COLLEGE of AMERICAN PATHOLOGISTS

Protocol for the Examination of Specimens From Patients With Neuroblastoma*

Version: Neuroblastoma 3.1.0.3   **Protocol Posting Date:** August 2016

This protocol is NOT required for accreditation purposes

*This protocol applies to neuroblastoma and related neuroblastic tumors.

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Accreditation Requirements
The use of this protocol is recommended for clinical care purposes, but is not required for accreditation purposes.

CAP Laboratory Accreditation Program Protocol Required Use Date: Not applicable

Important Note
First priority should always be given to formalin-fixed tissue for morphologic evaluation. Special studies (eg, ploidy analysis, fluorescence in situ hybridization for *MYCN* status) are critical to the molecular workup of neuroblastoma and require at least 100 mg of viable, snap-frozen tissue as the second priority for workup (Note A).

For more information, contact: The Children's Oncology Group Biopathology Center,
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Summary of Changes
v3.1.0.3 - Minor revision to note for INPC - *Ganglioneuroblastoma, intermixed (Schwannian stroma-**rich**), any age*

Surgical Pathology Cancer Case Summary

Protocol posting date: August 2016

NEUROBLASTOMA: Resection, Biopsy

Note: This case summary is recommended for reporting neuroblastic tumors, but is not required for accreditation purposes.

Select a single response unless otherwise indicated.

Specimen
___ Adrenal/periadrenal
___ Retroperitoneal, nonadrenal
___ Thoracic paraspinal
___ Cervical region
___ Other (specify): _____
___ Not specified

Procedure (Note B)
___ Resection
___ Incisional biopsy
___ Other (specify): _____
___ Not specified

Tumor Size
Greatest dimension: ___ cm
+ Additional dimensions: ___ x ___ cm
___ Cannot be determined (explain): _____

Patient Age
___ <18 months
___ ≥18 months and <5 years
___ ≥5 years

Histologic Type (select all that apply) (Note C)
___ Neuroblastoma
___ Ganglioneuroblastoma
      ___ Nodular subtype# (specify number of nodules): _____
      ___ Intermixed subtype
___ Ganglioneuroma
      +___ Maturing
      +___ Mature
___ Neuroblastic tumor, unclassifiable
___ Cannot be determined (explain): _____

#*Note: For nodular (composite) ganglioneuroblastomas with more than 1 nodule, degree of differentiation and mitotic-karyorrhectic index (MKI) must be given for each nodule. Please indicate the differentiation and MKI for the least favorable nodule in the case summary below. Classification of additional nodules can be described in the Comment.*

Degree of Differentiation (neuroblastic component) (Note D)
___ Undifferentiated
___ Poorly differentiated
___ Differentiating
___ Cannot be determined (explain): _____
___ Not applicable

+ Data elements preceded by this symbol may be clinically important but are not yet validated or regularly used in patient management.   2

Mitotic-Karyorrhectic Index (MKI) (neuroblastic component) (Note E)
___ Low (<100 per 5000 cells; <2%)
___ Intermediate (100-200 per 5000 cells; 2%-4%)
___ High (>200 per 5000 cells; >4%)
___ Cannot be determined (explain): _____
___ Not applicable

Treatment History (Note F)
___ No known preoperative therapy
___ Preoperative therapy given
___ Not specified

+ Treatment Effect
+___ No known preoperative therapy (not applicable)
+___ Not identified
+___ Present
        + Percent tumor necrosis: ____%
        + Percent therapy-induced cytodifferentiation: ____%
+___ Cannot be determined

International Neuroblastoma Pathology Classification (INPC) (select all that apply) (Note G)
Note: INPC applies to untreated primary tumors and tumors in metastatic sites provided that there is sufficient material to classify histologically. Classification based on limited material (biopsy or incomplete resection) may be subject to sampling error and should be noted, accordingly. Bone marrow biopsy is useful only for evaluation of degree of neuroblastic differentiation but is not eligible for MKI determination.
___ Favorable histopathology[#]
    [#]*Note: The following histologic type, differentiation, MKI, and age groupings denote a favorable histopathology*
        classification.
        Neuroblastoma (Schwannian stroma-poor)
            Poorly differentiated subtype, low or intermediate MKI, <18 months old
            Differentiating subtype, intermediate MKI, <18 months old
            Differentiating subtype, low MKI, <5 years old
        Ganglioneuroblastoma, nodular (list least favorable nodule)
            Poorly differentiated subtype, low or intermediate MKI, <18 months old
            Differentiating subtype, intermediate MKI, <18 months old
            Differentiating subtype, low MKI, <5 years old
        Ganglioneuroblastoma, intermixed (Schwannian stroma-rich), any age
        Ganglioneuroma (Schwannian stroma-dominant), mature or maturing, any age
___ Unfavorable histopathology[##]
    [##]*Note: The following histologic type, differentiation, MKI, and age groupings denote an unfavorable histopathology*
        classification.
        Neuroblastoma (Schwannian stroma-poor)
            Undifferentiated subtype, any MKI, any age
            Poorly differentiated subtype, high MKI, any age
            Poorly differentiated subtype, low or intermediate MKI, >18 months old
            Differentiating subtype, high MKI, any age
            Differentiating subtype, intermediate MKI, >18 months old
            Differentiating subtype, low MKI, >5 years old
        Ganglioneuroblastoma, nodular (list least favorable nodule)
            Undifferentiated subtype, any MKI, any age
            Poorly differentiated subtype, high MKI, any age
            Poorly differentiated subtype, low or intermediate MKI, >18 months old
            Differentiating subtype, high MKI, any age
            Differentiating subtype, intermediate MKI, >18 months old
            Differentiating subtype, low MKI, >5 years old
___ Not applicable secondary to previous chemotherapy
___ Cannot be determined secondary to insufficient material

Tumor Extent (Note H)

Primary Tumor
___ Cannot be determined (explain): _____
___ Encapsulated
___ Extracapsular extension without adjacent organ involvement
___ Extension into adjacent organs
___ Extension into spinal canal

Regional Lymph Nodes
___ No nodes submitted or found

Lymph Node Examination (required only if lymph nodes are present in the specimen)

Number of Lymph Nodes Involved
Specify: ____
       Specify site (if known): _____
___ Number cannot be determined (explain): _____

Number of Lymph Nodes Examined
Specify: ____
___ Number cannot be determined (explain): _____

Distant Metastasis (required only if confirmed pathologically in this case)
___ Present
       Specify site(s), if known: _____

Ancillary Studies (Note I)

MYCN Amplification Status (required for all tumors except ganglioneuroma)
Note: Results of MYCN amplification information may not be available to the pathologist at the time of the report.
___ Not amplified
___ Amplified
___ Gain
___ Cannot be determined
___ Pending

\+ Other (specify): _____

+ Additional Pathologic Findings (Notes J and K)
\+ Specify: _____

+ Comment(s)

Explanatory Notes

A. Submission of Tissue

Molecular testing is crucial for accurate risk stratification and clinical decision making. In addition to the tissue taken for histologic examination as described below, the International Neuroblastoma Pathology Committee recommends sampling a neuroblastic surgical specimen for biologic studies as follows[1]:

A minimum of 2 samples (A and B, each 1 x 1 x 1 cm) should be taken, preferably from morphologically different areas. Samples A and B are split into 4 pieces:

1	2
3	4

A,B 1   Make at least 10 touch preparations (air-dried, unfixed, and, if necessary, stored at –20°C) for fluorescence in situ hybridization (FISH) (for *MYCN*, chromosome 1p) and image cytometry

A,B 2   Put in sterile culture medium (for *MYCN*, chromosome 1p, ploidy, cytogenetics, culture and drug sensitivity, etc)

A,B 3,4 Snap-freeze in liquid nitrogen or at –70°C (for molecular biology studies and immunohistochemistry) (also snap-freeze residuum of A,B 1)

The above recommendations are applicable when the entire or a large proportion of the tumor is resected, or when 1 or more large biopsy specimens are available. If the amount of tumor tissue is restricted, morphologic diagnosis is the prime consideration. Imprints (for FISH study of *MYCN*) should always be made from fresh tumor tissue.

If, as a minimum procedure, only core biopsies are performed, they should be multiple (2 to 4, for formalin fixation and snap-freezing), preferably concomitant with fine-needle aspiration specimens for FISH study of *MYCN*. A minimum of 100 mg snap-frozen tissue may be necessary for ploidy study by flow cytometry. Such specimens are usually not sufficient for prognostic evaluation histopathologically.[1]

B. Procedures

Core needle biopsies can obtain sufficient material for special studies and morphologic diagnosis, but sampling problems may limit tumor subtyping or grading, especially in tumors that are heterogeneous (ie, ganglioneuroblastoma, nodular type). Histologic classification based on limited material should be noted in these cases. Grading can be performed on samples from metastatic sites, provided that the specimen is large enough to be representative. When handling an excision specimen, sections should be obtained from central and peripheral areas of the tumor according to common guidelines (at least 1 tumor section per centimeter in the longest dimension and sections from all inked surgical margins).[1] All grossly visible nodules or hemorrhagic foci should be individually sampled.

C. Histopathologic Type

It is recommended that the International Neuroblastoma Classification[1,2] described below be used when describing tumor samples.

There are 4 specific categories in this group of tumors:
    Neuroblastoma (Schwannian stroma-poor)
    Ganglioneuroblastoma, nodular (composite, Schwannian stroma-rich/stroma-dominant and stroma-poor)
    Ganglioneuroblastoma, intermixed (Schwannian stroma-rich)
    Ganglioneuroma (Schwannian stroma-dominant)

Neuroblastoma (Schwannian Stroma-poor) Category
Microscopically, tumors in the neuroblastoma category are composed of neuroblastic cells that form groups or nests separated by delicate, often incomplete stromal septa without or with limited Schwannian proliferation (comprising <50% of the tumor).[1]

Differential Diagnosis
The differential diagnosis of neuroblastoma usually also includes the pediatric small round blue cell tumors: Ewing sarcoma (including peripheral primitive neuroectodermal tumor [pPNET]), alveolar rhabdomyosarcoma, Wilms tumor, desmoplastic small round cell tumor, lymphoma, and myeloid leukemia. A cell surface glycoprotein, p30/32 (product of the *MIC2* gene detected by CD99 antibodies), common in peripheral primitive neuroectodermal tumor Ewing sarcoma and lymphomas, usually is negative in neuroblastoma; both neuroblastoma and Ewing sarcoma are frequently positive for PGP9.5 and NB84. In contrast, tyrosine hydroxylase commonly is positive in neuroblastoma and negative in Ewing sarcoma. Muscle-specific markers, such as desmin, myogenin, and MyoD1, are often positive in rhabdomyosarcomas, but negative in neuroblastoma; additionally, rhabdomyosarcoma cells often show morphologic evidence of muscle differentiation. Although the blastemal component of a Wilms tumor may mimic neuroblastoma, the former often exhibits WT1 positivity in addition to epithelial and mesenchymal components. Finally, lymphomas usually stain for multiple lineage-specific hematopoietic markers, whereas neuroblastomas are negative for these proteins. Undifferentiated neuroblastoma cells may, on rare occasions, express vimentin. Neuroblasts are also typically positive for synaptophysin and neuron-specific enolase, although these are less specific. Schwann cells are positive for S100 protein.

Electron Microscopy
Ultrastructural studies are still of value in the diagnosis of relatively undifferentiated neuroblastoma, where the diagnosis is not readily evident by light microscopic study or urinary catecholamine study, especially given the variable specificity of immunostaining. Diagnostic criteria include dense core granules of neurosecretory type and cell processes (primitive neurites) containing typically arranged microtubules.

Ganglioneuroblastoma, Nodular (Composite Schwannian Stroma-Rich/Stroma-Dominant and Stroma-Poor) Category[#]
Tumors in the ganglioneuroblastoma, nodular category are composed of multiple clones: 1 or more nodules of neuroblastic cells set within a background of ganglioneuroblastoma, intermixed, or ganglioneuroma-like tissue.[3]

Ganglioneuroblastoma, Intermixed (Schwannian Stroma-Rich) Category[#]
Ganglioneuromatous (Schwannian stroma-rich) component of the tumor exceeds 50%. Neuroblastic component is present in an intermixed or randomly distributed pattern of microscopic neuroblastic nests. The neuroblastic component consists of cells in various stages of differentiation (neuroblasts, differentiating neuroblasts, maturing ganglion cells) and has varying amounts of neuropil. Macroscopic hemorrhagic nodules are absent.

Ganglioneuroma (Schwannian Stroma-Dominant) Category
Two subtypes are included; neuroblastic cells (differentiating neuroblasts, maturing and mature ganglion cells) in the tumor tissue do not form microscopic nests but are individually distributed in the Schwannian stroma.

Maturing Subtype
Schwannian stroma is predominant with minor, scattered groups of differentiating neuroblasts or maturing ganglion cells along with completely mature ganglion cells. There are no islands of neuropil.

Mature Subtype
Schwannian stroma is predominant with exclusively completely mature ganglion cells. May have neuritic fascicular processes accompanied by Schwann cells and perineurial cells. Satellite cells may accompany mature ganglion cells. There is a complete absence of a neuroblastomatous component, including no islands of neuropil.

Neuroblastic Tumor, Unclassifiable
Neuroblastic cells evident; sample insufficient for categorization into 1 of the 4 basic types. A small biopsy taken from a large tumor can result in this designation.

Ganglioneuroblastomas are highly variable in both number of neuroblasts and their extent of differentiation. Variability is seen between tumors, between microscopic fields in the same tumor, and occasionally between the primary and metastatic tumor. Ganglioneuroblastoma diagnostic criteria include (a) mature Schwannian stromal component with individually scattered mature and/or maturing ganglion cells and (b) a neuroblastic component.

D. Degree of Differentiation
Neuroblastomas (Schwannian stroma-poor) and the neuroblastic component of nodular-type ganglioneuroblastomas are further classified into 1 of 3 subtypes[1]:

<u>Undifferentiated Subtype</u>
Neuropil absent; no tumor cell differentiation; diagnosis relies heavily on ancillary techniques, such as immunohistochemistry, electron microscopy, and/or molecular/cytogenetic analysis.

<u>Poorly Differentiated Subtype</u>
Neuropil evident in background; less than 5% of tumor cells show features of differentiating neuroblasts (ganglion cell-like) with synchronous differentiation of the nucleus (enlarged, vesicular with a single prominent nucleolus) and the cytoplasm (conspicuous, eosinophilic or amphophilic, and twice the diameter of the nucleus).

<u>Differentiating Subtype</u>
Greater than 5% of tumor cells show evidence of differentiation (may be accompanied by mature ganglion-like cells), and neuropil is usually abundant; some tumors can show substantial Schwannian stromal formation, frequently at their periphery, and a transition zone between neuroblastomatous and ganglioneuromatous regions can develop (although this zone lacks well-defined borders and comprises less than 50% of the tumor).

E. Mitotic-Karyorrhectic Index
The mitotic-karyorrhectic index (MKI)[1,4] is the number of mitotic and karyorrhectic nuclei per 5000 neuroblastic cells. It is a useful prognostic indicator for tumors in the neuroblastoma (Schwannian stroma-poor) category and should be determined as an average of all tumor sections available. The method described by Joshi et al[5] can be used to calculate MKI without the need to count 5000 cells. In summary, cellular density is usually estimated under low power, and the tumor is classified as either a dense (700 to 900 cells per 400X high-power fields [HPFs])#, moderate (400 to 600 tumor cells per HPF)#, sparse (100 to 300 cells per HPF)#, or mixed category (a mixed tumor has variable cellularity under different HPFs). Once categorized, random HPFs are chosen to count mitotic and karyorrhectic cells. High-power fields on specimens in the mixed category are selected to be proportional to the cellular density in the specimen; for example, in a sample with 70% dense cellularity and 30% sparse cellularity, 70% of the HPF should be in dense areas and 30% in sparse areas. In highly cellular tumors, the MKI can be determined in 6 to 8 HPFs, whereas in tumors with low cellularity and prominent neuropil, 20 or more HPFs may be necessary. Specimens are assigned to 1 of 3 prognostic categories:

(1) Low MKI — Less than 100 mitotic and karyorrhectic cells/5000 tumor cells, or less than 2% of tumor consisting of mitotic and karyorrhectic cells

(2) Intermediate MKI — 100 to 200 mitotic and karyorrhectic cells/5000 tumor cells, or 2% to 4% of tumor consisting of mitotic and karyorrhectic cells

(3) High MKI — Greater than 200 mitotic and karyorrhectic cells/5000 tumor cells, or more than 4% of tumor consisting of mitotic and karyorrhectic cells

Numbers of neuroblastic cells in each HPF (denominator for MKI determination) can vary, based on the type of microscope used (some practice is required for assessing the number of neuroblastic cells per HPF on a given microscope). The range of cells per HPF listed in parentheses in the above discussion are for a standard microscope setup with regular oculars. With a super-wide-field type of ocular, there may be an increased number of cells (1200 to 1500 cells per HPF in a dense category).

F. Treated Tumors
Neuroblastic tumors treated with chemotherapy typically show 1 of 3 responses: (1) maturation of the neuroblastic component, with increased Schwannian stromal content and a shift along the spectrum from neuroblastoma

towards ganglioneuroma; (2) necrosis of tumor cells with areas of hemorrhage (including hemosiderin-laden macrophages), calcifications, and fibrosis/stromal overgrowth; or (3) no significant effect. The International Neuroblastoma Pathology Classification (INPC) does not apply to treated tumors and should not be used. Rather, a diagnosis of "neuroblastoma (or whatever the original classification was) with treatment effect" should be rendered, and the histologic features enumerated. Any residual foci of undifferentiated or poorly differentiated neuroblasts should be commented upon; the percentage of viable tumor can be estimated, although the clinical significance of this value is dubious.

Important note: Once the International Neuroblastoma Pathology Classification INPC has been applied to a tumor based on pretreatment pathologic evaluation, the favorable/unfavorable histology designation never changes, regardless of posttreatment clinical or pathologic changes.

G. Prognostic Groups

The International Neuroblastoma Pathology Classification (INPC)[2] uses age, neuroblastic maturation, Schwannian stromal content, and MKI as prognostic indicators. Unfavorable indicators include undifferentiated neuroblastoma (especially in older patients) and high MKI. An important revision was added in 2003.[3] The original INPC classified all tumors in the category of ganglioneuroblastoma, nodular, as unfavorable.[2] The revised INPC distinguishes 2 prognostic subsets in this category, favorable and unfavorable, by applying the same age-linked histopathology evaluation to the nodular (neuroblastoma) components[3] (Table 1).

Table 1. International Neuroblastoma Pathology Prognostic Classification (INPC)

Age	Favorable Histology Group	Unfavorable Histology Group
Any	Ganglioneuroma (Schwannian stroma-dominant) • maturing • mature Ganglioneuroblastoma, intermixed (Schwannian stroma-rich)	Neuroblastoma (Schwannian stroma-poor) • undifferentiated and any mitotic-karyorrhectic index (MKI)
<1.5 y	Neuroblastoma (Schwannian stroma-poor) • poorly differentiated and low or intermediate MKI • differentiating and low or intermediate MKI	Neuroblastoma (Schwannian stroma-poor) • poorly differentiated and high MKI • differentiating and high MKI
1.5 y to <5 y	Neuroblastoma (Schwannian stroma-poor) • differentiating and low MKI	Neuroblastoma (Schwannian stroma-poor) • poorly differentiated and any MKI • differentiating and intermediate or high MKI
≥5 y	Ganglioneuroblastoma, nodular (composite, Schwannian stroma-rich/stroma-dominant and stroma-poor), favorable subset[#]	Neuroblastoma (Schwannian stroma-poor) • any subtype and any MKI Ganglioneuroblastoma, nodular (composite, Schwannian stroma-rich/stroma-dominant and stroma-poor), unfavorable subset[#]

[#] The neuroblastic nodule(s) of the ganglioneuroblastoma, nodular subtype are graded with the INPC age-linked histopathology evaluation and based on that evaluation classified as favorable or unfavorable. For multinodular tumors, each nodule is graded separately and the least favorable nodule determines the classification.

H. Staging

Given the multiple systems for staging and the increasing importance of pretreatment imaging characteristics, the pathologist is not required to report on staging for patients with neuroblastoma. The existing staging systems are described below and can be included in the Comment section if desired.

International Neuroblastoma Staging System (INSS)

The International Neuroblastoma Staging System (INSS) has traditionally been used to stage patients.[5] The core of this clinical staging is the size of the primary tumor, locoregional lymph node status, and the presence or absence of distant metastases.

Stage 1	Localized tumor with complete gross excision, with or without microscopic residual disease; representative ipsilateral lymph nodes negative for tumor microscopically (nodes attached to and removed with the primary tumor may be positive).
Stage 2A	Localized tumor with incomplete gross excision; representative ipsilateral nonadherent lymph nodes negative for tumor microscopically.
Stage 2B	Localized tumor with or without complete gross excision, with ipsilateral nonadherent lymph nodes positive for tumor. Enlarged contralateral lymph nodes must be negative microscopically.
Stage 3	Unresectable, unilateral tumor infiltrating across the midline, with or without regional lymph node involvement; or localized unilateral tumor with contralateral regional lymph node involvement; or midline tumor with bilateral extension by infiltration (unresectable) or by lymph node involvement. The midline is defined as the vertebral column. Tumors originating on 1 side and crossing the midline must infiltrate to or beyond the opposite side of the vertebral column.
Stage 4	Any primary tumor with dissemination to distant lymph nodes, bone, bone marrow, liver, skin, and/or other organs (except as defined for stage 4S).
Stage 4S	Localized primary tumor (as defined for stage 1, 2A, or 2B), with dissemination limited to skin, liver, and/or bone marrow (limited to infants less than 1 year of age). Marrow involvement should be minimal (ie, less than 10% of total nucleated cells identified as malignant by bone biopsy or by bone marrow aspirate). More extensive bone marrow involvement would be considered to be stage 4 disease. A meta-iodobenzylguanide (MIBG) scan (if performed) should be negative for disease in the bone marrow.

International Neuroblastoma Risk Group (INRG) Staging System (INRGSS)

Recently, a new clinical staging system, the INRGSS, has been proposed and increasingly adopted.[6] Unlike the INSS, which relies on postsurgical data, the INRGSS relies only on pretreatment imaging, patient age, and clinical extent of disease. The INRGSS can be summarized as localized disease (stage L1), regional disease (stage L2), metastatic disease (stage L3), and "special stage" (stage MS, similar to the INSS stage 4S). However, this schema relies heavily on image-defined risk factors* and may be difficult for pathologists to implement.

Stage L1
- Localized tumor not involving vital structures as defined by the list of image-defined risk factors and confined to one body compartment

Stage L2
- Locoregional tumor with presence of 1 or more image-defined risk factors

Stage M
- Distant metastatic disease (except stage MS)

Stage MS
- Metastatic disease in children younger than 18 months with metastases confined to skin, liver, and/or bone marrow with minimal marrow involvement as described in stage 4S, above.

* Image-Defined Risk Factors

Ipsilateral tumor extension within 2 body compartments
- Neck-chest, chest-abdomen, abdomen-pelvis

Neck
- Tumor encasing carotid and/or vertebral artery and/or internal jugular vein

- Tumor extending to base of skull
- Tumor compressing the trachea

Cervico-thoracic junction

- Tumor encasing brachial plexus roots
- Tumor encasing subclavian vessels and/or vertebral and/or carotid artery
- Tumor compressing the trachea

Thorax

- Tumor encasing the aorta and/or major branches
- Tumor compressing the trachea and/or principal bronchi
- Lower mediastinal tumor, infiltrating the costo-vertebral junction between T9 and T12

Thoraco-abdominal

- Tumor encasing the aorta and/or vena cava

Abdomen/pelvis

- Tumor infiltrating the porta hepatis and/or the hepatoduodenal ligament
- Tumor encasing branches of the superior mesenteric artery at the mesenteric root
- Tumor encasing the origin of the coeliac axis, and/or of the superior mesenteric artery
- Tumor invading 1 or both renal pedicles
- Tumor encasing the aorta and/or vena cava
- Tumor encasing the iliac vessels
- Pelvic tumor crossing the sciatic notch
- Intraspinal tumor extension whatever the location provided that:
- More than one-third of the spinal canal in the axial plane is invaded and/or the perimedullary leptomeningeal spaces are not visible and/or the spinal cord signal is abnormal

Infiltration of adjacent organs/structures

- Pericardium, diaphragm, kidney, liver, duodeno-pancreatic block, and mesentery
- Conditions to be recorded, but not considered image-defined risk factors.

Multifocal primary tumors
Pleural effusion, with or without malignant cells
Ascites, with or without malignant cells

Risk Groups

Risk group assessment can be defined by clinical and biological variables. A simplified approach is described using a compendium of biologic and clinical risk factors (Table 2).[7] Also included is a risk-grouping scheme for clinical trials of the Children's Oncology Group Neuroblastoma Studies (Table 3) based on the combination of clinical stage, age at diagnosis, *MYCN* status, histopathology classification, and DNA index. According to this scheme, patients are classified into the low-, intermediate-, or high-risk group. As for the patients in the intermediate-risk group, protocol assignment for treatment of the individual cases is determined by further subclassification based on the combination of the above-mentioned risk factors and presence or absence of 1p deletion and/or 11q loss of heterozygosity (LOH). A newer risk-stratification system for the International Neuroblastoma Risk Group (INRG) is also shown (Table 4).

Table 2. Biologic and Clinical Risk Factors and Groups in Neuroblastoma

Parameter	**Low Risk**	**Intermediate Risk**	**High Risk**
MYCN status	Normal	Normal	Amplified (>10 copies)
Ploidy	Hyperdiploid	Near-diploid	Near-diploid
	Near-triploid	Near-tetraploid	Near-tetraploid
11q, 14q loss of heterozygosity (LOH)	Rare	Common	Rare

Parameter	Low Risk	Intermediate Risk	High Risk
Age	Usually <1 y	Usually >1 y	Usually 1 to 5 y
Stage (INSS)	1, 2, 4S	Usually 3 or 4	Usually 3 or 4
Stage (INRGSS)	L1, MS	L2	M
Expected survival rate#	Greater than 95% with surgery alone	~90% with various intensities of chemotherapy	~40%

Based on the experience of Children's Oncology Group Neuroblastoma Studies/Protocols.

Abbreviations: INSS, International Neuroblastoma Staging System; INRGSS, International Neuroblastoma Risk Group Staging System.

Table 3. Risk Grouping Scheme Using the International Neuroblastoma Staging System (INSS)

Study	INSS Stage	Age	*MYCN*	Ploidy#	INPC	Other
Low Risk	1	any	any	any	any	
Low Risk	2a/2b	any	not amp	any	any	resection >50%
Intermediate Risk	2a/2b	0 – 12 y	not amp	any	any	resection <50% or biopsy only
High Risk	2a/2b	any	amp	any	any	any degree of resection
Intermediate Risk	3	<547 d	not amp	any	any	
Intermediate Risk	3	>547 d – 12 y	not amp	any	FH	
High Risk	3	any	amp	any	any	
High Risk	3	≥547 d	not amp	any	UH	
High Risk	4	<365 d	amp	any	any	
Intermediate Risk	4	<365 d	not amp	any	any	
High Risk	4	365-<547 d	amp	any	any	
High Risk	4	365-<547 d	any	DI=1	any	
High Risk	4	365-<547 d	any	any	UH	
Intermediate Risk	4	365-<547 d	not amp	DI>1	FH	
High Risk	4	≥547 d	any	any	any	
Low Risk	4s	<365 d	not amp	DI>1	FH	asymptomatic
Intermediate Risk	4s	<365 d	not amp	any	any	symptomatic
Intermediate Risk	4s	<365 d	not amp	DI=1	any	asymptomatic or symptomatic

Study	INSS Stage	Age	*MYCN*	Ploidy[#]	INPC	Other
Intermediate Risk	4s	<365 d	not amp	any	UH	asymptomatic or symptomatic
Intermediate Risk	4s	<365 d	missing	missing	missing	asymptomatic or symptomatic
High Risk	4s	<365 d	amp	any	any	asymptomatic or symptomatic

[#] Ploidy: DNA index (DI) greater than 1 (hyperdiploid) or equal to 1 (diploid); hypodiploid tumors (with DI less than 1) will be treated as a tumor with DI greater than 1.

Abbreviations: INPC, International Neuroblastoma Pathology Classification; NA, not applicable; amp amplified; FH, favorable histology; UH, unfavorable histology.

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Table 4. Risk Grouping Scheme for the International Neuroblastoma Risk Group (INRG) System

INRG Stage	Age	Histologic Category/ Tumor Grade	*MYCN*	Unbalanced 11q aberration	Ploidy	Pretreatment Risk Group
L1		GN maturing GNB intermixed	NA			A Very Low
		Any, except GN maturing or GNB intermixed	NA			B Very Low
			Amp			I Intermediate
L2		GN maturing GNB intermixed	NA			A Very Low
	<18 mo (<547 d)	Any, except GN maturing or GNB intermixed	NA	No		D Low
				Yes		J Intermediate
	≥18 mo (≥547 d)	GNB nodular, differentiating NB, differentiating	NA	No		E Low
				Yes		K Intermediate
		GNB nodular, poorly differentiated or undifferentiated NB, poorly differentiated or undifferentiated	NA	(Any)		K Intermediate
	(Any)		Amp			O High
M	<18 mo (<547 d)		NA		Hyperdiploid	F Low
	<12 mo (<365 d)				Diploid	G Low
	12-18 mo (365-<547 d)				Diploid	H Low
	<18 mo (<547 d)		Amp			P High
	≥18 mo (≥547 d)					Q High
MS	<18 mo (<547 d)		NA	No		C Very Low
				Yes		N Intermediate
			Amp			R High

Abbreviations: GN, ganglioneuroma; GNB, ganglioneuroblastoma; NA, not applicable; Amp, amplified, NB,neuroblastoma.

I. Molecular Classification/Genetics

MYCN Amplification

The most prognostically relevant genetic alteration in neuroblastoma is *MYCN* amplification. *MYCN* gene amplification is associated with high-risk neuroblastic tumors and poor patient prognosis. *MYCN* is a proto-oncogene located on the short arm of chromosome 2, the amplification of which leads to inhibiting cellular differentiation and promoting cellular proliferation and apoptosis/karyorrhexis.[8] Not surprisingly, amplification is associated with undifferentiated and poorly differentiated neuroblastomas with a high mitotic-karyorrhectic index.[9,10]

MYCN overexpression usually occurs by gene amplification in 1 or both of the following ways: (1) gene duplication adjacent to the usual locus on 2p, forming homogeneously staining regions (HSRs) seen on chromosomal banding patterns; and (2) formation of double minutes, small, circular extrachromosomal fragments of DNA that harbor copies of the *MYCN* gene and are replicated during mitosis. These mechanisms can occur individually or simultaneously in a given tumor cell.

The *MYCN* status of a given neuroblastic tumor can be determined by FISH within a relatively short period of time after the surgery/biopsy using touch preparation slides or formalin-fixed, paraffin-embedded sections (Note A). A double-staining procedure is required in order to compare the number of chromosome 2 and *MYCN* signals in the same tumor nuclei. Additional *MYCN* signals associated with a similar increase in the number of chromosome 2 signals does not represent *MYCN* amplification. *MYCN* status is defined as "amplified" when *MYCN* signals exceed chromosome 2 signals by 3 times or more in the given tumor cell nuclei. The prognostic significance of tumors showing increased *MYCN* signals, but not more than 3 times that of chromosome 2 signals (*MYCN* gain), is yet to be determined.

Recent studies have identified a subset of neuroblastic tumors with "discordance" between the genotype (*MYCN* amplification status) and the phenotype (differentiation, MKI, and histologic classification).[11] In cases with amplification of the *MYCN* gene but favorable histologic features (differentiating neuroblasts and/or low-intermediate MKI), the cells do not produce active N-myc protein and lack the classic "bulls eye" nucleoli.[11] In cases that lack *MYCN* amplification but have unfavorable histologic features (undifferentiated neuroblasts and/or high MKI), C-myc protein is often being expressed instead.[12]

MYCN amplification is also correlated with advanced-stage tumors often having chromosome 1p deletions, especially del 1p36.3.[13] The deletion of 14q has also been shown to be unfavorable, as have loss of 11q and gain of 17q.[14]

ALK Mutation and Amplification

Recent studies have demonstrated mutations in the anaplastic lymphoma kinase (*ALK*) gene in a subset of neuroblastic tumors, as well as in the germline of patients with a familial predisposition to this disease.[15-17] About 8% to 10% of tumors have *ALK* mutations, and about 25% have gene amplification or protein overexpression; these aberrations are all associated with higher risk and worse prognosis.[18-20] Although ALK immunohistochemistry does not always correlate with expression status, gene sequencing (especially of the kinase regions and mutational hotspots) is sometimes performed in treatment-refractory patients who might be candidates for tyrosine kinase inhibitors.

ATRX

Although mutations in the alpha-thalassemia/mental retardation X-linked syndrome (*ATRX*) gene are only found in 2% to 3% of all neuroblastic tumors, the vast majority of high-stage tumors in older children and adolescents have *ATRX* mutations (whereas congenital and infantile tumors only exceedingly rarely have them).[20,21] The *ATRX* gene product plays a role in telomere maintenance, and tumor cells with mutated *ATRX* have longer-than-usual telomeres, prolonging their survival.[22]

DNA Index

Determination of DNA index by flow cytometry is also important; however, a minimum of 100 mg and preferably 1 g of fresh tumor is typically required for this purpose (Note A). A DNA index near diploid/tetraploid is unfavorable, while hyperdiploid (near triploid) tumors have a better prognosis. However, the prognostic effects of DNA index are reported to be limited to those patients diagnosed at younger than 1 year of age.[14]

Others
Additional genetic abnormalities have clinicopathologic significance in neuroblastic tumors. Higher expression of TrkA (high-affinity nerve growth factor receptor) portends a good prognosis; *MYCN*-amplified tumors usually have a lower expression of TrkA.[1] *PHOX2B* mutations are frequently seen in familial neuroblastomas, but only rarely in sporadic tumors.[23] Finally, segmental chromosomal aberrations (especially 1p deletion, 11q deletion, and/or 17q gain) are associated with high-risk tumors, whereas alterations in the numbers of whole chromosomes are associated with lower risk tumors.[24,25] The increasing availability of comparative genomic hybridization (CGH) has made this analysis more common, but it has yet to be adopted as standard-of-care.

J. Clinical Presentation
The clinical presentation of neuroblastoma may provide valuable information in assessing biologic risk. The abdomen is the most common primary site of neuroblastoma, with more than 76% of tumors arising either in the adrenal glands or, less commonly, in the paravertebral sympathetic chains.[5]

The posterior mediastinum is the second most common primary site, and respiratory symptoms predominate. Cervical neuroblastoma presents as a mass with or without Horner syndrome.[26] All neuroblastomas, regardless of biologic risk, can extend along radicular nerves, through spinal foramina, and into the epidural space, forming a dumbbell-shaped mass. Because the spinal cord extends to the level of the T12 to L1 vertebrae, tumors above this level are more likely to cause cord compression and paralysis, bladder and bowel dysfunction, or numbness. Similarly, neuroblastomas primary in the pelvis may present with constipation or urinary symptoms, including dysuria, infection, flank pain, or urinary retention.[27]

The opsoclonus-myoclonus syndrome is the best example of a paraneoplastic manifestation of neuroblastoma. This is thought to occur due to cross-reactivity between antineuroblastoma antibodies and the Purkinje cells of the cerebellum. Although patients with opsoclonus-myoclonus syndrome usually have an excellent prognosis for their tumor, up to 70% of such patients will have permanent neurologic deficits despite complete tumor resection.[28]

K. Special Studies
Imaging
The most useful imaging study is computerized axial tomography (CT scan) performed with simultaneous administration of oral and intravenous contrast agents.[29] This provides excellent information about the primary tumor, including location, vascular encasement, and the status of regional lymph nodes. Hepatic and bony metastases can be visualized, as well as pulmonary metastases (the latter is an extremely rare site for dissemination).[5] Magnetic resonance imaging (MRI) can give valuable information about vascular and hepatic involvement, and can help to determine tumor resectability.

A diphosphate bone scan and an MIBG scan are requisite to assess the bone and bone marrow for distant disease.[30] Approximately 85% of neuroblastomas will take up MIBG.[5] A positive bone scan or bone survey indicates cortical bone involvement and is a negative prognostic factor.

Serum Chemistry
Serum chemistry assays are useful to help predict prognostic risk. These include serum lactic dehydrogenase (LDH), neuron-specific enolase (NSE), and ferritin.[31] Ferritin levels are the most important diagnostic marker of the 3, with an elevation above normal (before transfusion) associated with a worse prognosis. Reference ranges are dependent on the individual laboratory, but an upper normal limit of 142 ng/mL frequently is reported.[32] Serial LDH levels correlate with disease activity, and pretreatment values of more than 1000 U/L are associated with a worse prognosis.[33] Serum levels of NSE more than 30 ng/mL also are associated with a worse prognosis.[34]

Endocrine Markers
Urinary catecholamine secretion is increased in neuroblastoma and is useful as a confirmatory diagnostic marker. Serial determinations are used to assess therapeutic response and identify recurrence. Vanillylmandelic acid (VMA) and homovanillic acid (HVA) are the 2 catecholamine metabolites commonly measured[35] via high-performance liquid chromatography. In 1 study,[36] the sensitivity and specificity of HVA for detection of neuroblastoma were 72% and 98%, respectively; corresponding figures for VMA were 80% sensitivity and 97% specificity. Urinary catecholamines may not be elevated in undifferentiated neuroblastomas.

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