Sponsored by

Neuroblastoma Histopathology Reporting Guide

Family/Last name

Given name(s)

Date of birth DD – MM – YYYY

Patient identifiers

Date of request DD – MM – YYYY

Accession/Laboratory number

Elements in **black text** are CORE. Elements in **grey text** are NON-CORE.

☐ indicates multi-select values ◯ indicates single select values

SCOPE OF THIS DATASET

CLINICAL INFORMATION (Note 1)

◯ Information not provided

Age

◯ Information not provided
◯ <18 months
◯ ≥18 months and <5 years
◯ ≥5 years

Preoperative treatment

◯ Information not provided
◯ No known preoperative therapy
◯ Preoperative therapy given, *specify*

Previous biopsy

◯ Information not provided
◯ No previous biopsy
◯ Previous biopsy
- ◯ Core needle biopsy
- ◯ Excisional/open biopsy
- ◯ Other, *specify*

Known cancer predisposition syndrome, *specify*

Other clinical information, *specify*

OPERATIVE PROCEDURE (Note 2)

◯ Not specified
◯ Resection
◯ Excisional/open biopsy
◯ Core needle biopsy
◯ Fine needle aspiration (FNA)
◯ Bone marrow aspirate/core biopsy
◯ Other, *specify*

SPECIMEN DIMENSIONS (Note 3)

mm x mm x mm

TUMOUR SITE (Note 4)

◯ Not specified
◯ Adrenal/periadrenal
◯ Retroperitoneal, nonadrenal
◯ Thoracic paraspinal
◯ Cervical region
◯ Other, *specify*

⬇

◯ Primary site
◯ Metastatic site

TUMOUR DIMENSIONS (Note 5)

(Applicable to primary resections only)

Greatest dimension mm

Additional dimensions mm x mm

◯ Cannot be assessed, *specify*

BLOCK IDENTIFICATION KEY (Note 6)

(List overleaf or separately with an indication of the nature and origin of all tissue blocks)

HISTOLOGICAL TUMOUR TYPE (select all that apply) (Note 7)

(Value list based on the World Health Organization Classification of Paediatric Tumours (2023))
(Not applicable for tumours post chemo/radiotherapy)

◯ Ganglioneuroma
◯ Ganglioneuroblastoma, intermixed
◯ Neuroblastoma
◯ Ganglioneuroblastoma, nodular
◯ Other, *specify*

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DEGREE OF DIFFERENTIATION (Note 8)
(Applicable to neuroblastoma or nodules of ganglioneuroblastoma, nodular, that have not had chemo/radiotherapy)

- ◯ Undifferentiated
- ◯ Poorly differentiated
- ◯ Differentiating
- ◯ Cannot be determined, *specify*

MITOTIC-KARYORRHECTIC INDEX (MKI) (Note 9)
(Applicable to neuroblastoma and ganglioneuroblastoma, nodular tumour tissue that have not had chemo/radiotherapy)

- ◯ Low (<100 per 5,000 cells; <2%)
- ◯ Intermediate (100-200 per 5,000 cells; 2-4%)
- ◯ High (>200 per 5,000 cells; >4%)
- ◯ Cannot be determined, *specify*

PROGNOSTIC CLASSIFICATION (Note 10)
(Based on the International Neuroblastoma Pathology Committee classification)
(Not applicable for tumours post chemo/radiotherapy)

- ◯ Favourable
- ◯ Favourable, based on review of limited material
- ◯ Unfavourable
- ◯ Cannot be determined, *specify*

TREATMENT EFFECT (Note 11)

- ◯ Not identified
- ◯ Present

LYMPH NODE STATUS (Note 12)

- ◯ Cannot be assessed
- ◯ No nodes submitted or found

Number of lymph nodes examined

- ◯ Not involved
- ◯ Involved

Number of involved lymph nodes

- ◯ Number cannot be determined

Location of involved lymph nodes, *specify*

ANCILLARY STUDIES (Note 13)

- ◯ Not performed
- ◯ Performed

***MYCN* status**

- ◯ Not applicable
- ◯ Cannot be determined
- ◯ Pending
- ◯ Not amplified
- ◯ Amplified
- ◯ Gain

DNA content

- ☐ DNA ploidy

Record method

- ◯ Results pending
- ◯ DNA index 1.0 (diploid)
- ◯ DNA index >1.0 (hyperdiploid)
- ◯ DNA index, *specify value*

AND/OR

- ☐ CNV

Record method

Record result

- ◯ Results pending

Immunohistochemistry, *specify antibodies and results*

Other (e.g., ALK), *record test(s), methodology and results*

Representative blocks for ancillary studies, *specify those blocks best representing tumour and/or normal tissue for further study*

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ADDITIONAL MATERIAL FOR BIOLOGICAL/GENETIC ANALYSIS (Note 14)

Material available

- ◯ No
- ◯ Yes
  - ◯ Not histological assessed
  - ◯ Histologically assessed

Viable tumour present

- ◯ No
- ◯ Yes

%	viable tumour present
%	neuroblastic cells
%	Schwann cells
%	other cells (e.g., lymphoid cells, fibroblasts)

HISTOLOGICALLY CONFIRMED DISTANT METASTASIS (Note 15)

- ◯ Not applicable
- ◯ Not identified
- ◯ Present (select all that apply)
  - ☐ Liver
  - ☐ Skin
  - ☐ Bone marrow

Percentage of haemopoietic bone marrow replaced by tumour

- ◯ Cannot be determined
- ◯ ≤5%
- ◯ >5%, *specify* ____ %

- ☐ Bone, *specify site(s)*
- ☐ Soft tissue, *specify site(s)*
- ☐ Other, *specify site(s)*

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Definitions

CORE elements

CORE elements are those which are essential for the clinical management, staging or prognosis of the cancer. These elements will either have evidentiary support at Level III-2 or above (based on prognostic factors in the National Health and Medical Research Council (NHMRC) levels of evidence[1]). In rare circumstances, where level III-2 evidence is not available an element may be made a CORE element where there is unanimous agreement by the Dataset Authoring Committee (DAC).

Non-morphological testing e.g., molecular or immunohistochemical testing is a growing feature of cancer reporting. However, in many parts of the world this type of testing is limited by the available resources. In order to encourage the global adoption of ancillary tests for patient benefit, International Collaboration on Cancer Reporting (ICCR) includes the most relevant ancillary testing in ICCR Datasets as CORE elements, especially when they are necessary for the diagnosis. Where the technical capability does not yet exist, laboratories may consider temporarily using these data elements as NON-CORE items.

The summation of all CORE elements is considered to be the minimum reporting standard for a specific cancer.

NON-CORE elements

Non-core elements are those which are unanimously agreed should be included in the dataset but are not supported by level III-2 evidence. These elements may be clinically important and recommended as good practice but are not yet validated or regularly used in patient management.

Key information other than that which is essential for clinical management, staging or prognosis of the cancer such as macroscopic observations and interpretation, which are fundamental to the histological diagnosis and conclusion e.g., macroscopic tumour details, may be included as either CORE or NON-CORE elements by consensus of DAC.

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Scope

The dataset has been developed for the pathological reporting of biopsy and resection specimens of paediatric peripheral neuroblastic tumours.

Most such tumours in adults are benign ganglioneuromas; there is a paucity of evidence to support classification and treatment guidelines of neuroblastomas and ganglioneuroblastomas in patients over 10 years old due to their rarity.[2]

Treatment, especially traditional chemotherapy and radiotherapy, can alter the histologic appearance and composition of neuroblastic tumours. These changes may or may not reflect the original classification of the tumour or its original or future biologic behaviour. Thus, much of this dataset is not suitable for use with post-treatment tumours.[3-6]

In those rare cases where more than one primary tumour is present, separate datasets should be completed for each neoplasm.

The authors of this dataset can be accessed here.

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Note 1 – Clinical information (Core and Non-core)

Clinical information can be provided by the clinician, included in the pathology request form, pathology report, or patient medical record.

Age (Core)

More than 50 years ago, Breslow and McCann reported that both age and stage were strongly prognostic of survival in children with neuroblastoma.[7] This work led to a convenient cut-off of 365 days to define favourable versus unfavourable age. Decades later, London et al (2005),[8] analysed the influence of age on outcome in 3,666 patients in an effort to identify a statistically optimal age cut-off. Although the prognostic contribution of age to outcome was determined to be continuous in nature, statistical support for an age cut-off of 547 days was identified, and this cut-off has subsequently been integrated into cooperative group neuroblastoma risk classification systems.

Tumour histology is another powerful prognostic marker in neuroblastoma. Age is an integral component of the Shimada histologic classification, first described in 1984,[9] and the International Neuroblastoma Pathology Classification (INPC), a system that is based on the Shimada classification with minor modifications.[3] Interestingly, the review of 227 cases by the INPC Committee in 1999 demonstrated the prognostic significance of an age cut-off of 1.5 years, which is similar to the cut-off reported by London and colleagues.[8] Another prognostic age cut-off of five years was also identified.

Preoperative treatment (Core)

The morphologic appearance of a peripheral neuroblastic tumour may change over time, either spontaneously or in response to treatment. Stage MS disease in infants may spontaneously regress, with tumour cells undergoing apoptosis and/or differentiation, and in some cases leaving little if any histologic evidence of their prior existence.[10] Undifferentiated and poorly differentiated neuroblastomas, as well as ganglioneuroblastomas, may undergo maturation in response to therapy, with increased differentiation of neuroblasts, increased stromal content, and lower mitotic-karyorrhectic index (MKI).[4] The INPC classification was designed to categorise and risk-stratify pre-treatment neuroblastic tumours and, because of the potential for therapy-induced changes, is not suitable for use with post-treatment tumours. The clinical significance of post-therapy histologic features, if any, remains unknown.[4,5]

Prior biopsy (Non-core)

Knowledge of a previous biopsy is not only useful because it suggests likely administration of therapy in the interval period, but because comparison with the previous sampling can yield important clues about the identity of the tumour. For example, an initial biopsy that has mature ganglion cells in a stroma-rich background and a second biopsy with poorly differentiated neuroblasts and scant stroma would suggest a ganglioneuroblastoma, nodular. Additionally, canonical 'biopsy site changes' such as hemosiderin-laden macrophages, fibrosis, and inflammation could be explained by a prior procedure. The heterogeneity of peripheral neuroblastic tumours can be problematic on small biopsies and, thus, all information from all biopsies should be taken into account.

Cancer predisposition syndromes (Non-core)

An inherited predisposition to neuroblastoma has been estimated in 1-2% of patients with newly diagnosed neuroblastic tumours,[11,12] and a larger number of cases are likely to be identified with increased use of next generation sequencing of germline DNA. The genes most commonly altered in inherited neuroblastoma are *ALK* and *PHOX2B*. Other cancer predisposition syndromes (CPS) that may include neuroblastic tumours are RASopathy syndromes, Beckwith-Wiedemann syndrome, Li-Fraumeni syndrome and DNA repair disorders.

Characteristics that may suggest CPS include a family history of neuroblastic and/or other tumours, bilateral and/or multifocal tumours. A subset of these CPS may also have associated findings. Clinical features that suggest a *PHOX2B* germline mutation include a personal or family history of Congenital Central Hypoventilation Syndrome (CCHS) and/or Hirschsprung disease. Since patients with CPS may have multifocal tumours, it is possible that the histology and biomarkers may differ between specimens and thus require separate analyses.

Other clinical information (Non-core)

Although a diagnosis of a neuroblastic tumour can almost always be made on pathologic grounds, the patient's clinical history and presentation can be contributory. The results of ancillary testing such as urine catecholamines (homovanillic acid and vanillylmandelic acid), ^{123}I-*meta*-iodobenzylguanidine scintigraphy, and other imaging studies can support a diagnosis of neuroblastoma. Common clinical presentations depend on the sites of disease and may include respiratory distress, abdominal pain, constipation, hepatomegaly, rash, and fevers. Multiple paraneoplastic syndromes can be associated with neuroblastic tumours, including opsoclonus myoclonus ataxia, cerebellar ataxia, Horner syndrome, Hirschsprung disease/CCHS, rapid-onset obesity with hypoventilation, hypothalamic dysfunction, autonomic dysregulation (ROHHAD), and vasoactive intestinal peptide (VIP)-associated secretory diarrhoea or VIPoma syndrome.

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Note 2 – Operative procedure (Core)

The role of surgery in the treatment of neuroblastoma is evolving.[13-15] Beyond the universal need to obtain adequate tissue for establishing the diagnosis and performing ancillary biologic studies, the purpose and utility of surgery vary according to the risk classification of the patient. Although the previous International Neuroblastoma Staging System (INSS)[16] required lymph node sampling, it is not a part of the currently employed International Neuroblastoma Risk Group Staging System (INRGSS).[17] Furthermore, there is conflicting data as to the specific impact of the degree of surgical resection on overall survival. The majority of children diagnosed with early stage/very low and low risk tumours can be treated with surgery alone and/or low doses of chemotherapy, and in some cases just observation, if disease is localised with no image-defined risk factors (INRGSS L1 tumours). The goal of initial treatment for intermediate risk patients is to achieve a reduction in tumour burden (usually 50% or more). This can be achieved through any combination of surgery or chemotherapy. In contrast, children presenting with advanced stage/high risk neuroblastoma often require a multimodal approach to treatment: neoadjuvant chemotherapy, surgery, immunotherapy, myeloablative therapy, radiotherapy, and post-consolidation immunotherapy. In most studies of advanced stage neuroblastoma, the degree of primary tumour resection has shown no impact on local control or long-term outcomes unless metastatic disease can be controlled.[13,18] Thus, the aim should be gross total resection without mutilation and surgical sequelae. For these reasons, tumour presence at surgical margins is not usually assessed pathologically.

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Note 3 – Specimen dimensions (Non-core)

The size of the specimen received for pathologic examination indicates how representative it may be of the overall tumour histology, guides the number of sections to be submitted for histologic evaluation, and estimates the availability of tissue for ancillary testing such as molecular or genetic analysis.[19,20]

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Note 4 – Tumour site (Core)

The anatomic site of a specimen is an important part of any anatomic pathology report, but is especially critical for neuroblastic tumours in order to help correlate gross and microscopic pathologic findings with image-defined risk factors such as invasion of specific visceral organ structures and infiltration of adjacent organs or tissue, as well as to define metastatic disease.[17] Anatomic site may also correlate with clinical presentation such as sequelae of tumour growth from compression or invasion of adjacent structures, as well as the development of paraneoplastic syndromes.

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Note 5 – Tumour dimensions (Core and Non-core)

Tumour size is not a consideration in the INRGSS.[17] However, knowledge of the size of the resected tumour in the clinical laboratory may be useful in correlating with the tumour size as determined by imaging, especially when assessing potential therapeutic response using the International Neuroblastoma Response Criteria which incorporates the Response Evaluation Criteria in Solid Tumours (RECIST) algorithm, which uses the greatest dimension of the tumour.[17,21,22] It may also indicate the availability of tissue for ancillary testing such as molecular or genetic analysis.

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Note 6 – Block identification key (Non-core)

The origin/designation of all tissue blocks should be recorded. This information should ideally be documented in the final pathology report and is particularly important should the need for internal or external review arise. The reviewer needs to be clear about the origin of each block in order to provide an informed specialist opinion. If this information is not included in the final pathology report, it should be available on the laboratory computer system and relayed to the reviewing pathologist. It may be useful to have a digital image of the specimen and record of the origin of the tumour blocks in some cases.

Recording the origin/designation of tissue blocks also facilitates retrieval of blocks for further immunohistochemical or molecular analysis, research studies or clinical trials.

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Note 7 – Histological tumour type (Core)

Histologic diagnosis of neuroblastic tumours is based on the 2023 World Health Organization (WHO) Classification of Paediatric Tumours, 5th edition (Table 1).[6]

Table 1: World Health Organization classification of neuroblastic tumours.[6]

Descriptor	ICD-O codes[a]
Ganglioneuroma	9490/0
Ganglioneuroblastoma, intermixed	9490/3
Neuroblastoma	9500/3
Ganglioneuroblastoma, nodular (and other composite neuroblastic tumours)	9490/3

[a] These morphology codes are from the International Classification of Diseases for Oncology, third Edition, second revision (ICD-O-3.2).[23] Behaviour is coded /0 for benign tumours; /1 for unspecified, borderline, or uncertain behaviour; /2 for carcinoma in situ and grade III intraepithelial neoplasia; /3 for malignant tumours, primary site; and /6 for malignant tumours, metastatic site.

Neuroblastoma is often used as a generic term for all categories of peripheral neuroblastic tumours. According to the International Neuroblastoma Pathology Committee classification, four categories are defined in this group of tumours based on the degree of neuroblastic differentiation and the degree of Schwannian stromal development. They are Neuroblastoma (Schwannian stroma-poor), Ganglioneuroblastoma, intermixed (Schwannian stroma-rich), Ganglioneuroma (Schwannian stroma-dominant) and Ganglioneuroblastoma, nodular (composite, Schwannian stroma-rich/stroma-dominant and stroma-poor).[3,24,25] The presence or absence of Schwannian stromal development is noted in parentheses after the specific tumour category.

Because chemotherapy and radiotherapy can alter the histologic appearance of neuroblastic tumours (usually by inducing differentiation or maturation of the neuroblasts and increasing the relative proportion of stroma), the WHO and INPC classification systems cannot be used for post-treatment tumours.[3-6] In this situation, histologic classification is not applicable, and tumours should be diagnosed along the lines of 'neuroblastic tumour, post-treatment'. Tumours should never be reclassified after treatment (see also **Note 1 CLINICAL INFORMATION and Note 11 TREATMENT EFFECT**).

Ganglioneuroma (Schwannian stroma-dominant) category

Tumours in this category are predominantly composed of Schwannian stroma where maturing and mature ganglion cells are individually distributed or forming small clusters in the stroma. Completely mature ganglion cells are covered with satellite cells. For the sake of avoiding confusion, differentiating neuroblasts and ganglion cells in the peripheral neuroblastic tumours are defined based on the presence or absence of naked neuritic processes (neuropile) detectable around their cytoplasm by haematoxylin and eosin (H&E) stained section. Neuritic processes of the differentiating neuroblasts are still segmentally naked and not incorporated in the cytoplasm of Schwannian stromal cells, whereas no naked neuritic processes are detected around ganglion cells since they are immediately and completely incorporated and covered by Schwannian stromal cells. Accordingly, no microscopic foci of neuroblastic cells with detectable naked neuritic processes are found in the tumours of this category.

Ganglioneuroblastoma, intermixed (Schwannian stroma-rich) category

Tumours in this category are characterised by the presence of a ganglioneuromatous component with Schwannian stroma development (see below) exceeding 50% of the tissue, where microscopic nests of neuroblastoma are present in an intermixed or randomly distributed pattern. In those microscopic nests, the tumour cells are in various stages of differentiation (often predominantly differentiating neuroblasts) with clearly identifiable naked neuritic processes (neuropile, not covered by Schwann cells) around their cytoplasm. Grossly visible haemorrhagic/necrotic nodules suggestive of aggressive neuroblastomatous

growth are absent. Tumours in this category are histologically one step behind the final stage of complete maturation towards ganglioneuroma.

Neuroblastoma (Schwannian stroma-poor) category

Tumours in this category are composed of neuroblastic cells showing various degrees of differentiation. Tumour cells form groups or nests completely or incompletely demarcated by septal tissue where no or limited Schwannian proliferation (comprising less than 50% of tumour tissue) is observed.

Ganglioneuroblastoma, nodular (composite, Schwannian stroma-rich/stroma-dominant and stroma-poor) category

Tumours in this category are composed of multiple clones demonstrating distinct histologies: one or more Neuroblastoma (Schwannian stroma-poor) nodules set within a background of either Ganglioneuroblastoma, intermixed (Schwannian stroma-rich) or Ganglioneuroma (Schwannian stroma-dominant). The neuroblastoma nodules are usually grossly identifiable and often haemorrhagic/necrotic and fragile. In contrast, the Ganglioneuroblastoma, Intermixed/Ganglioneuroma component is tan-yellow and solid/elastic.

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Note 8 – Degree of differentiation (Core)

Based on the grade of neuroblastic differentiation, all tumours in the Neuroblastoma (Schwannian stroma-poor) category and neuroblastoma components of Ganglioneuroblastoma, nodular (composite, Schwannian stroma-rich/stroma-dominant) category are further classified into one of three subtypes (see below) for prognostic distinction (Favourable Histology versus Unfavourable Histology) according to the INPC.[24] Just as for histologic subtype, degree of differentiation should not be determined for post-treatment tumours.

Undifferentiated subtype

Tumour cells in this subtype are primitive with a high nuclear:cytoplasmic ratio and no morphologic evidence of neuroblastic differentiation and no neuritic process production. Accordingly, the diagnosis relies heavily on ancillary tests, such as immunohistochemistry and/or molecular/cytogenetic analysis. Some tumours in this subtype present a ‘starry-sky’ appearance.

Poorly differentiated subtype

Tumour cells also show a high nuclear:cytoplasmic ratio, and the diagnosis is supported by identifying neuritic process production of the tumour cells. Classical Homer Wright rosette formation can be seen in some tumours of this subtype. Less than 5% of tumour cells may show features of differentiating neuroblasts (see below).

Differentiating subtype

More than 5% of tumour cells demonstrate the appearance of differentiating neuroblasts with synchronous differentiation of the nucleus (enlarged, vesicular and often eccentrically located with a single prominent nucleolus) and the cytoplasm (conspicuous and twice the diameter of nucleus, eosinophilic or amphophilic with or without detectable Nissl bodies). Differentiating neuroblasts usually produce a larger amount of neuritic processes than poorly differentiated neuroblasts.

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Note 9 – Mitotic Karyorrhectic Index (MKI) (Core)

Mitotic-karyorrhectic index (MKI) is the number of mitotic nuclei and karyorrhectic nuclei per 5,000 neuroblastic cells. Based on the MKI, all tumours in the Neuroblastoma (Schwannian stroma-poor) category and neuroblastoma components of Ganglioneuroblastoma, nodular (composite, Schwannian stroma-rich/stroma-dominant) category are further classified into one of three classes; i.e., Low MKI (<100/5,000 cells), Intermediate MKI (100-200/5,000 cells), and High MKI (>200/5,000 cells), for the prognostic distinction (Favourable Histology versus Unfavourable Histology) according to the INPC.[3,24,26,27]

Following is the step-wise method recommended for determining MKI class:

1. First, estimate background number (denominator) of neuroblastoma cells in a 400X high power field (HPF) of your microscope; note that because the result is a ratio/index the precise area of an HPF is not relevant. The tumour may show uniform cellularity or present different cellular densities from area to area: For example, a densely cellular area may contain 1,000-1,500 cells per 400X HPF (area 0.2mm^2); a moderately cellular area may contain approximately 800 cells per 400X HPF; and a sparsely cellular area may contain <500 cells per 400X HPF.
2. Scan the tumour tissue by low power view, and determine whether the given tumour has a uniform cellular density or different cellular densities from area to area.
3. Pick representative areas based on the proportion of cellular densities, and count mitotic and karyorrhectic nuclei from each microscopic field (400X) of the given tumour. Karyorrhectic nuclei showing nuclear fragmentation, but not simply hyperchromatic nuclei, are included in the counting.
4. After having counted the total number of mitotic and karyorrhectic nuclei from the representative areas, the MKI is determined by averaging the number per 5,000 (denominator) tumour cells.

Specimens with less than 5,000 cells available for counting (core biopsies with a small proportion of tumour, for example) or with extensive geographic tumour necrosis, are unsuitable for MKI calculation and should be reported as 'cannot be determined'. Furthermore, MKI determination on bone marrow metastases is discouraged, as it has not been shown to correlate with the MKI of the primary tumour nor to be prognostically useful. MKI should not be determined for post-treatment tumours.

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Note 10 – Prognostic classification (Core)

The age of the patient and the histologic type (**Note 7 HISTOLOGICAL TUMOUR TYPE**), degree of differentiation (**Note 8 DEGREE OF DIFFERENTIATION**), and MKI (**Note 9 MITOTIC-KARYORRHECTIC INDEX**) of a peripheral neuroblastic tumour are utilised to categorise it as favourable or unfavourable histology according to the INPC classification system (Table 2).[3,24,25] Pathologic classification strongly correlates with prognosis and is used to risk-stratify patients for therapeutic protocols.[3,8,15,24-27] Ganglioneuroblastoma, nodular, and other composite neuroblastic tumours are classified by applying the INPC schema to the highest grade nodule or component of the tumour. When a biopsy or partial resection is performed, there exists a risk of failing to sample an unfavourable nodule or component of these two tumour types; thus, in such cases, a disclaimer about limited sampling should be made. Prognostic classification should not be determined for post-treatment tumours.

Table 2: International Neuroblastoma Pathology Committee Classification of neuroblastic tumours.[3,24,25]

Histologic type	Differentiation	Mitotic-karyorrhectic index (MKI)	Age	Histology
Neuroblastoma	Undifferentiated	Any	Any	Unfavourable
	Poorly differentiated	Low or Intermediate	<18 months	Favourable
			≥18 months	Unfavourable
		High	Any	Unfavourable
	Differentiating	Low	<5 years	Favourable
			≥5 years	Unfavourable
		Intermediate	<18 months	Favourable
			≥18 months	Unfavourable
		High	Any	Unfavourable
Ganglioneuroblastoma, nodular (and other composite neuroblastic tumours)	Classify according to highest grade nodule or component of tumour			
Ganglioneuroblastoma, intermixed	NA	NA	Any	Favourable
Ganglioneuroma	NA	NA	Any	Favourable

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Note 11 – Treatment effect (Non-core)

Assessment of the amount of residual viable tumour after neoadjuvant systemic treatment and type of histologic response may represent desired information for clinicians in terms of estimation of efficacy of treatment, even though there is limited data about the clinical significance or influence on patient prognosis. Treatment effect does not solely consist of necrosis. Calcifications, fibrosis, and tumour cell differentiation/ maturation should also be considered as response. The amount of remaining viable tumour may be estimated in percent on each histological slide to obtain an average score reflecting the overall response.

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Note 12 – Lymph node status (Core)

As in most cancer types, lymph node status has historically been an important aspect of neuroblastoma staging (for the INSS), and is therefore regarded a core element in the reporting of these tumours. This includes whether or not such lymph nodes were submitted for pathological investigation, how many lymph nodes were submitted and from what location, and whether they harbored metastatic tumour or not. The INRGSS specifies that nodal status is not used to distinguish L1 from L2 disease and that positive locoregional lymph nodes are still compatible with L2 disease, in contrast to distal lymph node metastases that qualify as stage M.[17] For instance, lower mediastinal nodes in combination with an upper abdominal primary tumour and a pelvic primary tumour with inguinal positive lymph nodes would both still be classified as stage L2 disease. It is evident from the above that close collaboration between surgeon and pathologist is crucial to

arrive at the correct staging. Chemotherapeutic effect indicative of the prior presence of tumour within a lymph node, such as calcifications, hemosiderin-laden macrophages, or foci of acellular neuropil should be considered lymph node involvement by tumour.

Although no pertinent literature currently exists on this subject, it might also be relevant to describe the histological aspect of lymph node metastases, in relation to the aspect of the primary tumour, specifying the degree of differentiation and of therapy effect. This, however, is not a requirement for this dataset.

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Note 13 – Ancillary studies (Core and Non-core)

MYCN*: MYCN* amplification is the single most important prognostic marker in neuroblastic tumours and is associated with poorly differentiated or undifferentiated tumours with high MKI.[28] *MYCN* is a proto-oncogene which inhibits cell differentiation and promotes division and apoptosis, biologic activities which correlate well with the histologic features (high MKI) often seen in *MYCN*-amplified tumours. Additionally, large prominent nucleoli within tumour cells ('bull's eye nucleoli') are associated with *MYCN* amplification.[29-31] Amplification occurs through duplication of the *MYCN* gene locus on chromosome 2p via the formation of double minutes (dmin), small circular extrachromosomal DNA fragments that contain the *MYCN* gene; these may subsequently be reintegrated into the chromosome as heterogeneously staining regions.[32,33] Amplification status is best assessed by fluorescence *in situ* hybridization on formalin-fixed paraffin-embedded or frozen tissue (or less commonly, air-dried touch preparation slides). Signals from a *MYCN*-specific probe are compared to those from a centromeric chromosome 2 probe; tumours with a *MYCN*:control signal ratio of 4 or more are 'amplified', while those with a ratio of more than 1 but less than 3 are considered to have *MYCN* 'gain,' the clinical significance of which is less clear.[29] Notably, some labs may now use copy number analyses (e.g., CGH or SNP arrays) or next generation sequencing approaches to detect *MYCN* amplification status. Analyses of large numbers of patients have also identified two unique subsets of patients with genotype-phenotype discordance: those who have *MYCN* amplification but favourable histology tumours and a good prognosis, and those without *MYCN* amplification who nonetheless have unfavourable histology tumours and dismal prognosis.[29,30] The former appear to not produce functional N-myc protein despite having amplification of the gene, and the latter are often associated with C-myc protein expression or other molecular abnormalities instead.[34,35] C-myc expression is not currently used in the Children's Oncology Group or International Neuroblastoma Risk Group classification systems.[36,37] Depending on the availability of such testing and the laboratory's workflow, the results of *MYCN* amplification status analysis may not be known to the pathologist at the time of diagnosis.

DNA content: DNA index is a prognostic factor in infants (<1 year old) with neuroblastoma. A DNA index near diploid/tetraploid is unfavourable, whereas hyperdiploidy is favourable.[38] Most laboratories currently use next generation sequencing or microarrays to determine the DNA index of tumour cells, but some may use the more traditional flow cytometry approach.

Immunohistochemistry*:* Peripheral neuroblastic tumours can usually be diagnosed based on the morphology of their neuroblastic and stromal components, especially in tumours with significant differentiation and/or neuropile content. However, for undifferentiated tumours and in biopsies with limited sampling, immunohistochemistry can be a useful adjunct. Phox2B is one of the most sensitive and specific markers for neuroblastoma, although it is also positive in other neural crest-derived tumours such as paraganglioma/pheochromocytoma.[39,40] Additional stains typically positive in neuroblastic tumours include PGP9.5, CD56, and NB84. The neuroblastic cells are usually positive for synaptophysin and neuron-specific enolase and the Schwannian stromal cells are usually positive for S100 (although these stains are of limited utility due to their nonspecific nature).

Other ancillary studies: Additional molecular and genetic aberrations may be of prognostic or other clinical significance, and more are being discovered as our knowledge expands; however, their use and availability may vary between laboratories. The anaplastic lymphoma kinase (*ALK*) gene is mutated in 8-10% of sporadic neuroblastic tumours, amplified in about 2% of sporadic tumours, and mutated in the germline of patients with genetic predisposition syndromes that include an increased risk of neuroblastoma (see **Note 1 CLINICAL INFORMATION**).[41,42] Unfortunately, ALK immunohistochemistry does not correlate well with gene mutation or amplification status, so sequencing of the kinase regions and/or known mutational hotspots is required. *ALK* aberrations are associated with higher risk disease but may also be amenable to treatment with tyrosine kinase inhibitors such as lorlatinib.[43]

Next generation sequencing assays have been useful in identifying the association of specific segmental chromosomal aberrations, including 1p deletion, 11q deletion, and 17 q gain with high risk tumours.[44] Likewise, abnormalities in the telomere maintenance/alternate lengthening of telomeres (ALT) pathways can prolong cell survival and recent evidence suggests these alterations are associated with more unfavourable or higher risk disease. Such genes include alpha-thalassemia/mental retardation X-linked syndrome (*ATRX*), with mutations found more commonly in high risk tumours in older children, and increased expression and/or rearrangements of the telomere reverse transcriptase (*TERT*) are associated with a poorer prognosis.[45,46] Although not yet widely used for directing clinical care, ALT pathways can be analysed by immunohistochemistry.

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Note 14 – Additional material for biological/genetic analysis (Core)

Determination of *MYCN* amplification status is a critical part of risk stratification for peripheral neuroblastic tumours and additional molecular/genetic testing can be helpful in directing clinical care, especially in patients with recurrent or refractory tumours (see **Note 13 ANCILLARY STUDIES**). Thus, documenting the availability of residual tissue for such testing, including whether it contains sufficient viable tumour for meaningful analysis, is important for the patient's ongoing care.

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Note 15 – Histologically confirmed distant metastases (Core)

Documentation of known metastatic disease is an important part of the pathology report and is part of the INRGSS.[17] Such information, if available, should be recorded with as much detail as is available, including the site, whether the specimen is a histopathology or cytopathology specimen and with reference to any relevant prior surgical pathology or cytopathology specimens. Of note, the International Neuroblastoma Response Criteria Bone Marrow Working Group has proposed a standardised approach to assessing marrow involvement by neuroblastoma which, while not part of the INRGSS, includes useful guidelines.[47] These include sampling at least two sites (usually bilateral iliac crests), estimating the percent marrow involvement by disease in each specimen (by surface area for cores and by cell number for aspirates), using immunohistochemistry/immunocytochemistry for more sensitive detection, and performing RT-PCR to assess minimal disease.

If distant sites are sampled and pathologically shown to be negative, metastatic disease is 'not identified', whereas if sampling is not performed, this section is 'not applicable'.

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