SIOP Europe
the European Society for Paediatric Oncology

European Reference Network
for rare or low prevalence complex diseases
Network Paediatric Cancer (ERN PaedCan)

LOW-RISK NEUROBLASTOMA
STANDARD CLINICAL PRACTICE RECOMMENDATIONS

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INTRODUCTORY PAGES

- Low-risk neuroblastic tumours
- Low-risk neuroblastoma
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1. Synopses - Low-risk neuroblastoma

Diagnosis tests

- Complete blood count, biochemistry, serum LDH
- Renal function evaluation (Including using the Schwartz formula)
- Urine catecholamines
- Histological Diagnosis/ Pathology
- Biological studies (MYCN, genomic copy number profile)
- Bilateral bone marrow aspirates and trephine biopsies
- Ultrasound, CT or MRI
- Scintigraphy MIBG (18F-FDG PET/CT preferably or 99mTc MDP if MIBG non-avid)
- Audiogram (for patients receiving carboplatin)
- Echocardiogram (for patients receiving anthracycline)

Groups of treatment

➢ Ganglioneuroma and ganglioneuroblastoma intermixed	Surgery
➢ Neonatal neuroblastoma discovered as neonatal adrenal mass	Observation until progression or until regression. If the mass persists at 1 year from diagnosis, surgery
➢ L1 without MYCN amplification	Surgery
➢ L2 without MYCN amplification and ≤ 18 months	
▪ No life-threatening symptoms and NCA	Observation until progression or development of LTS
▪ With life-threatening symptoms and NCA	Chemotherapy until LTS disappear
▪ With/without life-threatening symptoms with presence of SCA	Chemotherapy ± Surgery
➢ Ms* without MYCN amplification in ≤ 12 months	
▪ Without life-threatening symptoms and NCA	Observation until progression or development of LTS
▪ With life-threatening symptoms and NCA	Chemotherapy until LTS disappear
▪ With or without life-threatening symptoms with SCA	Chemotherapy ± Surgery

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NOTE: The current recommendation for patients with segmental chromosomal aberrations (SCA) is treatment with mild chemotherapy.

* Ms according to SIOPEN, without lytic bone lesions/CNS/lung or pleura (see Appendix A1.1)

Life-threatening symptoms (LTS)

The presence of any of these symptoms is an indication for chemotherapy.

Intraspinal neuroblastoma (See Appendix 3)

Patients who either have symptoms of spinal cord involvement or have a spinal tumour component that occupies more than one third of the spinal canal on the axial plane and/or the perimedullary spaces are not visible and/or the spinal cord signal is abnormal.

Systemic upset

- Pain requiring opiate treatment
- Gastrointestinal
  - Vomiting needing nasogastric/IV support
  - Weight loss >10% body weight

NOTE: diarrhea with VIP does not respond to chemotherapy and is a definite indication for surgery

- Respiratory distress without evidence of infection
  - Tachypnoea
  - Supplementary oxygen requirement
  - Ventilatory support
- Cardiovascular System
  - Hypertension
  - IVC (inferior vena cava) involvement +/- leg oedema
- Renal
  - Impaired renal function, creatinine increased x 2 ULN[1]
  - Poor urine output, less than 2mls/kg/hour
  - Hydroureter/hydronephrosis
- Hepatic
  - Abnormal liver function >2 ULN[1]
  - Evidence of DIC (disseminated intravascular coagulation)
  - Platelets <50 x 10^9/l
- Bladder/Bowel dysfunction secondary to a mass effect

[1]ULN = Upper Limit Normal

A very large tumour volume, with concerns of possible tumour rupture and/or the possible rapid development of significant systemic problems.

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NOTE: some of these symptoms will require immediate treatment with chemotherapy. In these cases, the definitive biopsy to obtain material for the analyses of the *MYCN* gene status and the genomic copy number profile should be delayed until the patient is fit enough to have a biopsy, which is likely to be either within 7 days of the chemotherapy administration or just before the second course of chemotherapy is given. If tumour material for the genomic copy number profile is obtained after the initiation of chemotherapy, the genomic copy number profile will be considered non-informative (no genomic copy number profile result) and the patient will not be eligible for a genomic copy number profile result treatment stratification.

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1. BACKGROUND AND RATIONALE

1.1 Introduction

This guideline includes clinical recommendations to diagnose and treat patients with low-risk neuroblastic tumours within Europe, aiming to define the current best practice as a standard of care for those patients not included in clinical trials and thus guaranteeing a homogeneous management of the disease.

It is based on definitions and strategies evaluated in prior studies carried out by the International Society of Paediatric Oncology Europe Neuroblastoma Group (SIOPEN) for non-high risk neuroblastoma patients as well as evidence-based international state-of-the-art obtained from literature review with results published by other researchers.

1.2 Background

Neuroblastoma (NB) is the most frequent extracranial solid tumour in childhood, representing 8 to 10% of all cases of childhood cancer. It has an incidence of 10 cases per million children per year. Most patients are diagnosed before the age of 5 years with a mean age at diagnosis of 22 months [1,2].

NB refers to a spectrum of neuroblastic tumours including neuroblastomas, ganglioneuroblastomas and ganglioneuromas that arise from neural crest-derived embryonic cells that form the sympathetic nervous system. NB accounts for 97% of all neuroblastic tumours. The origin and migration pattern of neuroblasts during fetal development explains the multiple anatomical sites where tumours develop (65% in the abdomen, 15% thorax, 5% pelvis, 3% cervical) [3,4]. Approximately 50% of cases are metastatic at presentation, generally with lymphatic, liver, bone, and bone marrow involvement. NB is characterized by an intriguingly heterogeneous clinical presentation and prognosis, with a very broad spectrum ranging from spontaneous regression to tumours so aggressive that they do not respond despite intensive and multimodal treatment [5]. For this reason, stratification according to risk factors is essential in its management and has been carried out and improved for decades, based on age (better prognosis in the youngest at diagnosis with an age cut-off at eighteen months), stage defined by the International Neuroblastoma Staging System (INSS) (localised disease better than metastatic) in the past and INGRSS currently and genetic characteristics (the distinctive genetic marker is the amplification of the MYCN gene, which indicates highly aggressive tumour behaviour). Furthermore, there are also clinical, biological and prognostic differences in relation to the location of the primary tumour [6,7].

NB risk stratification at diagnosis has evolved since the development of the INSS in 1988, the first internationally accepted staging system for NB with surgical/pathological information [8,9]. Imaging features determined before surgery (presence or absence of image-defined-risk factors, IDRF) were introduced in 2009 within a new staging system (INRGSS) proposed by the International Neuroblastoma Risk Group (INRG) Task Force [10] (See Appendix 1). Further studies including the analysis of more than 8000 patients resulted in the identification of relevant risk factors, which are now included in the current NB pre-treatment risk stratification classification system (see Appendix 1):

- The stage of the disease according to the INRG staging system

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- Age at the time of diagnosis
- Histologic category (ganglioneuroma, ganglioneuroblastoma intermixed, nodular ganglioneuroblastoma, neuroblastoma)
- Grade or how cells of the tumour are differentiated
- *MYCN* gene status
- Chromosome 11q status
- Tumour cell ploidy (DNA content of tumour cells)

Thus, it combines clinical, pathologic and genetic markers to predict the clinical behaviour of the tumour and how it will respond to treatment, classifying NB cases in 4 categories: very low-risk, low-risk, intermediate-risk and high-risk. Survival in the different NB risk categories is variable: low-risk children have a 5-year survival rate that is higher than 90%, intermediate-risk children have a 5-year survival rate between 50-75% and high-risk patients have a 5-year survival rate lower than 50%.

This guideline focuses on the low-risk neuroblastoma spectrum (See section 2). Among this variety of patients, congenital neuroblastoma is also included. Congenital neuroblastoma is a tumour with a tendency to spontaneously regress and has a benign clinical course despite a clearly malignant histological picture.

Localised resectable disease

LNESG1 and LNESG 2 studies: Over the past 15 years, the European group SIOPEN has developed a series of clinical treatment trials covering the whole clinical spectrum of neuroblastoma patients. The LNESG1 study has shown the feasibility of treatment by surgical resection only in patients with localised resectable disease of any age [10-13]. Guidelines were established indicating which patients should be operated on immediately at the time of diagnosis and which should undergo surgery after tumour reduction with chemotherapy by defining surgical risk factors based on the imaging characteristics, with a validation of the surgical risk factors defined as predictors of adverse surgical outcome in this cohort of 719 patients. However, a recently completed analysis from the International Neuroblastoma Risk Group (INRG) database showed less favourable Event Free Survival (EFS) and Overall Survival (OS) in patients with *MYCN* amplified INSS stage 1 and 2 tumours than in patients with non-amplified tumours (53%±8% and 72%±7% vs. 90%±1% and 98%±1% ($p<0.0001$), respectively). Within this cohort of patients with *MYCN* amplification, both EFS and OS were statistically significantly higher for patients whose tumours were hyperdiploid rather than diploid (EFS: 82%±20% vs. 37%±21% ($p=0.0069$) and OS: 94%±11% vs. 54%±15% ($p=0.0056$), respectively). No other clinical, pathological or biological variable evaluated or initial treatment approach had prognostic significance in this cohort [14,15].

Localised unresectable disease in infants <12 m without *MYCN* amplification were eligible for the INES 99.1 study. Among 120 patients included in the study, 79 without threatening-life symptoms received up front chemotherapy consisting of Cyclophosphamide/Vincristine (CV), 48 infants of whom then required second line chemotherapy because of no response, progression or surgical-risk factors remained. Surgery was attempted in 105 patients including 22 after CV alone. Five year Overall

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Survival and 5 years EFS were 99% ± 1% and 90% ± 3% with a median follow up of 6.1 years (range 1.6-9.1). Primary low-dose chemotherapy without anthracyclines was effective in 61% of infants presenting with an unresectable NB and no *MYCN* amplification, producing excellent survival rates without jeopardizing their long-term outcome [16].

On the assumption that most **infants <12 m with disseminated (4s/4 stage) neuroblastoma without *MYCN* amplification** have a favourable prognosis, two concomitant prospective trials (INES 99.2 and INES 99.3) were started in 1999. Up-front chemotherapy with Carboplatin/Etoposide (VP/Carbo) was given to patients presenting with LTS symptoms. For those with metastases to skeleton, lung, or CNS, CADO was also added. Surgery was to be performed only in the absence of surgical-risk factors in either scenario and in stage 4 cases if metastatic CR was achieved after chemotherapy. One hundred seventy infants with disseminated neuroblastoma without *MYCN* amplification, diagnosed between June 1999 and June 2004 in nine European countries, were eligible for either of the two studies. The 125 infants treated on trial 99.2 had a 2-year OS of 97.6% with no difference between asymptomatic and symptomatic patients (97.7% vs. 97.3%), patients without or with unresectable primary tumours (96.8% vs. 100%), and patients without or with positive skeletal scintigraphy without radiologic abnormalities (97.2% vs. 100%). The 45 infants treated on trial 99.3 had a 2-year OS of 95.6%. No patients died of surgical or chemotherapy-related complications. Infants with disseminated disease without *MYCN* amplification have excellent survival with minimal or no treatment. Asymptomatic infants with an unresectable primary tumour or positive skeletal scintigraphy without radiologic abnormalities may undergo observation alone [14].

Infants with disseminated disease (4s/4 stage) and *MYCN* amplification were enrolled in the INES 99.4 study. Induction chemotherapy using conventional drugs resulted in non-response or disease progression in 30% of the patients, and event free and overall survival were poor despite megatherapy, justifying new therapeutic approaches in this high-risk population [7].

Localised unresectable neuroblastoma in children > 12 months

The SIOPEN European Unresectable Neuroblastoma Study (EUNS) was open from 2001-2006, and recruited 160 eligible patients aged more than 12 months with *MYCN* non-amplified unresectable NB (presence of IDRF). All patients were to receive 6 courses of chemotherapy (3 of VP/Carbo and 3 of CADO) and underwent surgical resection of the primary tumour afterwards. The 5-year OS and EFS of the whole study were 87.6% and 76.4%, respectively. Age was confirmed to be the most important prognostic factor for OS and EFS. Both OS and EFS significantly improved in younger patients (age 12-18 months) vs. older patients (OS 100% vs. 70%, p=0.006, and EFS 78% vs. 62%, p=0.001). Histology also proved to be of significant prognostic value (central review in 112/160 cases). Histological subtyping, obtained in 101 patients, permitted classification according to INPC histology prognostic categories, 48 tumours (47.5%) were assigned to the favourable category and 53 (52.5%) to the unfavourable category. It was confirmed the excellent outcome with 5-year EFS and OS of 94% and 100%, respectively, in the favourable group and conversely, the 5-year EFS and OS remained

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unsatisfactory (52.2% and 71.6%, respectively) in the patients with unfavourable INPC. Of all of them, 46% had differentiating histology while 54% had poorly differentiated or undifferentiated histology. OS in the "differentiating" histology group reached 100% vs. 63% in the "poorly differentiated or undifferentiated" cases (p=0.004), and EFS was 91% vs. 62% (p=0.002). These results comply with the INRG conclusions [10].

Accordingly, patients with INRG stage L2 NB aged less than or equal 18 months in this guideline will be considered as having a "low risk" NB and will be treated with less chemotherapy than in the past or will be only observed.

1.3. Low and Intermediate Risk Neuroblastoma European Study (LINES)

The European study (EudraCT Number: 2010-021396-81) LINES (Low and Intermediate Risk Neuroblastoma European Study), groups together in a single protocol the treatment of all patients with "non-high risk" neuroblastoma (NB), with stratification into two groups: "low risk" and "intermediate risk". These two separate cohorts are included in one single protocol to enable patient data from these two groups to be entered into a common database, as the current prognostic classifications determining treatment may evolve further with subsequent more detailed molecular analysis of the tumours. We will go through the low-risk study.

Low-Risk Study

The low-risk group of patients includes NB patients without *MYCN* amplification with or without LTS in the following clinical situations:

- Children aged ≤18 months with localised neuroblastoma associated with image-defined-risk factors precluding upfront surgery (stage L2).
- Children aged ≤12 months with disseminated neuroblastoma without bone, pleura, lung or CNS disease (stage Ms, according to SIOPEN, see Appendix A1.1).

Observational study for neonatal adrenal masses

Knowledge on perinatal suprarenal masses, although based on a relatively large literature, is scattered amongst studies on very few cases with no methodical approach and often short follow up [17-19]. However, the incidence of adrenal tumours/masses has increased in the last decades due to the expanded use of prenatal ultrasonography in routine obstetric care and in the neonatal and early infancy care. As the differential diagnosis (See Appendix 5) of these masses ranges from benign (adrenal haemorrhage (AH) to malignant processes (neuroblastoma, adrenal carcinoma) and the optimal management of these masses has not been clearly defined, the SIOPEN group, through the LINES multicentre study, includes an observational sub-study for neonatal adrenal masses with surveillance, based on their results in the first multicenter European Trial for Infants with Neuroblastoma (INES) and the world-wide experience provided in the literature. Neuroblastoma at this age is an intriguing entity with a very good prognosis in most cases.

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The observational study for neonatal adrenal masses includes patients in the following clinical situation:

- Children aged less than or equal to 90 days when the adrenal mass is detected by ultrasound and/or MRI and the following conditions: it must not reach the midline, it must measure ≤ 5 cm at the largest diameter, there must be no regional involvement (MRI scan does not show evidence of positive ipsi/contralateral lymph nodes or other spread outside the suprarenal gland) and disease must be localised stage (no metastatic involvement).

1.4 Other collaborative groups approach in low-risk NB patients

The main difference between the SIOPEN approach in the management of these group of patients with respect to other collaborative groups such as the Children's Oncology Group (COG) and the German Society for Paediatric Oncology and Haematology (GPOH) is the age cut-off 12 or 18 months in the Ms stage and the inclusion of patients with segmental chromosomal aberrations (SCA) in L2 and Ms patients.

2. PATIENT GROUP

These treatment recommendations refer to patients with an established diagnosis of neuroblastoma according to the SIOPEN modified International Neuroblastoma Risk Group (INRG) and to the INSS criteria.

Low-risk neuroblastoma:

- Stage L2 without MYCN amplification. In this cohort of patients, we consider ≤18 months of age at diagnosis and all types of histology.
- Stage Ms (metastasis in liver and/or skin and/or bone marrow, according to SIOPEN and without lytic bone lesions/CNS/lung or pleura; see Appendix A1.1) without MYCN amplification and age ≤ 12 months at diagnosis.
- Stage L1 and INSS stage 1 without MYCN amplified.

- For non-metastatic neuroblastoma, the INRG Staging System [see Appendix 1] will be used.
  Stage L1: Locoregional tumour not involving vital structures as defined by the list of Image-Defined-Risk Factors (See Synopses section).
  Within Stage L1 tumours with MYCN amplification there are very occasionally INSS stage 1 [8] localised tumours with complete gross excision, with or without microscopic residual disease who have representative ipsilateral lymph nodes microscopically negative for tumour (nodes attached to and removed with the primary tumour may be positive) [9].
  Stage L2: Locoregional tumour with presence of one or more IDRFs.

- For metastatic neuroblastoma, the INRG Staging system will be used but modified, so that it is in accordance with the staging used in the previous trial for this group of patients INES 99.2-99.3 [14].

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Stage Ms: Metastatic disease confined to skin and/or liver and/or bone marrow <10% (or even other sites such as lymph nodes and/or testes), but not bone, lung, pleura or CNS, in infants <12 months. MIBG or technetium scintigraphy uptake to the skeleton may occur but there should be NO X-Ray or CT evidence of bone involvement.

3. DIAGNOSTIC CRITERIA

There are three steps to be done before treatment starts:

- Staging of neuroblastoma according to INRGSS criteria (including patients with ganglioneuroma and ganglioneuroblastoma intermixed).
  - Complete metastatic work-up with bone marrow evaluation (See section 3.2.5) and imaging: chest X-ray, abdominal US, CT or MRI of the primary tumour, MIBG scan (18F-FDG PET/CT preferably or technetium bone scan if primary tumour MIBG negative). Bone imaging is necessary to confirm symptomatic or isotope positive areas of bone and a CT skull is required if bony skull disease is suspected. MIBG scanning is an accurate method for detecting osteomedullary metastases, with sensitivity and specificity of 90% and 97%, respectively. SPECT enables better depiction of the small focal uptake, especially in areas close to intense physiologic uptake such as the liver and the bladder.
    Moreover:
    - If an MIBG lesion is seen also on X-ray (standard or CT) as “lytic lesion”, It has to be considered as a “true cortical lesion”.
    - If an MIBG lesion is not seen on X-ray (standard or CT), it has to be considered as a “bone marrow lesion”.
- Tumour tissue studied for confirmation of diagnosis with tumour cell content, *MYCN* status and genomic copy number profile.

NOTE: In case of emergency treatment, metastatic work up and biology studies should be carried out no later than the beginning of the second course.

3.2 Evaluation at diagnosis

3.2.1 Full history

With attention to: weight loss, diarrhea, pain, hypertension, mobility and bladder function. Describe duration of these symptoms.

3.2.2 Full clinical examination (including blood pressure)

Careful attention to the presence of:

- any skin lesions.
- absence of opsoclonus-myoclonus (See Appendix 4)
- hepatomegaly or “masses” in different regions (cervical, axillae, inguinal).
- signs of spinal cord involvement.

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- life threatening symptoms (See Synopsis section).
- Horner´s syndrome.

3.2.3 Haematology/Biochemistry

- Full blood count.
- Coagulation profile to rule out DIC.
- Renal and liver function biochemistry (Na, K, Ca, Mg, PO_4, urea, creatinine, glucose, bilirubin, transaminases).
- Serum lactic dehydrogenase (LDH).
- Urine catecholamine metabolites (VMA and HVA minimum), measured in mol/mmol of creatinine. It is recommended that dopamine is also measured. It can be performed in a single urine sample.
- Urine (preferred) or serum pregnancy test will be done on girls who are post-menarche.

3.2.4 Imaging

- AP chest X-ray.
- Abdominal ultrasound (US) for all primary masses. Baseline US to be used as reference for further follow-up.
- CT or MRI of primary tumour, with tumour volume calculated by elliptical approximation (D1 x D2 x D3 x 0.52). Tumour volume can be calculated also by common software algorithms which are implemented in most radiological workstations.
- 123I-MIBG Isotope scintigraphy. Using 123I-MIBG images are acquired 20-24 hours after the injection. Selected delayed images - never later than 48h - may be useful in the odd cases with equivocal findings. It is recommended to use the SIOPEN score in reporting the results.
- 18F-FDG PET/CT preferably if primary tumour MIBG negative. In case there is no availability for a 18F-FDG PET/CT perform ^{99m} Tc MDP (Technetium) scintigraphy.
- Bone radiographs of any symptomatic areas and of areas which are positive on isotope scan to confirm bone disease. If bone disease of the skull is suspected clinically or radiologically or in case of positive isotope scan of the skull, CT scan of the skull.

NOTE: If MIBG is non-avid it is recommended that a PET-FDG study is performed.

- If PET-FDG confirms the presence of a suspected bone metastasis, it is considered as a “True bone lesion”, and false negative MIBG lesion. On the contrary, if PET is negative, that particular lesion is not considered as present (true negative MIBG) [20,21].

Special considerations for neonatal adrenal masses:

The vast majority of fetal tumours are detected by obstetric US during the second and third trimesters. The technique is helpful in determining location, content, relationship to surrounding structures and the presence of heart failure, hydrops or associated malformations. Nevertheless, complementary

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examinations are necessary. Fetal MRI defines the exact extent of the tumour better than US, also complications within the tumour or caused by the tumour.

Ultrasonography (US)

A few parameters may be considered: structure, size, vascularisation, change in size and structure during observation.

- *Structure:* It may be cystic, solid, mixed. Cystic structure is associated with proven NB in 47% of cases while 74.5% of cases are solid. A mixed structure is associated with NB in 54% of cases, with the remaining cases associated with presumed other conditions mostly including AH [22].
- *Size:* Size does not appear to be a parameter suitable to differentiate AHs from NBs.
- *Vascularisation:* It has been proposed [23] that colour and flow Doppler sonography may be useful in differentiating between AH and perinatal NB. In a very limited series of cases it has been observed that in most cases AHs do not show vascularisation on Doppler studies, whereas solid NBs may show vascularisation. However, it also known that Doppler studies do not help in differentiating an AH from a haemorrhage occurring within a cystic NB [24].
- *Changes in echogenicity:* A neonatal solid adrenal mass which changes its echogenicity while it shrinks (usually: solid -> mixed -> cystic -> disappearing with possible residual calcifications) has been traditionally associated with a presumed AH.

The initial ultrasound has to be repeated at the reference centre and completed with abdominal MRI to rule out regional or metastatic involvement. Data on size and characteristics of the mass are important to be described.

CT scan

CT may be contributory in differential diagnosis when it shows a systemic vessel in a pulmonary sequestration, in case US failed to show it [25] however some pitfalls are well known. It may be also helpful to the diagnosis of NB when identifying subtle calcifications within the mass or in the adjacent retroperitoneum [22], and it is useful to evaluate local NB extension before surgical excision. In case of cystic lesions, rim enhancement may suggest the possibility of cystic NB [26].

MRI

In case of cystic lesions, MRI may be useful in determining the nature of the cyst content. Two different intensities may be demonstrated within the mass, with debris-fluid levels suggesting intracystic haemorrhage. However, MRI is unable to differentiate between adrenal haemorrhage and haemorrhage occurring within a cystic NB [27]. Similarly to CT, MRI may be useful if it demonstrates a systemic vessel within a solid suprarenal mass (possible subdiaphragmatic extralobar pulmonary sequestration (SEPS)), although this finding is not constant and pathognomonic of SEPS.

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I123-MIBG

There are no specific and clear recommendations in the group of very young children due to difficulties in performing the scan such as sedation, thyroid blockade or ionising radiation exposure. In general terms we recommend that an MIBG is done within the first 3-6 months from diagnosis to confirm the diagnosis of neuroblastoma and rule out metastasis. It is highly recommended that an MIBG scan is done in all cases of suprarenal masses with increased catecholamines. In cases of lesions smaller than 1 cm with negative catecholamines, MIBG scan can be avoided.

Urinary catecholamines

High levels of urinary catecholamines are helpful in confirming the diagnosis of NB. In patients with prenatally detected NB, urinary catecholamines will be normal in two thirds of them, with a negative predictive power of about 70% [22, 28-31].

Blood sampling

Blood counts, biochemistry including LDH.

A neonate in whom a suprarenal mass is visualized by ultrasound, either during pregnancy or neonatally (≤ 90 days after birth) should be referred to an experienced Paediatric Oncology centre.

3.2.5 Bone marrow

For staging procedures, the bone marrow should be studied according to INSS recommendations: bone marrow aspirations (from two separate evaluable sites, usually iliac crests) for morphology and bone marrow trephines (from two separate evaluable sites, usually iliac crests) are recommended where possible. If trephines are not done then it is recommended that 4 aspirates are taken (2 from each side). In case of children less than 1 year of age it can be difficult to get trephines, thus aspirates from both sides would be enough. In neonates it is recommended that bone marrow aspirates be taken from the tibia.

3.2.6 Pathology and biology of the primary tumour

It is essential that all children have a biopsy of the primary tumour, even in the presence of metastatic disease (providing this can be obtained with minimal trauma to the child) so that pathological and biological information can be obtained. If a biopsy of the primary is deemed to be too hazardous, for example in stage 4S with a small primary tumour and massively enlarged liver, then a Tru-Cut biopsy of the liver would suffice. In case of LTS that require initiation of emergency chemotherapy treatment before tissue sample is taken, perform the biopsy as soon as possible afterwards.

If skin nodules are present, a biopsy of these may be performed. For children with L2 tumours, an open biopsy is sometimes preferred, since the quality of the pathological and biological studies can be higher. For those centres which use multiple Tru-Cuts instead of open biopsy, sufficient amount of tumour material for pathological and biological studies must be guaranteed.

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The pathological analysis is histological grading according to INPC [32-34].
The biological analyses needed are the *MYCN* status and the genomic copy number profile obtained in a National Reference Laboratory (see section 1.2.7).

3.2.7 Molecular pathology

Derived from international efforts to enhance prognostic estimations, many studies have identified different tumour markers associated with overall and disease-free survival, including *MYCN* amplification, segmental chromosomal alterations in general and more specifically deletion of chromosomes 1p and 11q and gain of chromosome 17q [35-40]. Chromosome 1p loss was the first recurrent segmental aberration to be described, associated with *MYCN* amplified high stage NB, but also with prognostic power for non-amplified NB [38,39]. 17q gain was described as the most frequently detected aberration in *MYCN* amplified and non-amplified tumours. However, its prognostic impact is still controversial [40,41]. 11q loss is regarded as the prognostic most significant aberration in *MYCN* non-amplified tumours [36,37,39]. 1q gain was more recently identified as having prognostic significance [42]. And finally, 3p loss, despite being frequently associated with 11q deletions, were discovered to have some prognostic power in localized tumours [35,43,44].

All tumours with neuroblastic component in the tumour studied must have the MYCN oncogene status analysed by FISH, the standard method. All patients treated according to this guideline will have MYCN non-amplified tumours. In addition, it is highly recommended that analysis of the presence of segmental chromosomal abnormalities by genomic copy number profile is also performed.

3.2.7.1 MYCN oncogene status

The *MYCN* oncogene status (localized on chromosome 2p) is evaluated at diagnosis in all NB cases, with the exception of ganglioneuroma cases.
MYCN amplification (more than 5 copies of the *MYCN* gene per haploid genome) remains the most relevant outcome predictor. *MYCN* status has to be determined by a certified laboratory. It is found in around 20% of NB cases and is frequently associated with unfavourable histology: diploidy, 1p deletion and 17q gain while it is inversely associated with other genetic alterations such as 11q aberration and *ATRX* deletions or mutations [10,37,38,47,48].

3.2.7.2 Genomic copy number profile

Genomic copy number profile of the tumour can be done by Comparative Genomic Hybridization (array-CGH), single nucleotide polymorphisms (SNP array) or pangenomic next generation sequencing (NGS) techniques such as whole genome sequencing including low coverage WGS (lcWGS), WES or other NGS approaches if centrally reviewed.
Genomic profiling is classified as follows:

- **NCA genomic type:** Presence of numerical chromosomal alterations (NCA) only.
- **SCA genomic type:** Presence of any segmental chromosomal alteration (SCA) observed recurrently in neuroblastoma (deletion of chromosome 1p, 3p, 4p, or 11q; gain of 1q, 2p, or 17q) without or with numerical chromosomal alterations.

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- No result: No sample or sample containing an insufficient number of tumour cells, technical failure, prognostically non informative (presence of segmental alterations but none of those observed recurrently in NB; no chromosomal abnormality (neither NCA nor SCA) despite sufficient tumour cell content of the analysed sample).

3.2.8 Organ function

- Studies of cardiac function: base-line echocardiography prior to any anthracycline therapy is needed.
- Audiology studies are mandatory for patients > 1 year and highly recommended for patients < 1 year prior to receiving carboplatin [49].

3.2.9 Others

Assess carefully for the presence of life-threatening symptoms, opsoclonus myoclonus syndrome (OMS) and spinal cord compression (SCC) which would necessitate rapid initiation or treatment (See Synopsis section and Appendix 3 and 4, respectively).

4. TREATMENT DETAILS

4.1 Ganglioneuroma and ganglioneuroblastoma intermixed

Ganglioneuromas are benign mature neuroblastic tumours, which can display MIBG uptake and cause increased excretion of catecholamine metabolites in the urine [50]. The treatment of choice is surgical resection, even though surgery-related complications might be frequent due to invasive growth of ganglioneuromas [50,51]. For this reason, patients should only be treated in centers with expertise in paediatric complex surgical procedures. Chemotherapy is ineffective in treating ganglioneuromas.

Ganglioneuroblastoma intermixed tumors have previously been considered malignant. However, case reports and analysis of a series of patients with ganglioneuromas and ganglioneuroblastoma intermixed tumors revealed that postoperative progression was highly unlikely after incomplete resection [48,51-53]. Chemotherapy also tends to be ineffective, as in ganglioneuromas.

Patients with either ganglioneuromas or ganglioneuroblastoma intermixed tumours **should only receive surgical resection as treatment.** If complete resection involves mutilating surgery, then an incomplete resection of the tumour is acceptable and recommended. It is recommended to remove abnormal locoregional lymph nodes since it is difficult to differentiate between primary tumour and locoregional lymph node metastases without histological assessment. Chemotherapy is not recommended and should be avoided.

Minimal invasive surgery can be considered in tumours without IDRF, not located in the abdominal midline and of limited size.

MRI or a CT of the primary tumour site 1 month after surgery to assess the for possible tumor residue is advisable.

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4.2 Neonatal neuroblastoma discovered as neonatal adrenal mass (NAM)

Children who have no life-threatening symptoms are monitored at the prescribed intervals during the course of disease until the 12th month of observation. At that time-point, when the mass still persists it is recommended to perform a biopsy or resect the lesion to make a pathological and biological analysis. This surveillance will consist of physical examination, follow-up ultrasound and urinary catecholamine analysis. Tests will be done until week 12, at least in week 6 and in week 12.

NOTE: If the week 0 catecholamines were negative, then repeat catecholamines is only recommended if an increase in the size of the mass is seen.

- At this point, two features could be observed:
    - Initiation of regression: US and markers will be performed in weeks 18, 30 and 48.
    - If regression has not been observed, US and markers can be performed monthly until week 48.

NOTE: For regression of the tumour, no specific decrease in the size of the mass is required (the mass has to be undoubtedly smaller).

- If there is evidence of an increase of tumour volume (40% or more) and an increase in catecholamine metabolite excretion the children will go off the observational approach and follow the off-observation recommendations*, explained later.
- Suspicion of progressive disease (clinical symptoms/signs of progression or metastases) will prompt the cessation of the observational approach and the patient´s complete reassessment.
- If at month 12 the suprarenal mass still persists, pathological and biological analysis is recommended plus tumour excision if feasible (all L1, selected L2).
- If the suprarenal mass disappears, but the catecholamine metabolites persist elevated (evidence of neuroendocrine tumoral function), a diagnostic and staging procedure should be performed to rule out the presence of occult disease.
- In the case where complete regression is observed (at or prior to week 48) the patient should be followed at least, annually for 3 years and thereafter, according to local policies.

In all other cases, closer monitoring by ultrasound and urine catecholamines is recommended, based upon the individual criteria of the responsible centre, until stabilization is reached or surgery is planned.

*Children will be treated according to LNESG-2 algorithm.

Off-observation strategy.

When the patient is off the observation strategy, a full reassessment of the disease must be carried out in order to confirm the locoregional progression and the absence of metastases. The most appropriate surgical approach must be performed according to IDRF (L1, L2). In most of the cases, the mass will be L1 and complete excision can be achieved. If a neuroblastoma is confirmed pathologically, all biological studies have to be carried out and the patient treated according to stage and risk factors.

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4.3 L1 without MYCN amplification

Surgery

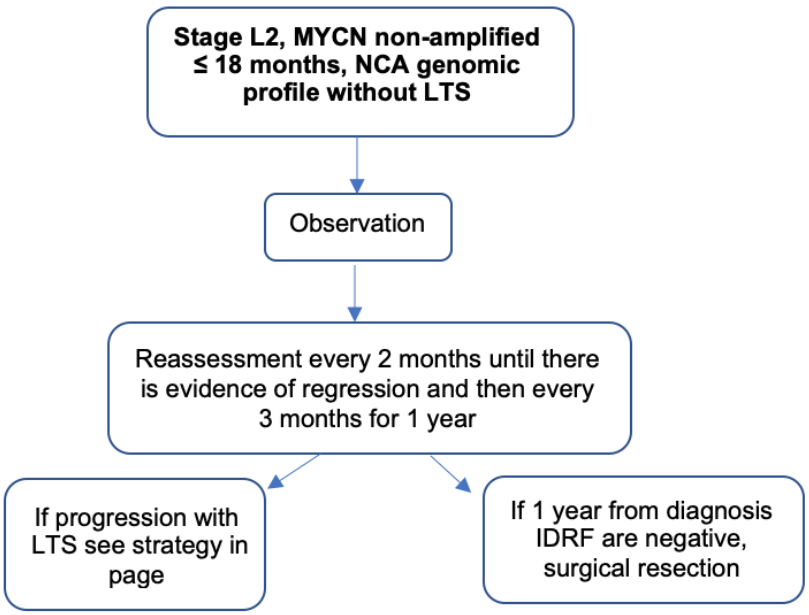
L1 tumours have no IDRF (See Appendix 2) and **complete surgery** is the best treatment.

Perform an MRI or a CT of the primary tumour 1 month after the surgery to assess the possible existence of residual tumour.

4.4 L2 without MYCN amplification and ≤ 18 months

Depends according to the presence or absence of LTS that risk patient´s life or organ function and the tumour genomic copy number profile type.

4.4.1 No life-threatening symptoms and NCA: observation.



Chemotherapy is indicated if progressive disease develops whilst being observed. The recommended chemotherapy regime is carboplatin-etoposide, since they are well-known drugs that can be used in infants with safety in an expert centre. In the unlikely case that after 2 courses, the patient develops LTS, chemotherapy will be switched to CADO.

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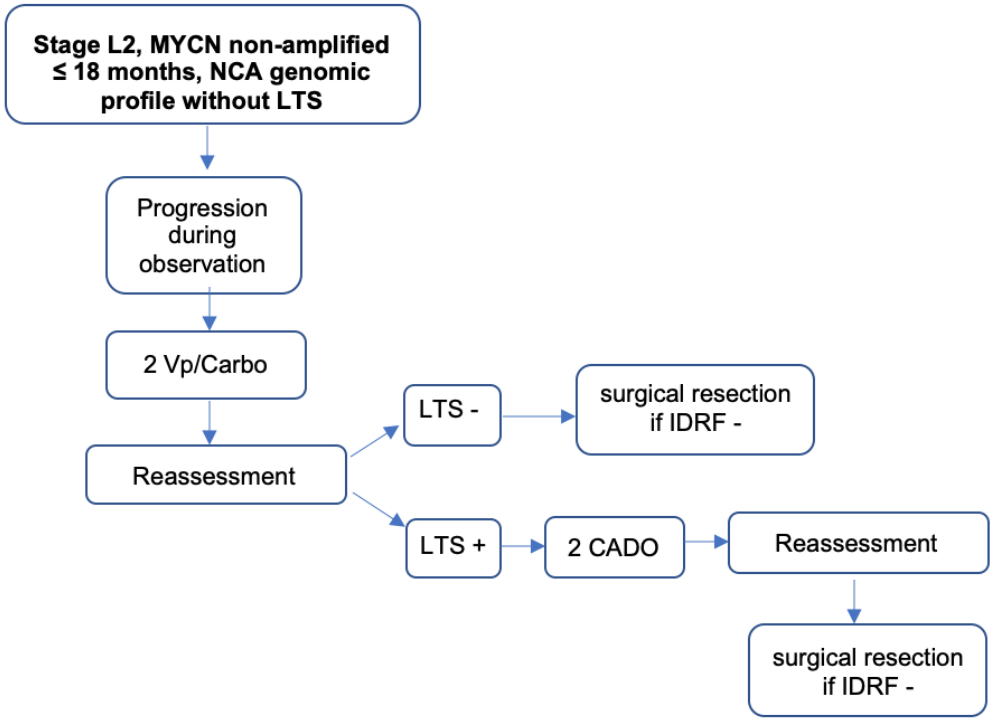
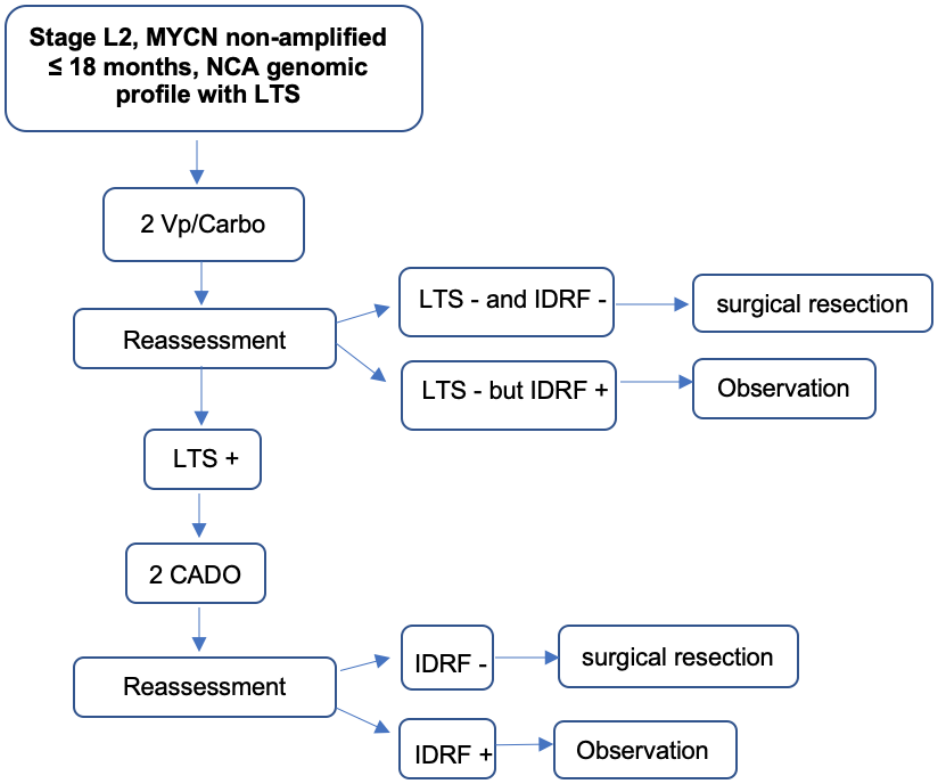


4.4.2 With life-threatening symptoms and NCA: chemotherapy.



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Treatment

- These patients will all receive chemotherapy with a minimum of 2 courses of Etoposide and Carboplatin (VP/Carbo).
- If the LTS persist after 2 courses of VP/Carbo they should receive CADO x 2. The aim is to resolve the life-threatening symptoms with the minimum number of courses of chemotherapy.
- A surgical resection of the tumour will be performed only if the IDRF become negative.

Refer to section 7 for detailed information regarding chemotherapy and dose modification.

Surgical management

A surgical resection of the primary mass in these low-risk infants (L2) should not be undertaken if IDRF are present during first-line treatment. The symptomatic patients who receive chemotherapy should have a surgical resection only when the tumour becomes IDRF negative. If the child remains symptomatic after chemotherapy discuss with the surgery team of your hospital/institution in a tumour board. It is recommended to remove abnormal locoregional lymph nodes since it is difficult to differentiate between primary tumour and locoregional lymph node metastases without the histological assessment. Minimal invasive surgery can be considered in tumours without IDRF, not located in the abdominal midline and of limited size.

Perform MRI or a CT of the primary tumour site 1 month after the surgery to assess the possible existence of residual tumor rest.

Investigations and assessment during treatment

Assessments for tumour volume are repeated after every two courses whilst on chemotherapy. See section 5.

For disease progression and management see section 6.

4.4.3 With or without of life-threatening symptoms with presence of SCA: chemotherapy.

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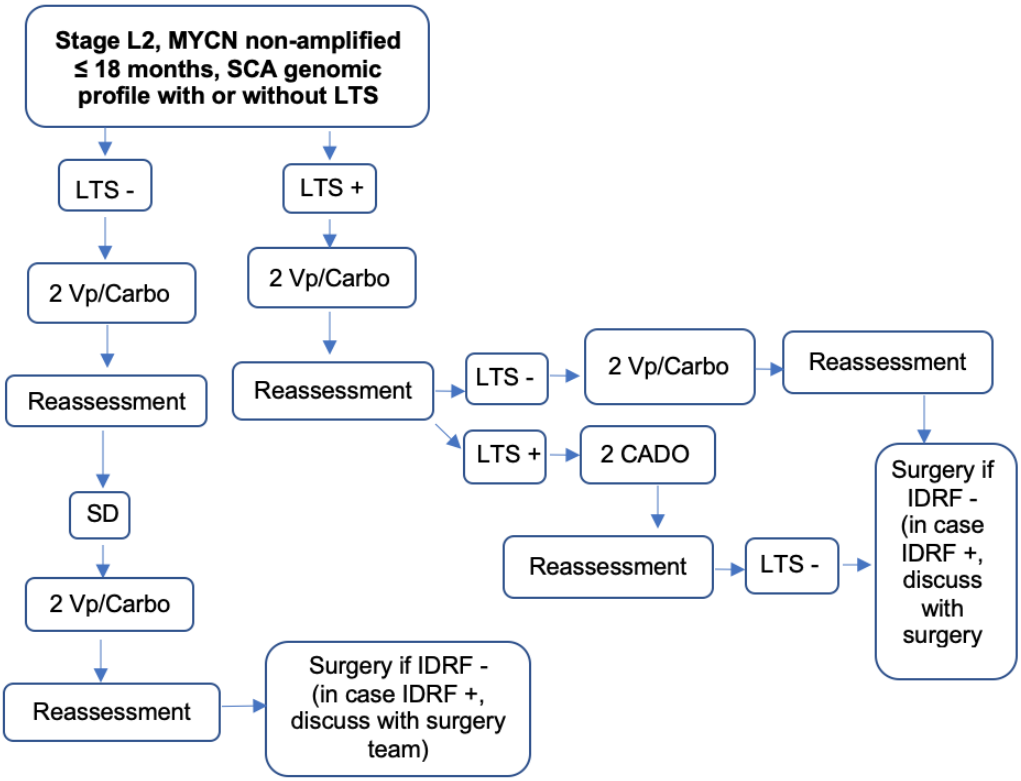

- Patients **without life threatening symptoms** will have four courses of VP/Carbo. The aim is for the IDRF to become negative so that a surgical resection can be performed. If the IDRF are still positive after 4 courses of VP/Carbo NO further courses of chemotherapy should be given and the patient should be observed.
- In patients **with life threatening symptoms** the aim is to resolve the life-threatening symptoms with four courses of chemotherapy, and for IDRF to become negative and to proceed to surgical resection.
- If the LTS persist and or are not resolving after 2 courses of VP/Carbo, 2 courses of CADO should be given.

Refer to section 7 for detailed information regarding chemotherapy and dose modification.

Surgical management

A surgical resection of the primary mass in low-risk infants (L2) should not normally be undertaken if IDRF are present during first-line treatment. However, the balance of risk versus benefit for these infants who have SCA genomic abnormalities may be influenced by the specific details of the risk factors. Thus, tumours that remain IDRF positive after chemotherapy should be discussed with the surgery team of your hospital/institution. If a tumour remains IDRF positive after chemotherapy this is not an absolute contraindication to surgery. Resection may still be recommended if evaluation of the primary tumour suggests that the risk to life, or of major functional loss, is less than the risk of leaving residual disease.

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It is recommended that abnormal locoregional lymph nodes are removed since it is difficult to differentiate between primary tumour and locoregional lymph node metastases without histological assessment.

Minimal invasive surgery can be considered in tumours without IDRF, not located in the abdominal midline and of limited size.

Performance of an MRI or a CT of the primary tumour site 1 month after the surgery to assess the possible existence of residual tumour..

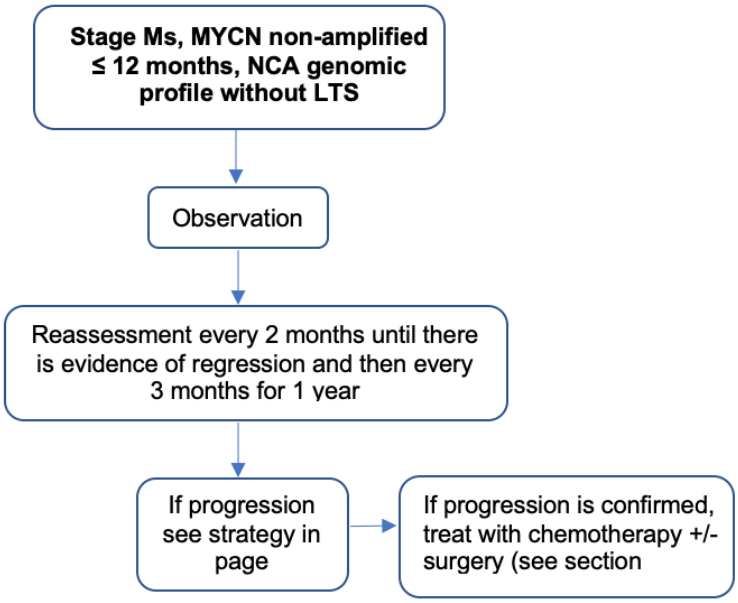
Investigations and assessment during treatment

Assessments for tumour volume are repeated after every two courses whilst on chemotherapy. For more details see section 5.

Disease progression and management see section 6.

4.5 Ms* without MYCN amplification in ≤ 12 months (* according to SIOPEN, without lytic bone lesions/CNS/lung or pleura, see Appendix A1.1)

4.5.1 Without Life-threatening symptoms and NCA: observation.



These patients should be observed to allow spontaneous regression of their tumour. Regular assessments will be undertaken.

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Surgical management

A surgical resection of the primary mass in low-risk infants (L2) should not normally be undertaken if IDRF are present during first-line treatment. However, the balance of risk versus benefit for these infants who have SCA genomic abnormalities may be influenced by the specific details of the risk factors. Thus, tumours that remain IDRF positive after chemotherapy should be discussed with the surgery team of your hospital/institution. If a tumour remains IDRF positive after chemotherapy this is not an absolute contraindication to surgery. Resection may still be recommended if evaluation of the primary tumour suggests that the risk to life, or of major functional loss, is less than the risk of leaving residual disease.

It is recommended that abnormal locoregional lymph nodes are removed since it is difficult to differentiate between primary tumour and locoregional lymph node metastases without histological assessment.

Minimal invasive surgery can be considered in tumours without IDRF, not located in the abdominal midline and of limited size.

Performance of an MRI or a CT of the primary tumour site 1 month after the surgery to assess the possible existence of residual tumour.

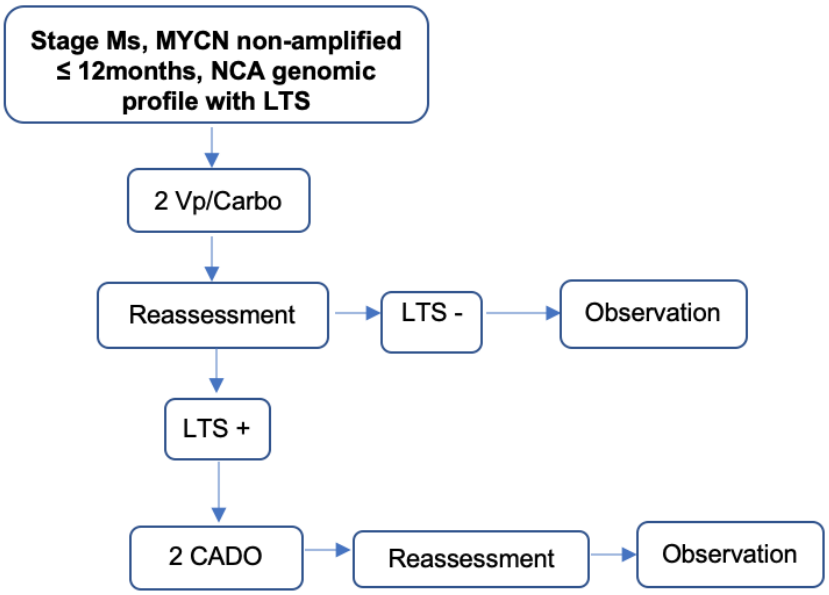
Investigations and assessment during treatment

Imaging of the tumour (in most cases this will be of the involved liver) will be undertaken every 2 months until there is evidence of regression and then every 12 weeks for one year, followed by annual assessments for 5 years.

Disease progression and management

In case of progression a full reassessment should be undertaken (See section 6).

4.5.2 With life-threatening symptoms and NCA: chemotherapy.



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- These patients will receive chemotherapy with a minimum of 2 courses of Etoposide and Carboplatin. The aim being to resolve the LTS with the minimum number of courses of chemotherapy.
- If the LTS persist after 2 courses of VP/Carbo, patients should receive 2 courses of CADO chemotherapy.

Refer to section 7 for detailed information regarding chemotherapy and dose modification.

Surgical management

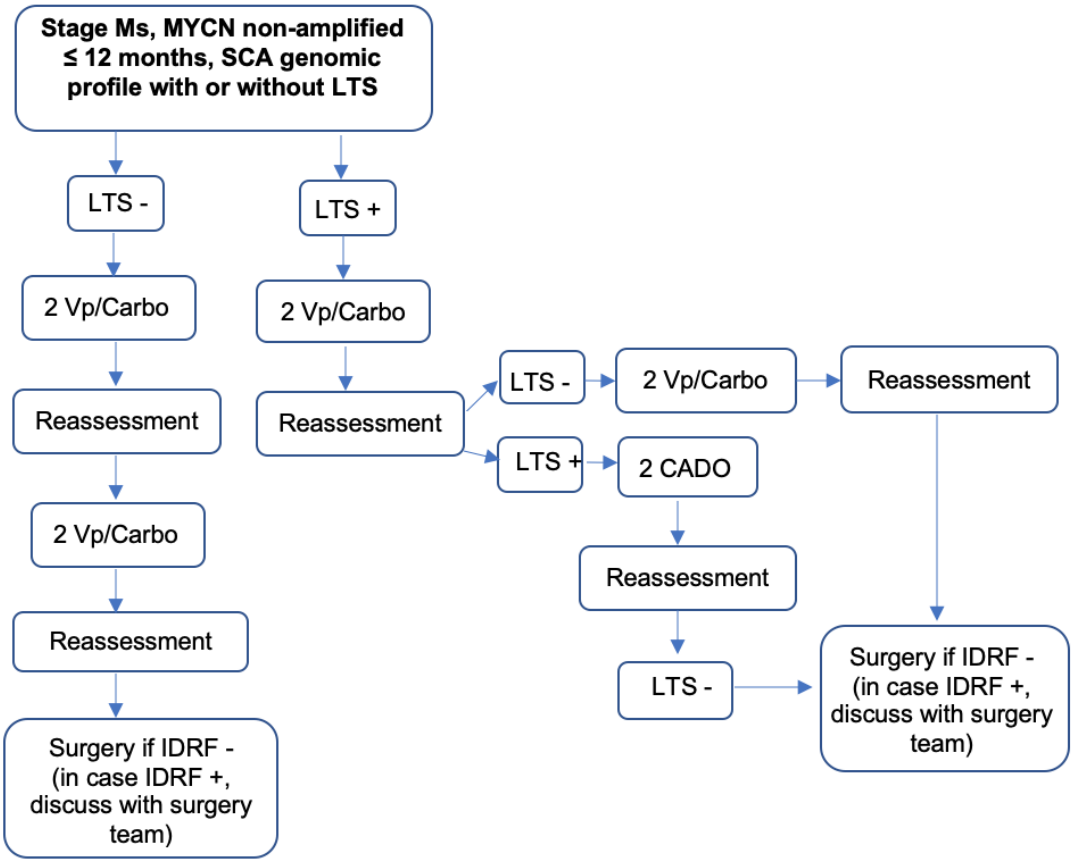
Resection of the primary tumour in stage Ms (according to SIOPEN, without lytic bone lesions/CNS/lung or pleura, see Appendix A1.1) is not indicated unless this is carried out as part of the initial diagnostic workup. It should never be carried out if there are image defined risk factors present. In patients with regressing tumours there is no evidence that a delayed resection is necessary.

Investigations and assessment during treatment

Assessments for tumour volume are repeated after every two courses whilst chemotherapy is being administered. For further details see section 5.

Disease progression and management see section 6.

4.5.3 With or without of life-threatening symptoms with SCA: chemotherapy.



Treatment

- All of these patients will be treated with a total of four courses of chemotherapy.
- When no LTS are present give VP/Carbo x 4.
- When LTS present give VP/Carbo x 2 followed by either 2 further courses of VP/Carbo if the symptoms have responded or are resolving or 2 courses of CADO if the LTS symptoms are not resolving with the initial 2 courses of VP/Carbo.

Refer to section 7 for detailed information regarding chemotherapy and dose modification.

Surgical management

A surgical resection of the primary mass in low-risk infants (L2) should not normally be undertaken if IDRF are present during first-line treatment. However, the balance of risk versus benefit for these infants who have SCA genomic abnormalities may be influenced by the specific details of the risk factors. Thus, tumours that remain IDRF positive after chemotherapy should be discussed with the surgery team of your hospital/institution. If a tumour remains IDRF positive after chemotherapy this is not an absolute contraindication to surgery. Resection may still be recommended if evaluation of the primary tumour suggests that the risk to life, or of major functional loss, is less than the risk of leaving residual disease.

It is recommended that abnormal locoregional lymph nodes are removed since it is difficult to differentiate between primary tumour and locoregional lymph node metastases without histological assessment.

Minimal invasive surgery can be considered in tumours without IDRF, not located in the abdominal midline and of limited size.

Performance of an MRI or a CT of the primary tumour site 1 month after the surgery to assess the possible existence of residual tumour.

Investigations and assessment during treatment

Assessments for tumour volume are repeated after every two courses whilst chemotherapy is being administered. For further details see section 5.

Disease progression and management, *see section 6.*

4.6 Special situations

Treatment recommendations for patients without genomic copy number profile result or histology neuroblastoma NOS.

Certain patients with neuroblastic tumours are not able to be classified with a clear definition of the histotype and are considered as neuroblastoma NOS (not otherwise specified). In these cases, it is recommended that another biopsy is taken in order to obtain a more accurate diagnosis and consequently stratify the patient to the appropriate stratification group. If a second biopsy is not available or gives the same result (NOS) the patient will receive “historic” treatment (INES).

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If the tumour biopsy is performed after the start of treatment the genomic copy number profile result will be considered non informative and cannot be used for treatment stratification. Thus, these patients will be managed in the same way as those with unknown genomic profile, with "historic" treatment.

Stage L2, ≤ 18 months, *MYCN* non-amplified without life threatening symptoms with *no genomic profile result* treat according to section 4.4.1
Stage L2, ≤ 18 months, *MYCN* non-amplified, *no genomic profile result*, with life threatening symptoms treat as in section 4.4.2
Stage Ms (according to SIOPEN, without lytic bone lesions/CNS/lung or pleura, see Appendix A1.1), ≤ 12 months, *MYCN* non-amplified, *no genomic profile result*, without life threatening symptoms treat as in section 4.5.1
Stage Ms (according to SIOPEN, without lytic bone lesions/CNS/lung or pleura, see Appendix A1.1), ≤ 12 months, *MYCN* non-amplified, *no genomic profile result*, with life threatening symptoms treat according to section 4.5.2

Dumbbell tumours

In case of dumbbell tumours with intraspinal neuroblastoma, these patients may be either asymptomatic or symptomatic with symptoms of spinal cord involvement. We recommended that patients who have a spinal cord component greater than one third of the diameter of the spinal canal are treated with chemotherapy even in the absence of signs or symptoms of spinal cord involvement (See Appendix 3).

If the symptoms resolve and the extraspinal component becomes resectable (i.e. IDRF negative) then a surgical resection of the extraspinal component should take place even though intraspinal disease remains. Macroscopic disease may be left in the intervertebral foramina, especially when there is a risk of leakage of spinal fluid and/or jeopardizing the blood supply of the spinal cord. There is no indication to surgically resect the residual spinal canal component of a dumbbell tumour.

Life-threatening symptoms (LTS)

In case of presence of LTS, it is recommended to initiate chemotherapy with Vp/Carbo as soon as possible as well as the necessary treatment support to stabilize the patient (e.g. diuretics, respiratory support).

Opsoclonus-myoclonus syndrome (OMS)

In case of OMS, see Appendix 4.

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5. ASSESSMENTS

5.1 Minimal Investigations during and at the end of treatment

Cycles number	At diagnosis	1	2	3	4	5	6	End of Treatment
Complete blood count, biochemistry, serum LDH	X	X	X	X	X	X	X	X
Renal function evaluation (Including the Schwartz's formula)	X	X	X	X	X	X	X	X
Urine catecholamines	X				X			X
Biological studies MYCN, Array-CGH /MLPA	X							
Bone marrow aspirates and trephine biopsy	X							X[1]
Ultrasound, CT or MRI	X		X[2]		X		X	X[3]
Scintigraphy MIBG (18F-FDG PET/CT preferably or 99mTc MDP if MIBG non-avid tumours)	X							X
Audiology (for patients receiving carboplatin)	X				X			X
Echocardiogram (for patients receiving anthracycline)	X				X			X

[1] In Ms (according to SIOPEN, without lytic bone lesions/CNS/lung or pleura, see Appendix A1.1)

[2] Preferable to use US to evaluate response

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[3] Evaluate with CT and/or MRI, comparable with imaging at diagnosis

In the observation cohorts: Imaging of the tumour (in most cases this will be of the involved liver) will be undertaken every 8 weeks until there is evidence of regression and then every 12 weeks for one year, followed by annual assessments for 5 years.

5.2 Investigations after completion of treatment

Tumour Assessment/Detection

As a minimum, imaging of the primary tumour site should be evaluated every 12 weeks in the first year after completion of treatment.

Toxicity Assessment

- Renal: GFR should be assessed at the end of treatment in patients considered to be at risk of renal toxicity.
- Auditory: Audiometry should be assessed at the end of treatment in those treated with carboplatin.
- Cardiac: Echocardiography should be carried out at the end of treatment in those patients that received an anthracycline.

5.3 Patient Follow-Up

In order to monitor disease status, e.g. absence of tumour in any site, the inactivity of the residual disease, if any, or early detection of relapse or progression of the disease, regular follow-up visits are recommended once the treatment is finished. Patient follow-up is also important to diagnose late developing toxicities from treatment. Long term follow-up in children with neuroblastoma should follow the different national policies for long term follow-up in children cured of a paediatric cancer.

The recommended assessment intervals are shorter in the first 5 years following treatment and lengthen after the 5-year mark, since the event rate is lower ≥ 5 years after diagnosis.
If relapse or progression is suspected complete disease staging based on MRI, 123I-MIBG scintigraphy, 18F-FDG PET-CT (for 123I-MIBG non-avid neuroblastomas) and bone marrow assessment is required.

5.3.1 Tumour assessment

In an asymptomatic patient, the following are recommended:

- Full Clinical examination (including blood pressure), neuron specific enolase (NSE) and catecholamine metabolite excretion at least every 6 months from 2nd year after end of treatment till 5th year and once a year since then 5 more years.
- Imaging of the primary site (x-ray, ultrasound, or CT or MRI scan as appropriate), at least every 6 months from 2nd year after end of treatment till 5th year and once a year since then 5 more years.
- Metastatic assessment: in case of residual skeletal MIBG positive, repeat MIBG scan every 4-6 months until negative or progression. If stable over 1 year, repeat it on a yearly basis for up

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to 5 years. In case of bone marrow disease at the end of treatment, perform bilateral bone marrow aspiration +/- trephine every 6 months until no abnormalities are detected.

5.3.2 Toxicity assessment

The toxicity assessment needs to be related to the treatment received by the patient, with regards to renal, audiological and cardiological follow-up. Those who have had extensive abdominal or pelvic radiotherapy may have prolonged thrombocytopenia.

Renal Follow-Up:

As per institutional practice.

Auditory Follow-Up:

Ototoxicity is usually permanent or irreversible. An adequate assessment at the end of treatment is strongly advised, and audiometry should be performed at 2 years and 5 years from diagnosis for those children treated with carboplatin.

If the child has sudden severe hearing loss which is not only a high-frequency loss then serious otitis media should be excluded and the audiometry repeated after 6 months.

Cardiac Follow-Up:

In this guideline, the cumulative dose of anthracycline does not exceed 240 mg/m2. Echocardiography every 5 years from diagnosis for children treated with anthracycline is recommended (accumulated dose of anthracycline less than 250 mg/m2 and no radiotherapy dose received with impact on the heart (radiation to chest, abdomen, thoracic or whole spine, TBI)).

Etoposide, second malignancy follow up:

It is important that any second malignancy occurring amongst the children treated with chemotherapy is registered in each country.

6. RECOMMENDATIONS IN CASE OF PROGRESSION

6.1 NAM

For the subset of patients with suprarenal neuroblastoma (MIBG positivity and/or elevated catecholamines) an event would be defined as having progressive disease (higher than L1) at the time of resection; where progression is defined as ≥ 40% increase in tumour volume. Progression/recurrence after resection will be defined as:

- Any new mass lesion, in a patient under observation for a suprarenal mass, which is subsequently biopsy proven neuroblastoma.
- Any new mass lesion, either local or distant, in a patient who has undergone resection of a suprarenal neuroblastoma, which is subsequently biopsy proven neuroblastoma.

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- MIBG positive for metastatic tumour.
- Bone marrow positivity.

These events should be managed according to the ERN current guidelines for non-high-risk neuroblastoma or high-risk neuroblastoma, following staging procedures and biological studies.

6.2 L2 without MYCN amplification and ≤ 18 months

Definition of progression

1. Development of new or different life-threatening symptoms (See Synopsis section).
2. Progression of localized disease (See Appendix 6)
3. Development of metastatic disease (bone, lung and pleura and CNS).

NOTE: Liver, bone marrow and skin metastases in a patient ≤ 12 months implies Ms disease according to SIOPEN, without lytic bone lesions/CNS/lung or pleura, see Appendix A1.1).

If any of the above occurs, this is an indication to undertake a **full reassessment** including:

1. Tumour biology – consider the need to reassess.
2. 3D imaging of local tumour, MIBG scan and further imaging if indicated as per pre-diagnosis investigations (See section 3.2.4)
3. Bilateral bone marrow aspirates and trephines.

The management of progression depends on which treatment was previously given.

- If *MYCN* amplification present follow high-risk protocol treatment recommendations
- **Localized progression:** If no *MYCN* amplification and no evidence of metastatic disease then treatment should be next chemotherapy in escalation of treatment:
  - if only VP/Carbo has been given treat with CADO x 2.
  - if CADO x 2 given, treatment is at the discretion of the centre, consider TVD or Cyclophosphamide/Topotecan.
  - Surgical management: If the IDRF become negative then a surgical resection of the primary tumour should be undertaken. If IDRF are present discuss with your surgery team. If a tumour remains IDRF positive after chemotherapy this is not an absolute contraindication to surgery. Resection may still be recommended if evaluation of the primary tumour suggests that the risk to life, or of major functional loss, is less than the risk from leaving residual disease.
- **Metastatic Disease:** If no *MYCN* amplification is present and there is metastatic disease in the bone, lung and/or CNS they need to be treated as stage M disease. The treatment they should then receive depends on their age when these metastases develop.
  - Patients ≤ 12 months should follow the intermediate-risk protocol treatment recommendations.
  - Patients > 12 months follow the high-risk protocol treatment recommendations. If no *MYCN* amplification is present and there is metastatic disease in the liver, bone marrow

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and/or skin this is Ms disease. The treatment they should then receive depends on their age when these metastases develop.

6.3 Ms (according to SIOPEN, without lytic bone lesions/CNS/lung or pleura, see Appendix A1.1) without MYCN amplification in ≤ 12 months not treated with chemotherapy (strategy of observation).

Definition of progression

1. Development of life-threatening symptoms (See Synopsis section).

NOTE: Local increase of tumour volume without the appearance of LTS is not an indication for starting chemotherapy.

2. Development of metastatic disease (bone, lung and pleura and CNS).

Reassessment (needed to re-establish stage)

1. Tumour biology – consider the need to reassess
2. 3D imaging of local tumour, MIBG scan and further imaging if indicated as per pre-diagnosis investigations (See section 3.2.4).
3. Bilateral bone marrow aspirates and trephines

Management of progression

- If *MYCN* amplification present treat on the high-risk protocol.
- **Progressive Ms (**according to SIOPEN, without lytic bone lesions/CNS/lung or pleura, see Appendix A1.1) **disease:** If no *MYCN* amplification is present, and LTS have appeared, treatment is with chemotherapy:
  - Start with 2 courses of VP/Carbo, aiming to resolve the LTS with the minimum number of courses of chemotherapy.
  - If the LTS persist after 2 courses of VP/Carbo give 2 courses of CADO.
  - A surgical resection of the primary tumour is not indicated.
- Patients without LTS should continue to be observed.
- **Metastatic disease (Stage M):** If no *MYCN* amplification is present and there is metastatic disease in the bone, lung and/or CNS they need to be treated as stage M disease. The treatment they should then receive depends on their age when these metastases develop.
  - Patients ≤ 12 months should go onto the intermediate-risk protocol.
  - Patients > 12 months go on to the high-risk protocol.

6.4 Ms (according to SIOPEN, without lytic bone lesions/CNS/lung or pleura, see Appendix A1.1) without MYCN amplification in ≤ 12 months treated with chemotherapy.

Definition of progression:

1. Development of new or different life-threatening symptoms (See Synopsis section).

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2. Progression of localised disease or previously known metastatic site (e.g. liver).
3. Development of new metastatic disease (bone, lung and pleura and CNS).

If any of the above occurs, this is an indication to undertake a **full reassessment** including:

1. Tumour biology – consider the need to reassess.
2. 3D imaging of local tumour, MIBG scan and further imaging if indicated as per pre-diagnosis investigations (See section 3.2.4).
3. Bilateral bone marrow aspirates and trephines.

Management of progression depends on which treatment was previously given.

- If *MYCN* amplification is now present follow the high-risk protocol treatment recommendations.
- **Progressive Ms (**according to SIOPEN, without lytic bone lesions/CNS/lung or pleura, see Appendix A1.1) **disease:** If there is no *MYCN* amplification and no evidence of metastatic stage M disease then treatment should be the next chemotherapy in the escalation of treatment:
  - if only VP/Carbo has been given treat with CADO x 2.
  - if CADO x 2 has been given, treatment is at the discretion of the trial coordinator. Consider TVD or Cyclophosphamide/Topotecan.
  - Surgical management: If the IDRF become negative then a surgical resection of the primary tumour should be undertaken. If IDRF are present discuss with your surgery team. If the tumour remains IDRF positive after chemotherapy this is not an absolute contraindication to surgery. Resection may still be recommended if evaluation of the primary tumour suggests that the risk to life, or of major functional loss, is less than the risk from leaving residual disease.
- **Metastatic Disease (Stage M):** If no *MYCN* amplification is present and there is metastatic disease in the bone, lung and/or CNS the patient needs to be treated as stage M disease. The treatment they should then receive depends on their age when these metastases develop.
  - Patients ≤12 months should follow the intermediate-risk protocol treatment recommendations.
  - Patients >12 months follow the high-risk protocol treatment recommendations.

7. DOSE MODIFICATIONS AND DELAYS

If chemotherapy is assigned to be given, two different regimens might be applicable: VP/Carbo or CADO. The chemotherapy regimens applicable in this protocol are described below.

It is very important to note that according to patient weight, the final drug dose might be calculated based on:

Body surface area (BSA) for patient weighing>10 kg or Weight for patients weighing ≤ 10 kg

For infants weighing below 5 kg, chemotherapy drug doses should be reduced by a further 33%.

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Chemotherapy courses should be given at the indicated intervals (see below) provided the absolute neutrophil count (ANC) is >1.0 x10^9/l and the platelet count (PLT) is >100 x 10^9/l. If the count has not recovered from the previous course of chemotherapy, treatment should be delayed for a week, and the count checked again.

VP/Carbo (Etoposide (VP16) and carboplatin)

Courses of VP/Carbo are given at 21-day intervals

DAY	1	2	3
Carboplatin	•	•	•
Etoposide	•	•	•

DRUG	Dose (mg/kg)	Dose (mg/m^2)	Administration
Carboplatin	6.6 mg/kg	200 mg/m^2	5% dextrose (5 ml/kg) over 1 hr daily x 3
Etoposide (VP16)	5.0 mg/kg	150 mg/m^2	0.9% saline (12.5 ml/kg) over 2 hrs daily x 3

Both these drugs are given on days 1-3 of each course.

It is advisable that a central line is inserted for chemotherapy.

In those patients where treatment is given via a peripheral line, great care should be taken to avoid extravasation.

Additional hydration is not required in the absence of vomiting, as long as oral intake is satisfactory. If this is not the case, then intravenous fluids at "maintenance" rates (depending on the age and weight of the child) should be given for the duration of chemotherapy.

CADO (Cyclophosphamide, doxorubicin and vincristine)

Courses of CADO are given at 21-day intervals

DAY	1	2	3	4	5
Cyclophosphamide	•	•	•	•	•
Doxorubicin				•	•
Vincristine	•				•

DRUG	Dose (mg/kg)	Dose (mg/m^2)	Administration
Cyclophosphamide	10 mg/kg	300 mg/m^2	5% dextrose (5 ml/kg) over 1 hr, daily x 5 days
Doxorubicin	1 mg/kg	30 mg/m^2	0.9% saline over 6 hours on days 4 and 5
Vincristine	0.05 mg/kg	1.5 mg/m^2 (max 2mg)	Bolus injection on days 1 and 5

A central line is essential and mandatory for the administration of doxorubicin over 6 hours. Hydration at the level of x2 maintenance or 3 liter/m^2 is required.

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Dose modifications:

If significant infective problems occur (CTCAE Grade 4), consider reducing the doses of myelosuppressive therapy by 20% for subsequent courses.

If an allergic reaction occurs during the administration of Etoposide, appropriate measures should be taken. However, the drug should be tried again with the next course at a slower rate and with steroid premedication.

In the case of marked ptosis or other neurological deficit (other than loss of tendon reflexes), consider reducing or omitting the next vincristine dose. In the case of CADO chemotherapy, the second vincristine of CADO should be postponed by one week.

If there is CTCAE Grade 2 renal toxicity see section 5.3.2, repeat GFR and modify the dose of Carboplatin.

8. SUPPORTIVE TREATMENT

Treatment according to this protocol should be restricted to institutions with experience in supporting patients with multisystem problems and who are familiar with the administration of combination chemotherapy.

A full range of supportive care and multidisciplinary approach should be available.

> The supportive care details that appear below are guidelines only. In cases where local institutional guidelines differ to these, the local guidelines maybe used; this is at the discretion of the local paediatric oncologists.

Life-threatening symptoms

In case of presence of LTS, it is recommended to initiate chemotherapy with Vp/Carbo as soon as possible as well as the necessary treatment support to stabilize the patient (e.g. diuretics, respiratory support in Ms (according to SIOPEN, without lytic bone lesions/CNS/lung or pleura, see Appendix A1.1) patients with massive hepatomegaly).

Anti-Emetics

Antiemetic therapy should be administered according to institutional guidelines, e.g. Ondansetron 5 mg/m² or 0.15 mg/kg (maximum single dose 8 mg) p.o./i.v. every 8 hours.

Hydration

Sufficient hydration (2 to 3 l/m² or twice maintenance) with appropriate electrolyte supplementation may be provided p.o. or i.v. in infants specially. Monitoring of blood pressure, cardiac and respiratory rates, body weight, and diuresis are mandatory, especially for the CADO treatment.

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Blood component therapy

Due to the risk of graft versus host reactions in newborn/ infants and in patients on chemotherapy all blood products (except fresh frozen plasma) should be irradiated with at least 15 Gy prior to transfusion, according to national policies. The use of leukocyte filters for leukocyte depletion (CMV negativity) is advised.

Red blood cells

As per institutional guidelines.

Platelets

As per institutional guidelines.

Central lines

The use of central lines is not mandatory for all patients. It is strongly recommended for the administration of the CADO chemotherapy.

Treatment guidelines for febrile neutropenia

All participating institutions must be familiar with managing of febrile neutropenia according to the accepted general principles of supportive care.

During episodes of fever and neutropenia patients need to be admitted to hospital for adequate diagnostic measures and appropriate treatment.

- If there is fever (>38^{O}C) and the neutrophil count is less than 1.0 X 109/L, then the centre´s usual combination of broad-spectrum antibiotics should be commenced following clinical and laboratory evaluation.
- If fever persists (>38^{O}C) for 72-96 hours despite broad-spectrum antibiotics, in the absence of defined source and site of infection, then antifungal therapy should be started, regardless of the clinical condition of the patient. Antifungal treatment can follow each treating Institution´s policy. Liposomal amphotericin can be an effective broad-spectrum treatment. Then, appropriate renal function monitoring and potassium supplementation should be instituted with its use. Itraconazole and voriconazole should not be considered since it should not be combined with vincristine. If no identified cause yet, complete clinical and laboratory investigation should be repeated. That could include a chest CT, an abdominal ultrasound and a cardiac ultrasound.

According to Local Institutional policy, antibiotic and antifungal treatment can be modified.

Pneumocystis pneumonitis prophylaxis

For patients receiving chemotherapy Pneumocystis carinii pneumonitis (PCP) prophylaxis is advisable, but may be given according to the recommendations of each national group. PCP prophylaxis is usually considered mandatory for patients receiving VP16/CARBO and CADO chemotherapy.

Patients should be considered for prophylactic sulfamethoxazole/trimethoprim (5mg TMP/kg/day divided in two equal doses and given orally 3 days a week). For sulpha-intolerant patients it is recommended that inhaled pentamidine or a preparation of trimethoprim only is used as prophylaxis. PCP prophylaxis

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using a pentamidine nebulizer at three-weekly intervals can be encouraged for children who are able to co-operate with jet inhalation (necessary to be effective) which is usually only the case for children of school age. Intravenous pentamidine can also be used.

Nutrition

Appropriate nutrition and adequate calorie intake should be aimed for all patients. This is especially important for young patients with significant hepatomegaly and stage IVs disease, as well as patients receiving VP16/ CARBO or CADO chemotherapy. The early start of supplementary nutrition is highly recommended.

Once a 10% weight loss occurs, the institution of nasogastric alimentation with a high caloric nutritional formula or parenteral nutrition via a central venous line if enteral feeds are not tolerated, according to local policies is recommended.

Renal function monitoring

Glomerular function – GFR

Serum creatinine should be monitored prior to each chemotherapy course. Glomerular function is to be assessed according to national/ group guidelines, applying either timed urine collection, isotope clearance, or the calculated creatinine clearance.

We recommend to use Schwartz's formula 1976 in children less than 2 years old, where creatinine clearance (Ccrea) can be calculated from single serum samples:

$$\text{Ccrea} = \frac{\text{F x height [cm]}}{\text{Crea serum [mg/dl]}} \quad \text{ml/min/1.73m}^2$$

where **F** is proportional to body muscle mass, hence depending on age and gender:

- LBW infants 0-12 months **F** = 0.33
- AGA infants 1-12 months **F** = 0.45
- Children (boys and girls) **F** = 0.55
- Adolescent girls **F** = 0.55
- Adolescent boys **F** = 0.70

In children older tan 2 years old it is preferred the use of Schwartz-IDMS, 2009 where creatinine clearance (Ccrea) can be calculated from single serum samples:

$$\text{Ccrea} = \frac{0{,}413 \times \text{height [cm]}}{\text{Crea serum [mg/dl]}} \quad \text{mL/min/1,73m}$$

To estimate the GFR based on the serum cystatin we recommend to use:

Schwartz, 2012 $70{,}69 \times CisC^{-0{,}931}$ mL/min/1,73 m^2

Active immunization

Vaccinations are not allowed during immunosuppressive treatment except for the annual flu vaccination which is recommended for those children older than 6 months in selected group of patients (follow each country/institution recommendations on annual flu vaccination campaign).

The active immunization will be resumed after the end of treatment in all patients following general instructions in the Pediatric Oncology field:

- Inactivated vaccines: between 3 and 6 months after the end of chemotherapy.
- Live vaccines: from 6 months after the end of treatment.
- If the treatment includes anti-B antibodies (e.g. rituximab): wait 6 months for any vaccine
- If the primary vaccination was complete prior to chemotherapy: administer a booster dose of all the vaccines on the schedule, starting at 3-6 months depending on the type of vaccine.
- If the primary vaccination was incomplete: there are two ways to act.
    - Complete revaccination, according to the child's age, from 3-6 months according to the type of vaccine.
    - Consider valid the doses administered before the disease and complete the vaccination schedule according to the guidelines for incomplete calendars.

Concomitant Medication

Concomitant use of azoles with vincristine is not recommended due to their interaction, rising the neuropathy potential toxicity of the vincristine.
https://reference.medscape.com/drug-interactionchecker

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APPENDIX 1 - TUMOUR STAGING

THE INTERNATIONAL NEUROBLASTOMA RISK GROUP (INRG) STAGING SYSTEM

A1.1 Modified INRGSS as used in this guideline

Stage	Description
L1	Localised tumour not involving vital structures as defined by the list of image-defined risk factors
L2	Locoregional tumour with presence of one or more image defined risk factors
M	Distant metastatic disease (except stage Ms)
Ms*	Metastatic disease in children younger than 12 months with metastases confined to skin, liver, and/or bone marrow

* Ms: metastatic disease confined to skin and/or liver and/or bone marrow (or even other sites such as lymph nodes and/or testes), but NOT bone, lung, pleura or CNS, in infants ≤12 months. MIBG or technetium scintigraphy uptake to the skeleton can occur but without bone lesions documented by X-ray and/or CT scan.

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A1.2 Original INRGSS

Stage	Description
L1	Localised tumour not involving vital structures as defined by the list of image-defined risk factors and confined to one body compartment
L2	Locoregional tumour with presence of one or more image defined risk factors
M	Distant metastatic disease (except stage Ms)
Ms	Metastatic disease in children younger than 18 months with metastases confined to skin, liver, and/or bone marrow

NOTE: see original paper for detailed criteria [10]. Patients with multifocal primary tumours should be staged according to the greatest extent of disease as defined in the table.

APPENDIX 2: IMAGE DEFINED RISK FACTORS (IDRF)

The Image Defined Risk Factors (IDRF) have been designed to guide surgical management of neuroblastoma at diagnosis, in particular to indicate whether biopsy or attempted resection is recommended as the first surgical procedure [10, 21].

The same system of risk factors can be applied later during the course of treatment and follow-up, although it is not designed for this purpose. If a tumour remains IDRF positive after chemotherapy in specific clinical situations as in sections 3.1.4.3 and 3.1.5.3 this may not be an absolute contraindication to surgery. Resection may still be recommended if evaluation of the primary tumour suggests that the risk to life, or of major functional loss from surgery, is less than the risk from leaving residual disease. In these situations, discuss with your surgery team.

A2.1 List of Image Defined Risk Factors

Ipsilateral tumour extension within two body compartments: Neck-chest, chest-abdomen, abdomen, pelvis.

- **Neck**
  - Tumour encasing carotid and/or vertebral artery and/or internal jugular vein
  - Tumour extending to base of skull
  - Tumour compressing the trachea
- **Cervico-thoracic junction**
  - Tumour encasing brachial plexus roots
  - Tumour encasing subclavian vessels and/or vertebral and /or carotid artery
  - Tumour compressing the trachea

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- **Thorax**
  - Tumour encasing the aorta and/or major branches
  - Tumour compressing the trachea and/or principal bronchi
  - Lower mediastinal tumour, infiltrating the costo-vertebral junction between T9 and T12
- **Thoraco-abdominal**
  - Tumour encasing the aorta and /or vena cava
- **Abdomen/pelvis**
  - Tumour infiltrating the porta hepatis and/or the hepatoduodenal ligament
  - Tumour encasing branches of the superior mesenteric artery at the mesenteric root
  - Tumour encasing the origin of the celiac axis, and/or of the superior mesenteric artery
  - Tumour invading one or both renal pedicles
  - Tumour encasing the aorta and/or vena cava
  - Tumour encasing the iliac vessels
  - Pelvic tumour crossing the sciatic notch
- **Intraspinal tumour extension whatever the location provided that:**
  - More than 1/3 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, duodenopancreatic block and mesentery.

Conditions recommended to be highlighted in the clinical history, but NOT considered IDRFs:

- Multifocal primary tumours
- Pleural effusion (with or without malignant cells)
- Ascites (with or without malignant cells)

A2.2 Relationship between the primary tumour and neighbouring vital structures

It is recommended that these relationships between the primary tumour and neighbouring vital structures should be systematically discussed in a multidisciplinary team including (at least) radiologists, nuclear physicians, surgeons and paediatric oncologists.

To ensure uniform staging the following terms should be used by radiologists for description of the relationship between a tumour and the neighbouring vital structures, i.e. structures that cannot be sacrificed without impairment of normal function such as major arteries, veins, and lymphatic vessels or major nerves. Other vital structures are the heart, diaphragm, lungs and airways, liver and biliary system, spleen, pancreas, kidney and the urinary tract, bowel and mesentery.

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A2.2.1 "Separation"

"Separation" means that a visible layer, usually fat, is present between the tumour and the neighbouring structure. When a tumour is separated from a vital structure, according to previous definitions, an IDRF is not present.

A2.2.2 "Contact" and related terms

"In contact" means that no visible layer is present between the tumour and the neighbouring structure. For vessels, a "contact" means that less than 50% of its circumference is in contact with the tumour (more than 50% being named "encasement"). The term "compression" is additionally used for veins with reduced diameter but still partially visible lumen. The term "compression" is also used for spinal cord and airways when a tumour is in contact and causes the short axis of the structure to be reduced. For other vital structures (neighbouring organs), a "contact" may be associated with "displacement" (abnormal anatomic location) or "distortion" (abnormal anatomic shape) of the structure, but neither with "total encasement" nor "infiltration". When a tumour is "in contact" with a vital structure, according to previous definitions, an IDRF is not present. However, "compression" of the trachea or the spinal cord is considered as an IDRF.

A2.2.3 "Encasement" and related terms

"Encasement" means that a neighbouring structure is surrounded by the tumour. A "total encasement" means that a vital structure (organ, vessel) is completely surrounded by the tumour.
"Encasement" of a vessel means that more than 50% of its circumference is in contact with the tumour. The additional term "stretched" means that a partially or totally encased or displaced vessel is elongated by an expanding tumour, i.e. an artery having a reduced diameter or a compressed vein with no visible lumen. When a tumour is encasing a vital structure, according to previous definitions, an IDRF is present.

A2.2.4 "Infiltration"

"Infiltration" means involvement of vital structures other than vessels (infiltration of the vessel wall can currently not be demonstrated by imaging): an infiltrating tumour has extension into a neighbouring organ causing the margins between the tumour and the infiltrated structure to be lacking or ill-defined. When a tumour infiltrates a vital structure, according to previous definitions, an IDRF is present.

A2.2.5 Compartments, uni- and multifocality

The majority of tumours occur in a single anatomic compartment. However, some tumours may extend into an adjacent compartment and increase the risk of injury of vital structures during surgery; these tumours should be classified L2 according to the INRGSS. This usually occurs with cervico-thoracic locations arising from the stellate sympathetic ganglion, or with lower mediastinal locations extending into the retroperitoneum along the aorta, or in the pelvic presacral locations extending upward in the abdomen around the aorta and/or IVC.

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An upper abdominal tumour with enlarged lower mediastinal nodes or a pelvic tumour with inguinal lymph node involvement is considered locoregional disease. However, nonregional (distant) lymph node involvement, such as retroperitoneal primary with supraclavicular lymph node, is metastatic disease (stage M).

Multifocal primary neuroblastoma is a rare situation. These tumours should be staged according to the greatest extent of disease. This special feature should be recorded but will not be considered, by itself, as an IDRF.

A2.2.6 Effusions

Isolated pleural effusion and ascites, even with malignant cells, are not considered IDRFs in the INRGSS. Although pleural disease is associated with reduced survival rates in metastatic patients, isolated pleural effusion or ascites is rare in patients with locoregional disease, and its impact on outcome is still unclear.

A2.3 IDRF assessment according to anatomic location

A2.3.1 Neck

Cervical NBs arise from the superior cervical sympathetic ganglion located behind the internal carotid artery or, more rarely, from the stellate ganglion. These tumours usually extend anteriorly and laterally displacing the carotid artery and internal jugular vein, medially compressing the airways and upward to the skull base along the carotid artery.

According to the INRGSS, surgical risk factors should be considered when the tumour encases the carotid and/or vertebral artery and/or internal jugular vein, extends to the base of skull, and/or compresses the trachea.

A rarer NB involving the cervico-thoracic junction and arising from the stellate ganglion may encase the brachial plexus roots and/or subclavian vessels and/or vertebral artery. Intraspinal extension may be also observed. All these conditions are considered IDRFs.

A2.3.2 Thorax

Thoracic neuroblastomas mostly arise from the paraspinal sympathetic trunks in the posterior mediastinum. Foraminal and intraspinal extensions (dumbbell tumours) are typically observed at this anatomical level. Spinal cord compression may be diagnosed on either neurological signs and/or on the basis of imaging findings. According to the INRGSS, an IDRF is present when 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 MRI signal is abnormal, i.e. imaging features usually observed in symptomatic patients.

A lower mediastinal tumour, infiltrating the costo-vertebral junction between T9 and T12 is still considered as an IDRF as there is a theoretical risk of medullar ischaemia by Adamkiewicz anterior spinal artery injury [53]. This artery originates in 85% of the cases between T9 and T12 on the left side, whereas in 15% of the cases it originates on the right side and is usually associated with a supplementary circulatory circuit. Tumour growth within this area induces the formation of collaterals.

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Therefore, arteriography of the medullary artery is not mandatory, since the preoperative risk is minimal, although it should be mentioned to the parents. On the other hand, an invasive procedure such as arteriography in very young patients is risky.
Tumours encasing the aorta and/or major branches and tumours compressing the trachea and/or principal bronchi usually cause major surgical difficulties. Therefore, these conditions are considered IDRFs.

A2.3.3 Abdomen

Abdominal neuroblastomas arise from either the adrenal gland and/or from sympathetic ganglions (celiac, superior and inferior mesenteric ganglions) and/or sympathetic fibres and plexuses located along the aorta and its main branches.
Relationships between the primary tumour and abdominal vessels usually represent the main limitation for surgery. Therefore, accurate analysis of all arteries and veins is a major issue in imaging reports. Main abdominal vessels (aorta, celiac axis, superior and inferior mesenteric arteries, renal arteries and veins, inferior vena cava, iliac arteries and veins, porta hepatis) should be clearly assessed and classified according to the previously defined terms. According to the INRGSS, tumours encasing the superior mesenteric artery, the origin of the celiac axis, one or both renal pedicles, the aorta, the inferior vena cava and/or the iliac vessels are considered IDRFs. Adjacent organs and structures should also be precisely assessed and classified according to the previously defined terms.
According to the INRGSS, the following are considered IDRFs: tumours infiltrating the porta hepatis or the hepatoduodenal ligament, diaphragm, kidney, liver, duodeno-pancreatic block, and/or the mesentery.
Paraspinal lumbar neuroblastomas rarely may be associated with foraminal and intraspinal extensions (dumbbell tumours) sharing the same pattern and neurological issues as mediastinal primaries.

A2.3.4 Pelvis

Pelvic neuroblastomas mainly arise from either the upper hypogastric sympathetic plexus and/or presacral sympathetic ganglions.
Pelvic neuroblastomas are frequently associated with foraminal/intraspinal extensions and associated with sacral and/or inferior lumbar radicular neurological signs, but not with spinal cord involvement. According to the INRGSS, an IDRF is present when more than one third of the spinal canal in the axial plane is invaded. However, intraspinal tumour extension below the level of L2 does not lead to spinal cord involvement but only to radicular involvement. This condition is usually not a contraindication for primary surgery of the extraspinal component and usually does not lead to emergency neurosurgery.
Relationships between mass and major pelvic vessels (aorta, inferior vena cava, primitive, internal and external iliac arteries and veins) should be clearly assessed and classified according to the previously defined terms. According to the INRGSS, tumour encasement of the aorta and/or inferior vena cava and/or iliac vessels is associated with IDRF.

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The involvement of the sciatic notch is considered an IDRF. The "sciatic notch" in the IDRF definition is a sagittal-oblique plane at the anatomic level of the greater sciatic notch, joining the spine of the ischium and the lateral border of the sacrum.

APPENDIX 3: SPINAL CORD COMPRESSION (SCC)

A3.1 Spinal cord involvement

The connections existing between the sympathetic nervous system and the spinal cord account for the ability of neuroblastoma to infiltrate the intervertebral foramina with occasional involvement of the spinal canal. Despite the fact that tumour growth almost invariably remains extradural, it may still cause spinal cord involvement which can progress to irreversible paraplegia. Prolonged compression of the spine leads to irreversible loss of motor neuron [54,55]. Early diagnosis and prompt treatment of spinal cord involvement in neuroblastoma is therefore of critical importance to limit permanent neurologic impairment. On the basis of MRI, 10-15% of children with neuroblastoma have documented foramina or intraspinal involvement, although only half of them present with neurological signs of spinal cord involvement, and very few develop paraplegia [56,57].

A3.2 Diagnosis and Evaluation of Spinal Cord Involvement

Dumbbell neuroblastoma tends to present at a younger age, is commonly associated with intrathoracic disease and is significantly more frequent amongst children with localized, rather than metastatic disease [58].

Early detection of spinal cord involvement can be difficult especially in younger children. The most common symptoms are back pain, reduced mobility of the legs and/or arms, sensory and sphincter dysfunction. The presence of a motor deficit is particularly important since children who develop complete motor loss usually experience little or no recovery. Infants with congenital dumbbell tumours have a particularly poor outcome with regards to neurological recovery.

The extent of motor loss can be graded as follows;

1 Capable of unassisted ambulation may have pain and/or difficulty with micturition.
2 Only can walk with assistance.
3 Antigravity strength alone.
4 Presence of trace movement alone.
5 Complete motor and sensory loss.

NOTE: most episodes of spinal cord involvement in neuroblastoma occur in infants, for whom this scale is only partially applicable.

MRI is the best method to detect infiltration of the intervertebral foramina and invasion of the spinal canal by neuroblastoma. Although a CT scan may be adequate in some cases, it is recommended that MRI be used wherever possible, particularly in the diagnostic work-up of infants with cervical, thoracic, or pelvic disease.

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Intraspinal tumour extension should be considered as IDRF provided that more than 1/3 of the spinal canal in the axial plane is invaded, and/or the perimedullary leptomeningeal spaces are not visible, and/or the spinal canal is abnormal (See Appendix 2).

A3.3 Treatment of spinal cord involvement

Treatment of symptomatic spinal cord involvement has evolved over the last few years but the optimal management of neuroblastoma-induced spinal cord compression is, however, still controversial [59]. While neurosurgery can achieve immediate decompression, it can also lead to collateral tissue alteration [60,61]. It requires very experienced surgeons and may carry the risk of late spinal deformities.
Chemotherapy is another alternative to laminectomy that reduces the intraspinal tumor volume within a few days without compromising the chance of neurological recovery [59-61] and it has been gaining prominence to the point of being the preferred initial intervention for patients with spinal cord compression.
Neurologic recovery has been correlated with the severity of the presenting neurologic deficits and the recovery for patients treated with chemotherapy or laminectomy similar. However, orthopaedic sequelae may be more frequent in children who were managed with laminectomy [57,60,61].

Other strategy used less and less is radiotherapy due to its potential disadvantages such as the reduction and alteration of the growth of vertebrae in the radiotherapy field and the increases of the risk of late secondary malignancy.

The main residual sequelae, although prompt intervention, are motor deficits, bladder or bowel disfunction or scoliosis [56].

The European experience within INES 99.1 recorded 40 cases of dumbbell tumours of which 25 had life threatening symptoms. Out of the 116 infant unresectable neuroblastomas 11 (27%) underwent a primary neurosurgical approach (unpublished data).

- Spinal cord involvement without symptoms
  - The regular use of MRI has increased the number of cases with documented infiltration of foramina (with or without invasion of the spinal canal). However, in the majority of cases, especially when the intraspinal component is modest (less than 33% of the diameter), there are no neurological symptoms.
  - There is very little, if any, evidence that an asymptomatic intraspinal tumour will grow any more after a resection of an extraspinal component. Information related to the few, well documented cases suggests that the intraspinal neuroblastoma in patients with no neurological symptoms tends to remain stable or even regress without specific treatment.
  - **If the spinal cord component occupies greater than estimated one third of the cross section surface area of the spinal canal, and/or the leptomeningeal spaces are not visible and/or the spinal cord signal is abnormal the recommendation of this**

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guideline is to treat these patients with chemotherapy even in the absence of signs or symptoms of spinal cord involvement.

- Spinal cord involvement **with** neurologic signs
  - Patients with localised neuroblastoma who present with signs of spinal cord involvement require urgent specific treatment. If neurological deficits are present and if there is clinical progression, rapid therapeutic decisions must be made in a matter of hours or at most within 1-2 days. The decision regarding whether to administer emergency chemotherapy to these infants with a neurological deficit should be taken after urgent discussions between the oncologist and the neurosurgeon. Laminectomy or laminotomy is preferable only in infants showing a very rapid neurologic deterioration. This, however, occurs infrequently.
  - **Once it has been decided that urgent chemotherapy is needed it should never be delayed** in order to obtain a pre-chemotherapy biopsy sample. There is no urgent indication to remove the extraspinal tumour (which is likely to be unresectable, and surgery runs the risk of worsening the neurological deficit). The tumour should be biopsied (by Tru-cut, fine needle, or open biopsy), when the patient is stable within 7 days of starting chemotherapy. Initial chemotherapy in this situation should never be postponed in order to try and obtain a biopsy.

Medical treatment of Spinal Cord Involvement is as follows:

- Dexamethasone 0.5 mg/kg I.V. bolus followed by 0.2 mg/kg/day I.V. in 3 divided daily doses. Chemotherapy is given using VP/Carbo. A second course should be given 21 days after the beginning of the first course. (See section 7 for further details about chemotherapy doses and administration).
- A further MRI scan should be obtained following the first course of chemotherapy. If either no improvement occurs or if deterioration of the neurological signs is observed, and the intraspinal component has shown no response to treatment (i.e. has not shrunk in size), laminotomy and an excision of the intraspinal component should be considered.
- If the symptoms persist and the tumour remains unresectable on reassessment with MRI after 2 courses of VP/Carbo, then alternative chemotherapy should be given according to the protocol, CADO.

- Some patients can have persistent neurological signs and symptoms from neurological damage caused at initial presentation of the SCC. If these neurological signs are stable over 2 courses of chemotherapy and the reassessment imaging does NOT show progressive disease it is generally not appropriate to continue on with extra courses of chemotherapy.

A3.4 Asymptomatic intraspinal residual tumour following chemotherapy

It is not necessary to remove any residual asymptomatic intraspinal tumour following chemotherapy.

All patients with symptomatic and asymptomatic intraspinal neuroblastoma should be registered in the international NB-SCI registry.

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APPENDIX 4: OPSOCLONUS MIOCLONUS SYNDROME (OMS)

Opsoclonus-myoclonus syndrome (Opsoclonus-myoclonus-ataxia syndrome, Dancing eye syndrome, Kinsbourne syndrome) is a rare syndrome characterized by three main symptoms, which are opsoclonus (rapid multi-directional conjugate eye movements), a movement disorder characterised by a jerky – sometimes frankly myoclonic - ataxia, accompanied by behavioural change consisting of irritability usually with sleep disturbance. These neurological signs may vary widely in their expression and are not necessarily all present together [62,63].

OMS is a rare paraneoplastic or possibly post-viral serious neurologic syndrome. In children, OMS is associated in more than half of cases with neuroblastoma [64,65]. Nevertheless, only in 2-3% of newly diagnosed NB patients OMS is observed [65].

The majority of children with OMS and neuroblastoma have favorable prognostic tumor characteristics and a high survival rate [66,67], the majority of children also experience developmental delays and long-term nervous system dysfunction such as language deficits and behavioral abnormalities [68-69].
The etiology of this condition is thought to be immune mediated, with antineuronal antibodies or immune cells against the tumour that cross-react with neural cells in the central nervous system, but the exact pathogenesis has not yet been elucidated. Furthermore, though immunosuppressive therapies may ameliorate the acute symptoms, no effective treatment to prevent the common neuropsychologic sequelae has been established.

All children with OMS should be evaluated for neuroblastoma. Most children identified with a neuroblastoma will need to undergo standard staging (See section 5) and surgical resection, followed by monitoring by the neuro-oncology team. Treatment of the neuroblastoma, while important in its own right, does not appear to alter the outcome of opsoclonus-myoclonus syndrome.

Symptoms precede the diagnosis of neuroblastoma in approximately one-half of patients. Symptoms usually fluctuate in severity and may have a prolonged course. A video illustrating opsoclonus is available at www.dancingeyes.org.uk

Prompt treatment is generally regarded as important. Therefore, treatment should start as soon as the diagnosis of OMS is established. In case of OMS with neuroblastoma, treatment start may, if it is felt to be clinically appropriate, be delayed until after resection of the tumour (but usually no more than 14 days from diagnosis).

Treatment start with the standard corticosteroid treatment with high-dose steroids such as dexamethasone pulses, and intravenous immunoglobulin (IGIV) as well as neuroblastoma risk-adapted chemotherapy (cyclophosphamide the majority of cases) [70]. The role of Rituximab still needs to be elucidated.

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See: Multinational European Trial for Children with the Opsoclonus Myoclonus Syndrome/ Dancing Eye Syndrome (NCT01868269)
https://clinicaltrials.gov/ct2/show/NCT01868269

APPENDIX 5: DIFFERENTIAL DIAGNOSIS OF ADRENAL NEONATAL MASSES (NAM)

- Adrenal haemorrhage (AH): it may present with physical and radiographic findings that are indistinguishable from other suprarenal masses. The incidence of adrenal haemorrhage is nearly 2 cases per 1000 live births; however, advances in prenatal ultrasonographic imaging may increase this figure. Because the majority of these haemorrhages spontaneously resolve it is important to clearly distinguish them from other suprarenal masses, in particular neuroblastoma, which can spontaneously resolve in the same time frame. When the adrenal haemorrhage is diagnosed in utero, the lesion is characterized by an echogenic mass, and it is more frequent on the right side. On follow-up, ultrasound of the mass appears increasingly hypo-echoic and usually involutes. Many lesions completely resolve, leaving only residual minor calcifications. However, the cyst may not regress and then other explorations might be necessary. If the diagnosis is not made antenatally, the infant rarely may present signs/symptoms of adrenal insufficiency. Most of the times, there will be hyperbilirubinemia, anaemia, scrotal hematoma. Boys are affected more frequently and the right side also more commonly. The aetiology is unknown, being related to birth trauma, foetal hypoxia, maternal hypotension and sepsis; but these factors might not be present at all. It has also been related to vascular factors (venous congestion, arterial insufficiency). Adrenal haemorrhage can be sequentially followed-up by ultrasound, without harming the baby [71].

- Enteric duplication cysts: they may present in the suprarenal location. However, they appear as early as the 16th week, allowing some means of discrimination from cystic neuroblastoma [72].

- Subdiaphragmatic extralobar pulmonary sequestration (SEPS): they are important to distinguish from neuroblastomas because they may not require surgery. These rare congenital lung abnormalities consisting of non-functioning pulmonary tissue that lack tracheobronchial communication and receive their blood supply from anomalous systemic arteries. They may be intra or extralobar. These latter ones occur between the diaphragm and the lower lobe of the lung. The majority of them are diagnosed before the age of 10 and frequently associated with congenital abnormalities such as diaphragmatic hernias. The typical sonographic appearance suggests a solid mass, which persists with stable size postnatally most of the times and occasionally regresses or grows.

- Adrenal cytomegaly [73]**:** This phenomenon is not well known yet. It is characterized by the presence of large polyhedral cells with eosinophilic granular cytoplasm and enlarged nuclei in the adrenal cortex. It is thought to be a degenerative process, but not a malignancy. It rarely forms cysts and can be detected during prenatal ultrasonographic examinations.

- Adrenocortical tumours: the incidence of adrenocortical tumours is approximately 3 per million and these tumours comprise less than 1% of all paediatric neoplasms. Most of them are functional and may be detected easily if there is sufficient clinical suspicion (hypertension, precocious puberty) though

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patients often present with a large abdominal mass. These tumours are more common in girls and might be associated with other syndromes like Beckwith-Wiedemann, hemihypertrophy. The family history may include adrenal carcinoma or other cancers (Li Fraumeni syndrome, Wilms, hepatocellular carcinoma) or not, since most likely they occur sporadically. The diagnosis of a functioning adrenocortical tumour can be confirmed by biochemical tests. Free cortisol levels in 24hour urine are the most common screening test.

Other rare diseases: congenital adrenal hyperplasia, can present with an increase in adrenal size and other signs/symptoms; pheochromocytoma and paraganglioma have been rarely described in neonates. Hydronephrosis, multicystic dysplasia of the upper pole of a duplex collecting system. Neonatal adrenal abscesses should also be taken into account in the differential diagnosis.

APPENDIX 6: RESPONSE EVALUATION

This study will use volume measurements for the tumour response assessment. Tumours do not necessarily grow or shrink in a rounded fashion and 3D evaluation is likely to be more accurate than uni or bidimensional measurement. The size of the tumour should be calculated using volume calculation. This may be obtained from the commonly used elliptical approximation (D1 x D2 x D3 x 0.52) or by common software algorithms which are implemented in most radiological workstations.

In this guideline the following definitions will be used for response evaluation criteria, based on:

Complete Response (CR):	Complete disappearance of all measurable disease.
Stable Disease (SD):	No tumour shrinkage to demonstrate any objective reduction in tumour volume and not a sufficient increase in tumour volume to qualify for PD.
Progressive Disease (PD):	An increase in tumour volume of >40% or the appearance of a new site of disease. If 3D measurements are not obtainable then PD is defined as an increase of 25% in the product of 2 dimensions.

The definition of partial response (PR) is not used in this protocol for determining treatment. However, PR will be used to assess the disease status of patients for data collection. Partial response is defined as any shrinkage of the primary tumour and/or a decrease in size or number of metastatic sites.

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