrine differentiation may be observed (Fig. 1H). Because of the large spectrum of histological patterns displayed, the distinction of MPNSTs from other malignant tumors of soft tissues may be difficult. The WHO classification therefore requires that, to be classified as a MPNST, a tumor has to either arise from a nerve or from a pre-existing benign PNST or show perineurial or Schwann cell differentiation by EM or immunohistochemistry.

Histologically, GEM grade III nerve sheath tumors are similar to the human MPNSTs and are likewise defined as tumors that display high cellularity, anaplasia, and frequent mitotic figures; that either arise from a peripheral nerve or a lower grade PNST; or that show differentiation along nerve sheath lines by immunohistochemistry or EM (Fig. 2E). As in the human tumors, GEM grade III tumors may display divergent differentiation (GEM PNST with divergent differentiation, grade III) and histologically mimic other soft tissue tumors

(Fig. 2F). Therefore, as in the human MPNST with divergent differentiation, demonstration of perineurial or Schwann cell differentiation by EM or by immunohistochemistry is essential for diagnosis. The term “GEM PNST, grade III” is preferred to “GEM MPNST” because the word “malignant” is a clinical descriptor, and the clinical behavior of these murine lesions is still unknown.

**Murine Hyperplastic Lesions of the Peripheral Nerve.** Human hyperplastic peripheral nerve lesions consist of diffuse or nodular enlargements of the nerve in which one or more of the normal components are increased (30). Hyperplastic lesions are not included in the WHO classification, which is limited to classification of neoplastic lesions.

The GEM hyperplastic lesions are classified according to the normal nerve component involved. Diffuse “global” nerve hyperplasia is defined as diffuse enlargement of the involved nerve, which appears histologically normal except for its increased size and thickened endoneurium (Fig. 2G). Schwann cell hyperplasia is defined as a multifocal, diffuse process, in which involved nerves are enlarged due to increase of the Schwann cell component only (Fig. 2H).

**Approach to GEM PNST Classification.** Proper interpretation and definition of murine lesions are dependent on high-quality and uniform specimen processing (sampling, fixation, and staining) and analysis (immunostaining and EM). To facilitate and standardize the characterization of PNSTs in murine models, the panel developed a practical approach to the handling of these specimens. These recommendations do not replace but rather complement the previous recommendations made at the workshop that focused on central nervous system GEM tumors (28).

Table 5 Recommendations for mouse work-up for PNSTs<sup>a</sup>

Grade I lesions: perform S-100:
Diffuse positivity → GEM schwannoma
Focal positivity → GEM neurofibroma
Negative → EM is essential.
With Schwann cell or perineurial differentiation on EM → GEM neurofibroma
No Schwann cell or/and perineurial cell differentiation on EM → tumor is not a GEM PNST and should be classified according to EM features or markers as fibrosarcoma, rhabdomyosarcoma, spindle cell carcinoma, etc.
Grade II or III lesions: perform S-100, desmin, actin:
Diffuse S-100 positivity → GEM schwannoma
With other markers positive → GEM schwannoma grade II or III with divergent differentiation (Triton tumor)
With focal S-100 positivity → GEM PNST grade II or III
With other markers positive → GEM PNST grade II or III with divergent differentiation.
Negative for S-100 → EM (essential).
With Schwann cell or perineurial differentiation on EM → GEM PNST grade II or III
Without Schwann cell or perineurial differentiation on EM → not a GEM PNST and should be classified according to EM features or markers as fibrosarcoma, rhabdomyosarcoma, spindle cell carcinoma, etc.

<sup>a</sup> PNST, peripheral nerve sheath tumor; EM, electron microscopy.

Peripheral nerve tumors in the mouse present as soft tissue tumors; their origin from a peripheral nerve may not be easily recognized, and it is therefore advisable that sampled lesions be removed with adjacent structures to enable microscopic examination. The size of the tumor and its location and relationship to adjacent structures (association with a nerve, its infiltrating or sharp margin) should be carefully noted.

Because the classification of GEM PNSTs is based on the cellular differentiation of tumor cells (schwannian, fibroblastic, perineurial, or divergent), immunohistochemistry and EM are essential for classification. A tissue sample should be submitted in glutaraldehyde for EM on every tumor. It is strongly recommended that in new, uncharacterized murine models, EM be performed on all lesions. Once a murine model has been characterized, immunohistochemical analysis may suffice for the analysis of grade I lesions, and EM may be reserved only for the S-100 protein-negative cases (Appendix C).

In grade III GEM lesions, a panel of immunohistochemical stains is recommended to highlight divergent differentiation and exclude other types of soft tissue tumors. Immunostaining for S-100 protein (Schwann cell) and actin and desmin (muscle) should be performed. Lesions that are negative for these markers should be explored further by ultrastructural studies. Additional immunohistochemical stains may be necessary to exclude tumors when soft tissue tumors arise in specific sites. Finally, to facilitate the review of immunostained slides by pathologists and other scientists, either as panels in future workshops or as individual consultants, 3,3'-diaminobenzidine should be used as a substrate of the detection system (rather than immunofluorescence), and standard murine immunohistochemical protocols, as are currently available at the Mouse Models of Human Cancer Consortium website,<sup>18</sup> should be followed.

## Discussion

Review of the available NF mouse models has illustrated that their lesions have recapitulated, to various degrees, the human tumor counterparts. These improved mouse models of NF now provide an invaluable platform for rigorous preclinical trials of innovative therapeutic approaches. Pathological validation of these tumors, an essential step in the characterization of these models, is best achieved by a panel, which provides the opportunity for pathologists with different areas of expertise (neuropathology, soft tissue pathology, EM, and veterinary pathology) to review and compare all available models. This type of systematic and comprehensive evaluation is valuable for formulating a classification with consistent and comprehensible terminology, as well as for providing a platform for communication between mouse modelers and pathologists, and for drafting and disseminating recommendations for standardized specimen handling and work-up procedures.

In the present review, some histological differences and similarities were observed between murine and human PNSTs. For example, the infiltrative growth pattern of GEM grade I schwannomas contrasts with the well-circumscribed, pushing margin seen in human schwannomas. On the other hand, some of the murine lesions showed close histological similarities to the human counterparts (for example GEM grade I neurofibromas and human neurofibromas and grade III GEM PNSTs and human MPNSTs). The classification system proposed is a descriptive, morphology-based classification that may evolve and change as more information about the natural history of these murine tumors becomes available. Also, as more NF mouse models emerge, other histologically distinct lesions associated with specific genetic alterations may become apparent. Eventually, the histology, biological

behavior, and genetic alterations could be correlated and integrated into the GEM classification system and thus compared with the genetics, biology, and histology of NF-associated human lesions and corresponding sporadic human lesions. In this regard, it is recommended that future central reviews be planned to validate and analyze new models of GEM PNSTs, as well as other lesions in the NF mouse models.

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## Appendices

**Appendix A: Summary of the NF Mouse Models Presented at the Workshop (Table 3).**

**Appendix B: WHO Classification for Human PNSTs (Table 4).**

**Appendix C: Recommendations for Mouse Workup for PNSTs.** Initially, until models have been well characterized, it is recommended that all lesions be stained with H&E and S-100 immunostain and have EM analysis. Immunohistochemical stains to rule out common tumors in specific sites have been added (see Table 5).

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