



# HIGH-RISK NEUROBLASTOMA STANDARD CLINICAL PRACTICE RECOMMENDATIONS

## **DISCLAIMER:**

These ESCP guidance documents were produced by the relevant tumour group or specialist committee as recommendations on current best practice. The ESCP guidance documents are not clinical trial protocols.

The interpretation and responsibility of the use of ESCP guidance documents lies fully with the user who retains full responsibility for the use of these guidance documents and his actions and (treatment) decisions based thereon, such as, but without limitation thereto: checking and prescribing certain doses, checking prescriptions, etc. A user should never base its decision solely on the content of these guidance documents and should always check any other relevant medical information that is available and make appropriate use of all relevant medical information.

These guidance documents have been made publicly available by SIOP Europe – the European Society of Paediatric Oncology and the European Reference Network for Paediatric Oncology (ERN PaedCan). It is the responsibility of the user who downloads these documents to make sure that:

- their use within the Paediatric Clinical Unit / Hospital is approved according to the local clinical governance procedures.
- appropriate document control measures are in place to ensure that the most up to date locally approved versions are considered.
- any anomalies or discrepancies within the documents are immediately brought to the attention of the relevant special interest group chair and the European Clinical Study Group who has developed the ESCP guidance document.

Every care has been taken whilst preparing these documents to ensure that they are free of errors. Nonetheless, SIOP Europe and ERN PaedCan cannot be held liable for possible errors or mistakes in these guidance documents, nor can SIOP Europe and ERN PaedCan be held liable for any kind of damage resulting out of the use of these guidance documents.

## **INTRODUCTORY PAGES**

- High-Risk Neuroblastoma Study
- HR-NBL study
- Version 1.0, 3rd of February 2020

**This document has been developed by:**

- **Claudia Pasqualini**
- **Dominique Valteau-Couanet**
- **Ruth Ladenstein**

**Planned review date**

---

**Table of contents**

Table of Contents

<b>1. BACKGROUND AND RATIONALE</b> .....	<b>3</b>
1.2 Induction chemotherapy .....	3
1.3 High-dose chemotherapy .....	5
1.4 Surgery .....	6
1.5 Local radiation therapy .....	6
1.6 Maintenance treatment .....	7
<b>2. PATIENT GROUP</b> .....	<b>8</b>
2.1 Recommended criteria to enter the protocol .....	8
2.2 Recommended criteria to enter consolidation phase .....	9
2.3 Recommended criteria to perform local radiotherapy .....	10
2.4 Recommended criteria to enter maintenance treatment.....	10
<b>3. DIAGNOSTIC CRITERIA</b> .....	<b>10</b>
3.1 Imaging.....	10
3.2 Histopathology .....	10
3.2.1 General Remarks .....	10
3.2.2 Sectioning and securing tumour material in case of resected tumours.....	11
3.2.3 Sectioning and securing tumour material in case of surgical biopsies .....	12
3.2.4 Securing tumour material in case of core needle (tru cut) biopsies .....	12
3.2.5 Securing tumour material in case of fine needle aspirations (FNA) .....	13
3.2.6 Histology report .....	13
<b>4. BIOLOGICAL ANALYSIS</b> .....	<b>15</b>
<b>5.TREATMENT DETAILS</b> .....	<b>15</b>
5.1 Treatment overview .....	15
5.2 Induction chemotherapy .....	16
5.2.1 RAPID COJEC chemotherapy induction .....	16
5.2.2 TVD cycles .....	20
5.3 Peripheral blood stem cells harvest.....	21
5.4 Consolidation therapy regimen and ASCR .....	22
5.4.1 Busulfan .....	22
5.4.2 Melphalan.....	23
5.4.3 Supportive care during Busulfan-Melphalan .....	24
5.5 Autologous Stem Cell Rescue (ASCR) .....	24
5.6 Surgery .....	25
5.6.1 Definition of Procedures .....	26
5.6.2 Definition of Major Surgical Complications .....	26
5.6.3 Aspects of Surgical Procedures.....	27
5.7 Radiotherapy .....	28
5.7.1 Timing of radiotherapy .....	28
5.7.2 Planning and Dose of Radiotherapy .....	29
5.8 Maintenance phase.....	31
5.8.1 Treatment schedule.....	32
<b>6. ASSESSMENTS</b> .....	<b>33</b>
6.1 Disease assessment at diagnosis and during the treatment.....	33
6.2 Toxicity assessment during treatment .....	34

---

6.2.1 Overview of toxicity evaluation for RAPID COJEC induction before each course .....	34
6.2.2 Overview of toxicity evaluation during consolidation phase .....	35
6.2.3 Overview of toxicity evaluation during maintenance phase .....	35
<b>7. SUMMARY OF KNOWN ADVERSE EVENTS ASSOCIATED WITH DINUTUXIMAB BETA....</b>	<b>36</b>
<b>8. DOSE MODIFICATIONS AND DELAYS.....</b>	<b>39</b>
8.1 Induction.....	39
8.2 Busulfan-melphalan .....	40
8.3 Maintenance treatment .....	40
<b>9. PATIENT FOLLOW UP .....</b>	<b>42</b>
9.1 Timing of patient’s evaluation.....	42
9.2 Long term follow-up assessment .....	43
<b>10. REFERENCE LIST.....</b>	<b>45</b>
<b>APPENDIX 1 : TUMOUR STAGING .....</b>	<b>50</b>
<b>APPENDIX 2: CRITERIA FOR ADVERSE EVENTS.....</b>	<b>51</b>
<b>APPENDIX 3: HEARING.....</b>	<b>52</b>
<b>APPENDIX 4: PERFORMANCE SCALE .....</b>	<b>54</b>
<b>APPENDIX 5: INTERNATIONAL NEUROBLASTOMA RESPONSE CRITERIA .....</b>	<b>55</b>
<b>APPENDIX 6: BONE MARROW SAMPLING GUIDELINES .....</b>	<b>56</b>
<b>APPENDIX 7: NUCLEAR MEDICINE GUIDELINES.....</b>	<b>57</b>
<b>APPENDIX 8: HARVEST GUIDELINES.....</b>	<b>61</b>
<b>APPENDIX 9: DRUG INFORMATION .....</b>	<b>64</b>

---

## 1. BACKGROUND AND RATIONALE

Prognosis of patients with high-risk neuroblastoma (HR-NBL) remains poor despite multimodal treatment including induction chemotherapy, local treatment (surgery and radiotherapy), high-dose chemotherapy (HDC) followed by autologous stem cell rescue (ASCR) and maintenance treatment.

### 1.2 Induction chemotherapy

Induction chemotherapy is one of the mainstay aspects of multimodal treatment of HR-NBL. Over the last four decades different chemotherapy regimens have been evaluated in this setting by academic cooperative groups with increasing intensity and different combinations of conventional chemotherapeutics.

Until the 1990's, a number of different induction regimens were used by the various European national neuroblastoma groups, with no regimen showing clear superiority. The first randomized study conducted by the European Neuroblastoma Study Group (ENSG), between 1990 and 1999 (ENSG5) investigated the effect of dose intensity of induction therapy on EFS in patients over the age of 1 year with metastatic disease. Patients (n=262) were randomized to receive either COJEC (rapid) or OPEC/OJEC (standard) induction regimens.[Pearson A, Lancet Oncol 2008] Each regimen utilized the same drugs - cisplatin, carboplatin, etoposide, cyclophosphamide and vincristine - at the same dose, but the dose intensity (in mg/m<sup>2</sup> per week) of COJEC was 1.8 fold higher. Therapy in the COJEC arm was administered every 10 days, regardless of haematological recovery, whilst it was delivered every 21 days in the OPEC/OJEC arm, dependent on haematological recovery. In those patients who were responding after induction therapy and had achieved a bone marrow complete response (two aspirates and two biopsies), attempted surgical excision of the primary tumour was undertaken, followed by HDC with single agent Melphalan (180 mg/m<sup>2</sup>) and ASCR, and (from 1999) six months of 13-cis-RA. Complete (CR) and very good partial (VGPR) responses were achieved in 53% patients assigned to standard treatment and in 74% patients assigned to COJEC treatment (p=0.002); 10-year EFS was 18% for patients receiving standard and 27% for patients receiving COJEC (p=0.085). The intensified regimen was therefore adopted as the 'standard' induction regimen for the SIOOPEN/HR-NBL1 trial, and was administered to all patients recruited to the trial between 2002 and 2013.

Within the HR-NBL1 trial the addition of granulocyte colony stimulating factor (G-CSF) to COJEC induction was randomized (R0), showing a significantly reduced toxicity profile when G-CSF was used. In this randomized patient cohort, response in the bone marrow compartment was achieved in 70% of patients, response in the skeletal compartment (mIBG positive patients) in 75-80% of cases and tumour response ≥ partial response (PR) in 71-72% of patients with high risk neuroblastoma [Ladenstein R, J Clin Oncol 2010].

Approximately one third of children with high-risk neuroblastoma do not respond to first-line therapy. Topotecan is a specific topoisomerase-I inhibitor that has demonstrated good activity against

neuroblastoma *in vitro* and *in vivo*, both alone and in combination with other anti-tumour compounds. In a Phase II study carried out by the Italian Neuroblastoma Group, topotecan was given for 5 consecutive days prior to vincristine and doxorubicin administered simultaneously in a 48-hour continuous infusion. Of 25 patients so treated, 14 achieved PR after 2 cycles with a 56% major response rate. Toxicity was mainly myelosuppression with no toxic deaths recorded.

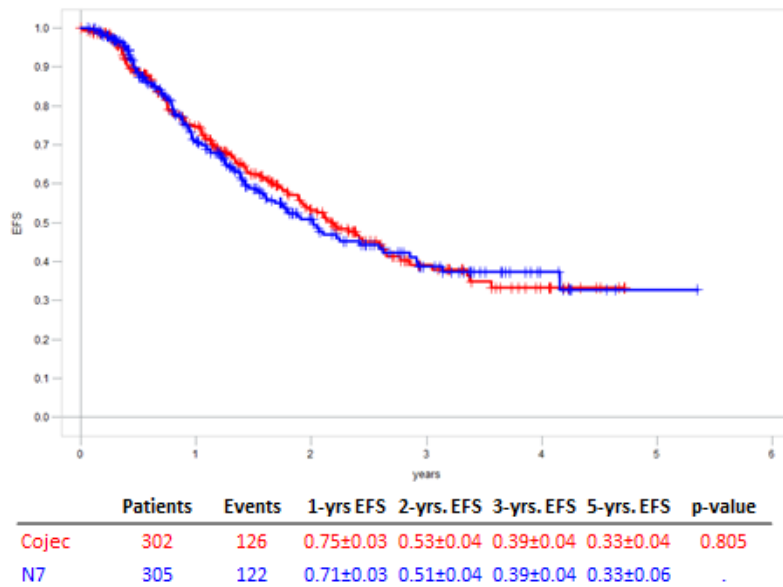
Based on these results, from April 2007 to October 2009, 65 patients with metastatic HR-NBL who had not achieved the SIOPEN criteria for HDC after induction received two courses of topotecan 1.5 mg/m<sup>2</sup>/day for 5 days, followed by a 48-hour infusion of vincristine, 2 mg/m<sup>2</sup>, and doxorubicin, 45 mg/m<sup>2</sup> (TVD). Following two courses of TVD, four (6%) patients had an overall CR, while 23 patients achieved a metastatic CR or a PR with  $\leq 3$  mIBG skeletal areas and no bone marrow disease and were eligible to receive HDC.[Amoroso L, Canc Res Treat 2018]

In North America, different induction regimens have been developed. The N7 regimen was initially developed at MSKCC and reported 83% of responses (CR and VGPR) to induction chemotherapy. The initial results reported by MSKCC (overall CR/VGPR of 83%) have not been replicated by 2 randomized studies conducted by the French (SFOP) and Austrian neuroblastoma groups, although both groups reported that patients achieving CR have higher long term EFS [Kohler JA, *Pediatr Blood Cancer* 2007; Valteau-Couanet D, *J Clin Oncol* 2005; Valteau-Couanet D, *Pediatr Blood Cancer* 2014]. The regimen had then to be shortened from 7 to 5 cycles (modified N7) due to increased frequency of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML).[Cheung NK, *Med Pediatr Oncol* 2001; Kushner BH, *J Clin Oncol* 2004, Mora J, *Clin Transl Oncol* 2015] From 2001, the COG adopted a modified N7 approach for induction chemotherapy for their COG A3973 trial that included two putatively non cross-resistant drug combinations: high-dose cyclophosphamide plus doxorubicin/vincristine (CAV) and high-dose cisplatin/etoposide (P/E). This trial reported CR or VGPR in 48-50% of patients. Five-year EFS was 38% and 5-year OS was 50%. [Kreissman SG, *Lancet Oncology* 2013] A pilot study conducted by COG showed the feasibility and tolerability of two cycles of Topotecan-Cyclophosphamide (T/C) during induction chemotherapy with 66% CR/VGPR rate. [Park JR, *J Clin Oncol* 2011] Following this study report, the first two cycles of CAV were substituted by T/C after closure of A3973. From 2007, the COG trial ANBL0532 included this modified approach.[Park JR, *J Clin Oncol* 2016] In its recent report, 45% of patients achieved CR/VGPR with 3-year EFS of 51% and 5-year OS of 68%.

In 2013, a new randomization (R3) was introduced into the SIOPEN/HR-NBL1 trial to compare COJEC with the modified N7 induction regimen. After the R3 randomization was closed for recruitment on June 2017, a data cut-off was performed (September 2017) to inform the design of the HR-NBL2 trial (Figure 1). The 3-year EFS was 39%  $\pm$  4% for COJEC vs 39%  $\pm$  4% for modified N7 (p=0.805), the rate of metastatic CR was 33% and 37%, respectively (p=0.492). The rate of grade 3/4 toxicities was higher in the N7 arm (mucositis, general condition, febrile aplasia, etc).

With RAPID COJEC having less acute toxicity than modified N7, RAPID COJEC was selected to be the SIOPEN reference induction regimen. If metastatic complete response was not achieved after RAPID COJEC, 2 additional cycles of TVD are administered in order to increase the number of patients eligible to HDC.

Figure 1: EFS of COJEC vs modified N7 (R3 results in SIOPEN/HR-NBL1)



### 1.3 High-dose chemotherapy

HDC followed by ASCR has improved outcomes in patients with HR-NBL in European and North America randomized trials, becoming the standard of care for these patients.[Matthay KK, N Engl J Med 1999; Berthold F, Lancet Oncol 2005; Pritchard J, Pediatr Blood Cancer 2005]

These trials explored the impact on survival of consolidation regimens consisting of HD carboplatin-etoposide-melphalan (CEM) and total body irradiation (TBI), MEC and HD melphalan. More recently, Matthay *et al.* published the long-term results of patients treated with CEM+TBI followed by ASCR and reported 5 year-EFS and OS rates of 30% and 39%, respectively.[Matthay KK, J Clin Oncol 2009] In the COG A3973 study, CEM was selected as the standard of care in the attempt to find an optimal regimen substituting for the TBI-containing once employed in the CCG 3891 protocol.

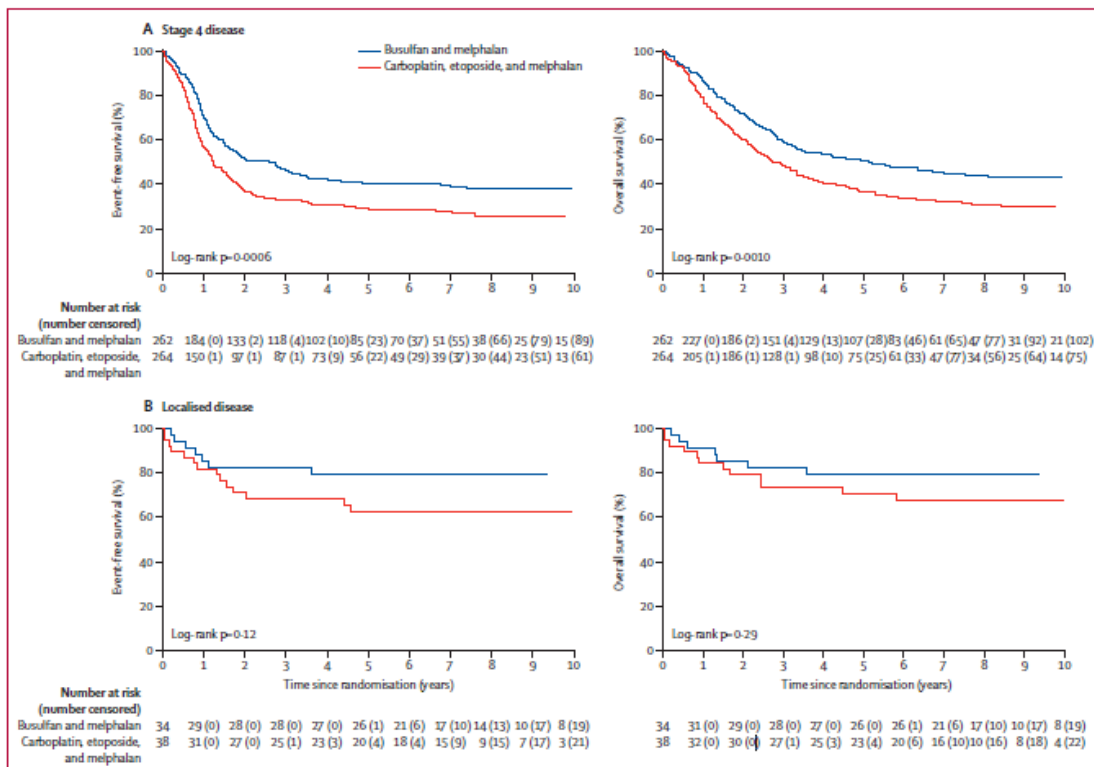
Bu-Mel was the conditioning regimen mainly used in Europe based on results showing a significant advantage of Bu-Mel in patients with high-risk neuroblastoma.[Hartmann O, Bone Marrow Transplant 1999] The long-term results of this single institution cohort of patients with HR-NBL treated with HD Bu-Mel containing regimens confirmed the benefit of this regimen, with 5-year EFS and OS rates of 35% and 40%, respectively.[Proust-Houdemont S, Bone Marrow Transplant 2016]

These data provided the rationale to widely implement the use of Bu-Mel, which was then compared with CEM in the HR-NBL1/SIOPEN randomized trial (R1). Of 1,577 patients with HR-NBL, 563 were



randomly assigned in a 1:1 ratio to either Bu-Mel or CEM following rapid induction therapy with COJEC (Figure 2). The trial was stopped because a pre-specified interim analysis showed a 49% EFS rate with Bu-Mel vs 33% for CEM ( $p < 0.001$ ). [Ladenstein R, Lancet Oncol 2017] The 3-year OS was 60% with Bu-Mel vs 48% for CEM ( $p = 0.003$ ), and the rate of relapse or progression was significantly lower in the Bu-Mel group (47% vs 60%;  $p < 0.001$ ). A multivariate analysis confirmed that the improved EFS was associated with Bu-Mel regimen. The toxicity was acceptable for both conditioning regimens. While the frequency of grade 3-4 infection, fever and renal toxicity was higher in the CEM arm, the rate of sinusoidal occlusive syndrome (SOS), which was reversible, was higher in the Bu-Mel arm. The rate of acute toxic death was 3% for Bu-Mel and 5% for CEM, and severe toxicity was not significantly different in the 2 arms. Therefore, HD Bu-Mel has now become the standard HD regimen in the SIOPEN HR strategy.

Figure 2: Bu-Mel vs CEM in R1/HR-NBL1 (adapted from Ladenstein R, Lancet Oncol 2017)



### 1.4 Surgery

This study aims to achieve complete primary tumour excision, ideally prior to HDC, to improve local control. Surgical issues are discussed in detail in section 5.6.

### 1.5 Local radiation therapy

External beam radiotherapy has a long history of use in neuroblastoma. Within the SIOPEN group, it is standard practice following induction chemotherapy, surgery and HDC.

In SIOPEN it has been the practice to give 21.6-Gy radiotherapy to all patients as a standard dose regardless of the disease extent and the quality of surgery. In the previous HR-NBL1/SIOPEN cohort, among 1297 patients, 200 patients experienced a local relapse, either as a unique site of relapse (“local only”, n=60) or with metastatic sites (“combined”, n=140). The 5-year cumulative incidence of local relapse (“local only” + “combined”) was  $23\% \pm 3\%$  in patients with macroscopic residual tumour, and  $15\% \pm 1\%$  in patients with complete resection. In these two groups, the 5-year EFS was  $38\% \pm 3\%$  and  $49\% \pm 2\%$ , respectively.

Radiotherapy guidelines are given in detail in section 5.7.

### 1.6 Maintenance treatment

SIOPEN recommends that patients with HR-NBL in the front-line setting receive maintenance therapy following induction chemotherapy, surgery, HDC/ASCR and local radiation. SIOPEN, as well as COG, focused on the development of strategies that incorporate anti-GD2 monoclonal antibodies into maintenance therapy.

Two main forms of anti-GD2 antibodies have been used in neuroblastoma clinical trials. ch14.18/SP2/O (dinutuximab) is a ch14.18 antibody produced in murine cells, while ch14.18/CHO antibody (dinutuximab beta) is a mouse-human chimeric monoclonal IgG1 antibody produced in a mammalian CHO cell line, both being specifically directed against the GD2.[Mujoo K, Cancer Res 1987]

Early phase clinical trials in Europe and North America used dinutuximab, the ch14.18/SP2/O version of the antibody.[Saleh MN, Hum Antibodies Hybridomas 1992; Yu AL, J Clin Oncol 1998; Handgretinger R, Eur J Cancer 1995] A phase II trial for children with metastatic neuroblastoma conducted by the GPOH compared dinutuximab (20 mg/m<sup>2</sup>/day for 5 days in six cycles every two months) with 12 months of low dose maintenance chemotherapy as consolidation treatment. Of 334 assessable patients, 166 received dinutuximab and 99 the low-dose chemotherapy, while 69 had no further maintenance treatment. Three-year OS was  $69\% \pm 4\%$  for dinutuximab vs  $57\% \pm 5\%$  for chemotherapy vs  $47\%$  for no additional therapy. However, the different treatments were not randomized and univariate analysis showed similar EFS for the 3 groups.[Simon T, J Clin Oncol 2004] COG tested the clinical efficacy of dinutuximab in the ANBL0032 trial. Based on preclinical and early phase trial results showing increased activity when combined with GM-CSF or IL-2, ANBL0032 was a phase III trial designed to test if the addition of dinutuximab with GM-CSF and IL2 to standard HR-NBL differentiation therapy with isotretinoin improved patient outcomes. Front-line patients were enrolled if they had achieved a CR/PR following induction chemotherapy and had undergone HDC/ASCT. They were randomized to receive 13-cis-RA alone for 6 cycles or 13-cis-RA for 6 cycles with 5 cycles of dinutuximab combined with GM-CSF or IL-2 in alternating cycles. The investigational therapy was associated with significant higher toxicities. At two years the EFS was  $66\% \pm 5\%$  vs  $46\% \pm 5\%$  and OS  $86\% \pm 4\%$

---

vs 75±5% for the investigational arm and the conventional arm, respectively. The interim assessment stopping rules were met and randomization was halted.[Yu AL, NEJM 2009]

SIOPEN has evaluated dinutuximab beta, the ch14/18/CHO antibody, in several successive trials. The benefit of IL-2 given in addition to dinutuximab beta was investigated in a prospective phase III trial in the context of HR-NBL1 trial.[Ladenstein R, J Clin Oncol 2016] Four-hundred and six patients with HR-NBL were randomized (R2) following induction chemotherapy, HDC/ASCR and local therapy. Patients received 5 cycles of dinutuximab beta (100mg/m<sup>2</sup>/cycle as 5 daily 8 hour infusions) alone or in combination with IL-2 (6 x 10<sup>6</sup> IU/m<sup>2</sup> on days 1-5 and 8-12 of each cycle). There was no statistical difference in outcome between the arms; 3-year EFS and OS of 60±4% and 66±4% for the dinutuximab beta alone arm versus 57±4% and 65±4% for the combination arm. Outcomes were favorable compared to historical controls (13-cis-RA as maintenance treatment), but no survival benefit was found with the addition of IL-2. Importantly the combination arm was associated with significantly more toxicity and as a result early termination (grade 3&4 allergic reactions 9% vs 20%, capillary leak rate 1% vs 14%, early termination rates 18% vs 44%). The Long-Terms Infusion (LTI) study was designed as a phase I/II dose-finding study, administering continuous infusion dinutuximab beta over 10 days (100mg/m<sup>2</sup>/cycle) in patients with relapsed/refractory neuroblastoma with the objective of determining a tolerable treatment schedule whilst maintaining satisfactory immunomodulatory efficacy. The 10-day continuous infusion schedule combined with IL-2 at a dose of 6 x 10<sup>6</sup> IU/m<sup>2</sup>/day was found to be tolerable.[Lode HN, J Clin Oncol 2016] The protocol met the primary efficacy endpoint; increased ADCC and tolerable antibody administration with significantly less pain. The objective clinical response rate was 40%.

One major issue with the R2/HR-NBL1 trial was the number of patients on the IL-2 containing arm who did not complete immunotherapy treatment as prescribed. With the improved tolerance and favorable immunomodulatory effects of the LTI schedule demonstrated in the LTI study, SIOPEN elected to adopt the LTI schedule into the HR-NBL1 trial and to randomize a decreased dose of IL-2 (R4) to clarify whether there is a benefit to adding IL-2 to dinutuximab beta. The R4 randomization demonstrated that IL-2 did not improve the impact on survival of dinutuximab beta, thus dinutuximab beta with 13-cis-RA, without IL-2, have become the SIOPEN standard of care for maintenance.

## **2. PATIENT GROUP**

### **2.1 Recommended criteria to enter the protocol**

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

- High-risk neuroblastoma defined as:

- Stage M neuroblastoma above 365 days of age at diagnosis (no upper age limit), any *MYC* status\*
- or
- L2, M or Ms neuroblastoma with *MYC* amplification, any age

## 2.2 Recommended criteria to enter consolidation phase

The validation of the eligibility to the consolidation phase with HD Bu-Mel will be performed at the end of induction after the disease evaluation and after surgery of the primary tumour for those patients who will receive surgery before HDC.

The patient will receive HDC if:

- Sufficient metastatic response, defined as:
  - Bone disease: MIBG uptake (or FDG-PET uptake for MIBG-nonavid tumours) completely resolved after RAPID COJEC or SIOPEN score  $\leq 3$  and at least 50% reduction in mIBG score (or  $\leq 3$  bone lesions and at least 50% reduction in number of FDG-PET-avid bone lesions for MIBG-nonavid tumours) after 2 cycles of TVD
  - Bone marrow disease: CR and/or minimal disease (MD) according to International Neuroblastoma Response Criteria [Park JR, JCO 2017; Burchill S, Cancer 2017]
  - Other metastatic sites: complete response after induction chemotherapy +/- surgery
- Acceptable organ function and performance status
  - Performance status  $\geq 50\%$
  - Haematological status: ANC  $> 0.5 \times 10^9/L$ , platelets  $> 20 \times 10^9/L$
  - Liver function: Alanine aminotransferase (ALT)  $\leq 3.0 \times ULN$  and blood bilirubin  $\leq 1.5 \times ULN$  (toxicity  $<$  grade 2). In case of toxicity  $>$  grade 2, *an Expert's advice is highly recommended to define the best approach for such patients.* Renal function: Creatinine clearance and/or GFR  $\geq 60$  ml/min/1.73m<sup>2</sup>. If GFR  $<$  60ml/min/1.73m<sup>2</sup>, *an Expert's advice is highly recommended to define the best approach for such patients.*
  - Cardiac function: Shortening fraction  $\geq 28\%$  or ejection fraction  $\geq 55\%$  by echocardiogram, no clinical congestive heart failure. Normal pulmonary artery pressure.
  - Normal chest X-ray and oxygen saturation
  - Absence of any toxicity  $\geq$  grade 3
- Sufficient collected stem cells available; minimum required:  $3 \times 10^6$  CD34+ cells/kg body weight stored in 2 separate fractions

Of note,

- For patients with insufficient metastatic response at the end of induction (“refractory disease”), *an Expert's advice is highly recommended to define the best approach for such patients.*

- 
- For patients 12-18 months old with stage M non-MYC amplified, and with numerical chromosomal alterations only, in case of metastatic complete response, the treatment will be completed by surgery, without neither HDC nor maintenance treatment.

### 2.3 Recommended criteria to perform local radiotherapy

- No evidence of disease progression after HDC/ASCR
- Interval between the last ASCR and radiotherapy should be ideally between 60 and 90 days
- Performance status  $\geq 50\%$
- Haematological status: ANC  $>0.5 \times 10^9/L$ , platelets  $> 20 \times 10^9/L$
- Acceptable liver function (if the liver is in the irradiated volume): Alanine aminotransferase (ALT)  $< 3.0 \times ULN$  and blood bilirubin  $< 1.5 \times ULN$  (toxicity  $< \text{grade } 2$ )

### 2.4 Recommended criteria to enter maintenance treatment

- No progressive disease
- Performance status  $\geq 50\%$
- Maintenance (starting with 13-cis-RA) should not start ideally later than day 120 post ASCR
- Haematological status: ANC  $>0.5 \times 10^9/L$ , platelets  $> 20 \times 10^9/L$  and haemoglobin  $> 7.0 \text{ g/dL}$
- Acceptable organ function:
  - Liver function: Alanine aminotransferase (ALT)  $< 3.0 \times ULN$  and blood bilirubin  $< 1.5 \times ULN$  (toxicity  $< \text{grade } 2$ )
  - Renal function: Creatinine clearance and/or GFR  $\geq 60\text{ml/min}/1.73\text{m}^2$  (toxicity  $< \text{grade } 2$ ).
  - Cardiac function: Shortening fraction  $\geq 28\%$  or ejection fraction  $\geq 55\%$  by echocardiogram, no clinical congestive heart failure.
  - Normal chest X-ray and oxygen saturation
  - Absence of any toxicity  $\geq \text{grade } 3$

## 3. DIAGNOSTIC CRITERIA

### 3.1 Imaging

Nuclear medicine guidelines are given in detail Appendix 7.

### 3.2 Histopathology

#### 3.2.1 General Remarks

The handling of the tumour tissue should always be performed by the pathologist who, besides the important task of making morphologic diagnoses and giving prognoses based on histopathologic findings, should choose the relevant tumour areas for molecular-genetic/biological analysis. The pathologist should assess:

(a) the **representativity** of the chosen areas and report the percental distribution of viable tumour tissue, necrosis and preserved non-tumour tissue (i.e. fibrosis), and

(b) the **tumour cell content**, i.e. the percentage of tumour cell nuclei compared to all preserved nuclei. This procedure will enable reliable interpretation of the molecular-genetic results. It can only be facilitated if the pathologist evaluates a frozen section from the sample chosen for molecular-genetic/biological analyses and/or paraffin sections of tissue areas adjacent to (i.e. mirroring) this sample.

In all instances, tumour material from different tumour areas (nodules are of special interest) ought to be taken for histologic and molecular-genetic/biological examination. The reason for this recommendation is based on the observation of tumour heterogeneities at the genetic level (i.e. for the *MYC* and/or the chromosome 1p status) and/or at the histologic level (ganglioneuroblastoma, nodular subtype according to the International Neuroblastoma Classification, INPC), both of which have prognostic implications.[Shimada H, Cancer 2001]

**Close co-operation between pathologists and biologists is therefore strongly recommended.**

Pathologists should inform the biologists if morphologically unfavorable looking areas are present in the paraffin embedded material but most likely not in the specimens selected for molecular-genetic/biological investigations. These areas should be specifically analyzed using paraffin material.

### 3.2.2 Sectioning and securing tumour material in case of resected tumours

Ink the surgical margin and cut the tumour in parallel slices of about 1 cm thickness. Inspect every cut surface and take at least two samples from morphologically different-appearing areas (1x1x1cm) if such are present. Stroma-poor tumour tissue is of main interest for the molecular biologist and often presents a soft, gelatinous or friable, gray or brownish staining cut surface due to bleeding between tumour cells. Very firm, light yellow or wittish areas usually represent Schwann cell stroma or fibrosis are of less interest but should be sampled for histological confirmation. Nodules with a cut surface darker than the surrounding tissue must always be sampled. Identify the samples specifically with capitals (A, B, etc.), or whatever system is the practice of each laboratory, and cut each of them into four pieces which are marked with numbers (i.e. tumour specimen A 1-3, specimen B 1-3). More material can be processed in the same way (C, D, etc.), but material from two different areas is the minimum. Check carefully for the presence of nodules.

- *Samples A1 and B1*: make **10 touch preparations** (at least 5) from a freshly cut surface which has not been in contact with absorbing wadding or the table top. The slides are air-dried and can, if necessary, be stored unfixed at -20°C for fluorescence based in situ hybridisation (FISH) and image cytometry (ICM). Storage of the slides for one week at room temperature does not adversely affect DNA quality but weakens or destroys most cell line or differentiation associated markers detected by antibodies (immunocytology) and is therefore not recommended. The

---

tissue pieces used for making touch preparations should be **fixed in formalin** for routine histologic examination. Make sure that paraffin sections are cut from the surface from which imprints were produced. This should facilitate a documentation of the cellular composition of the imprints and indicate the content of tumour cells normal cells, such as Schwann cells, lymphocytes, fibrovascular stroma etc.; amount of necrosis should be indicated as well. This information is crucial for the interpretation of the FISH, ICM and cytogenetic results.

- *Samples A2-3 and B2-3*: **snap freeze as soon as possible** in separate vials in liquid nitrogen or on dry ice and then stored in liquid nitrogen or in a -80°C freezer. Please indicate the time between tumour collection and freezing. Before using these for further analyses, making cryosections for the determination of the tumour cell content is mandatory.

The samples should be forwarded to the biology reference laboratory as soon as possible. After this procedure, the remaining part of the surgical specimen can be fixed in formalin and worked-up according to standard guidelines.

### 3.2.3 Sectioning and securing tumour material in case of surgical biopsies

Follow the same procedure as described above.

Cut the tissue specimen along the largest diameter. Make 10 touch preparations from the freshly cut surface, fix the piece used for imprinting in formalin and embed in paraffin for histological analysis. The other half of the biopsy is snap frozen in liquid nitrogen or on dry ice and stored in liquid nitrogen or in a -80°C freezer.

If several small tissue pieces are received which cannot be cut into smaller fragments about one third of the pieces should be fixed in formalin and embedded in paraffin for histological analysis, while two thirds should be snap frozen in liquid nitrogen or at -70°C carbon dioxide.

### 3.2.4 Securing tumour material in case of core needle (tru cut) biopsies

A minimum of four (preferentially five) core needle biopsies from different areas of the tumour should be received. If they are brought to the pathology department on a humidified filter paper they may be used for imprinting. If transported in PBS or in culture medium subsequent imprinting is often less successful because the transport medium may wash away cells from the tissue surface.

Two needle biopsies are fixed in formalin and embedded in paraffin for histological analysis. The two (or three) remaining biopsies are snap frozen in separate vials in liquid nitrogen or at -80°C freezer. Reporting of the tumour cell content on frozen sections is required for each needle biopsy as they may originate from different tumour areas with different histological composition. Preparation of cell suspensions from core needle biopsy is not recommended due to the paucity of the material.

---

### 3.2.5 Securing tumour material in case of fine needle aspirations (FNA)

Fine needle aspirations (FNA) yield cytological tumour cell samples and are generally not recommended because (a) the precise diagnosis and classification of the tumour according to the INPC is only possible on histological sections and (b) the material for biological investigations may be scarce. However, tumour localization and the clinical condition of the child may in certain cases exclude surgical or tru cut biopsies.

Prepare at least five punctures from different areas of the tumour. The first droplet of the aspirated material should be smeared on a glass slide, immediately stained (i.e. by Diffquick) and assessed for tumour content. The remainder should be transferred from the syringe into 0.5ml PBS. Depending on the available number of tumour cells in each vial, suspended tumour cells should be centrifuged on cytopins for cytomorphological, immunocytological and FISH analysis, spun down and snap frozen and/or used for analysis of ploidy by i.e. flow cytometry.

### 3.2.6 Histology report

#### At diagnosis

##### - *Resected tumour*

Morphologic classification: The tumour should be classified according to the International Neuroblastoma Pathology Classification.[Shimada H, Cancer 2001]

The mitotic rate and calcifications should also be indicated. The surgical margins of resection should be described, without making any conclusion as to whether the tumour residual is microscopic or macroscopic. The report must clearly indicate the estimated percentage of tumour cells, i.e. neuroblastic/ganglionic cells, versus Schwann cells and other normal cells contained in the samples used for the biological studies. A copy of the report should be submitted to the molecular biologist.

##### - *Surgical and core needle (tru cut) biopsy*

In case of limited biopsy material, it has to be kept in mind that the tumour material obtained is not necessarily representative of the whole tumour. For example, the biopsy could be taken from either a neuroblastic nodule or the ganglioneuromatous area of a nodular ganglioneuroblastoma. In such critical cases, the use of the following term, according to the INPC, is recommended: ‘neuroblastic tumour, unclassifiable’. This term relates to a tumour which belongs unequivocally to the peripheral neuroblastic tumour entity, but which cannot be allocated with certainty into one of the four basic categories which are neuroblastoma (Schwann cell stroma-poor), ganglioneuroblastoma intermixed (Schwann cell stroma-rich), ganglioneuroma (Schwann cell stroma-dominant), ganglioneuroblastoma nodular (Schwann cell stroma-rich/-dominant and stroma-poor). Other terms recommended by the INPC to be used for tumours giving rise to problems in classification, are: ‘neuroblastoma (Schwann cell stroma-poor), NOS’. This term is used for tumours with an unequivocal categorisation, but the subtype, i.e. undifferentiated, poorly differentiated, differentiating, cannot be assessed due to poor quality of the



---

sections, extensive haemorrhage, necrosis, crush artefacts, etc. ‘Ganglioneuroblastoma, NOS’ is used for a tumour with a stroma-rich/-dominant appearance containing areas of extensive calcification which may obscure a stroma-poor nodule.

### **After cytotoxic therapy**

#### *- Tumour material*

Sectioning of the tumour material in resected tumours or biopsies after cytotoxic therapy can be done following the same guidelines as for tumours resected or biopsied at diagnosis before cytotoxic therapy. However, for sampling, it must be remembered that necrotic areas and also calcifications can be massive. Therefore, it is essential to state exactly the percentage of viable tumour cells versus normal cells, and to indicate the amount of necrosis and calcification. It is known that both chemo- and radiotherapy can induce marked morphologic changes and can also induce cytodifferentiation and maturation (with development of a Schwann cell stroma), but most likely do not change the original genetic characteristics of the tumour. Therefore, assignment to the prognostic subgroups must not be made, although different areas of the tumour can be classified morphologically according to different categories and subtypes of the INPC. The final report made by the pathologist should always specify in the diagnostic line that the investigated tumour is a post-chemotherapy specimen.

#### *- Regional lymph node examination*

Biopsy of regional nodes is highly recommended whenever feasible despite their appearance. The histology report should include information on site and number of positive nodes, type of metastatic spread, i.e. presence of micrometastases (less than 2 mm), intranodal parcelled metastases, intranodal massive metastases, nodal metastases with extracapsular extension in localisations not adherent to the resected tumour specimen, and morphologic description of the tumour infiltrate.

### **Immunohistochemistry**

#### *Differential Diagnosis*

Neuroblastomas of undifferentiated subtype (according to the INPC) or artificially crashed biopsies of poorly differentiated neuroblastomas may look like any small blue round cell tumour and thus pose diagnostic difficulties. In these instances, the use of the following antibodies is recommended: MIC2 (CD99), desmin, myogenin, low molecular-weight cytokeratin, leukocyte common antigen (CD45), Tdt, and vimentin. These antibodies are usually negative in neuroblastic tumours. Positive markers are: CD56 (N-CAM), synaptophysin, NSE (monoclonal neuron specific enolase), NF (neurofilament triplet protein), tyrosine hydroxylase and Phox2B, the latter being the most specific marker for neuroblastic tumours. However, it has to be kept in mind that some of these markers, although often positive, may be negative in undifferentiated neuroblastomas. Although GD2 is positive in the large majority of neuroblastic tumours and useful for the detection of neuroblastic cells in bone marrow aspirates, anti-

---

GD2 antibodies which detect the antigen in FFPE material are presently not available. Anti-S-100 antibodies can be used to unequivocally distinguish Schwann cell stroma from fibrous tissue.

#### *Cytologic material*

For detection and quantification of tumour cells in bone marrow and fine needle aspirates, anti-GD2 for bone marrow, and anti-CD56, anti-GD2 and anti-CD45 for tumour material are recommended. [Burchill S, Cancer 2017]

## 4. BIOLOGICAL ANALYSIS

In HR-NBL, the following investigations (clinical decision making) will be performed on primary tumour tissue obtained at diagnosis:

- **MYC copy number status (all patients)**
- **For patients 12-18 months old with stage M and MYC non amplified tumours: Genomic copy number profile (high resolution aCGH and/or SNPα and/or lcWGS)**

## 5. TREATMENT DETAILS

### 5.1 Treatment overview

HR-NBL strategy consists in 4 main treatment phases (Figure 3):

- Induction Phase
- Consolidation Phase
- Local Treatment Phase
- Maintenance Phase

Figure 3. Treatment overview

## 5.2 Induction chemotherapy

Figure 4 depicts the schema of the RAPID COJEC induction chemotherapy regimens, including the time points for surgery and stem cell harvest.

*Figure 4: Induction chemotherapy schema*

Patients that are initially diagnosed with localised disease that start one course of Carboplatin-Etoposide as per intermediate/low-risk clinical trial recommendation and are subsequently identified as *MYC* amplified disease,, the chemotherapy course will replace the first course (A) of the RAPID COJEC induction.

### 5.2.1 RAPID COJEC chemotherapy induction

Three different courses (A, B, C) are given every 10 days regardless of neutrophil or platelet counts, except in case of uncontrolled infection.

*Table 1: RAPID-COJEC overview*

Day	0	10	20	30	40	50	60	70
Course	A	B	C	B	A	B	C	B
VINCRIStINE	↓	↓	↓	↓	↓	↓	↓	↓
CARBOPLATIN	↓				↓			
ETOPOSIDE	↓↓		↓↓		↓↓		↓↓	
CISPLATIN		←		←		←		←
CYCLOPHOSPHAMID E			↓↓				↓↓	
G-CSF (days of administration)	3→8	12→8	23→8	32→8	43→8	52→8	63→8	72→76 or until harvest

COURSE A starts on days 0 and 40, COURSE B on days 10, 30, 50 and 70 and COURSE C on days 20 and 60.

*G-CSF*: The use of G-CSF (5µg/kg/day subcutaneously) during RAPID-COJEC induction will start 24-48 hours (according to the course; see Table 1) after the end of chemotherapy and until the ANC is > 0.5 x 10<sup>9</sup>/L or 48 hours before the next planned course of chemotherapy. There should be an interval of at least 24 hours between the last G-CSF injection and the start of the next course of chemotherapy.

**COURSE A**  
Start on days 0 and 40

Course A (days)	1	2
Vincristine	<input type="checkbox"/>	
Carboplatin	<input type="checkbox"/>	
Etoposide	<input type="checkbox"/>	<input type="checkbox"/>

DRUG	Time	Dose	Administration
<b>DAY 1</b>			
VINCRIStINE	H0	1.5 mg/m <sup>2</sup> (max dose 2 mg)	As a single iv bolus or over 1 hour according to local policies
CARBOPLATIN	H1	750 mg/m <sup>2</sup>	Infused over 60 minutes iv in 5% dextrose

<b>ETOPOSIDE</b>	H2	175 mg/m <sup>2</sup>	Infused over 4 hours iv in 0.9% saline
<b>DAY 2</b>			
<b>ETOPOSIDE</b>	H0	175 mg/m <sup>2</sup>	Infused over 4 hours iv in 0.9% saline

The use of G-CSF (5µg/kg/day, subcutaneously) during RAPID-COJEC induction will start 24 hours after the end of chemotherapy (course A), and will be stopped the day prior to commencing the next course with an interval of at least 24 hours between the last G-CSF injection and the start of chemotherapy, i.e. days 3 to 8, and days 43 to 48.

**COURSE B**

**Start on days 10 - 30 - 50 – 70**

Course B (days)	1	2
<b>Vincristine</b>	<input type="checkbox"/>	
<b>Cisplatin</b>	<input type="checkbox"/> ➤	
<b>Hyperhydration</b>	<input type="checkbox"/> ➤	<input type="checkbox"/> ➤

DRUG	Time	Dose	Administration
<b>VINCRIStINE</b>	H0	1.5 mg/m <sup>2</sup> (max dose 2 mg)	As a single iv bolus or over 1 hour according to local policies
<b>PRE-HYDRATION</b>	H1	200 ml/m <sup>2</sup> /h	Infused over 3 hours before cisplatin: 0.9% sodium chloride with 10 mmol/l potassium chloride
<b>MANNITOL 20%</b>	H1	40ml/m <sup>2</sup>	Short infusion iv
<b>MANNITOL 20%</b>	H3.5	40ml/m <sup>2</sup>	Short infusion iv
<b>HYDRATION During cisplatin</b>	H4	125 ml/m <sup>2</sup> /h	Infused over 24 hours in parallel with cisplatin: 1.5 l/m <sup>2</sup> /24h of 0.9% sodium chloride 1.5 l/m <sup>2</sup> /24h of 5% glucose, 30 mmol/m <sup>2</sup> / 24h of potassium chloride, 2.5 mmol/m <sup>2</sup> /24h of calcium gluconate, 10 mmol/m <sup>2</sup> /24h of magnesium sulphate
<b>CISPLATIN</b>	H4	80 mg/m <sup>2</sup> /24h	Over 24 hours in 0.9% sodium chloride alongside the hydration

<b>POST-HYDRATION</b>	H28 - H52	125 ml/m <sup>2</sup> /h	1.5 l/m <sup>2</sup> / 24 hours of 0.9% sodium chloride 1.5 l/m <sup>2</sup> /24 hours of 5% glucose, 60 mmol/ m <sup>2</sup> /24h of potassium chloride, 2.5 mmol/ m <sup>2</sup> calcium gluconate, 10 mmol /m <sup>2</sup> /24h of magnesium sulphate
<b>MANNITOL 20%</b>	If needed	40ml/m <sup>2</sup>	If diuresis falls below 400 ml/m <sup>2</sup> /6 hours, Short infusion iv

During pre-hydration, the cisplatin infusion together with its parallel hydration and post-cisplatin hydration, a careful record of fluid input and output should be kept to prevent hydration overload and ensure diuresis. Magnesium supplementation during cisplatin treatment is recommended at a daily dose of 180mg/m<sup>2</sup>/day during the induction period but may need to be adjusted following monitoring of Mg levels. *Mannitol and magnesium are not to be given con-currently as these are not compatible.* The addition of calcium, potassium and phosphate may be modified according to serum levels. Furosemide should be avoided because of the increased risk of ototoxicity. To avoid fluid overload the total fluid intake should be no more than 4.5L/m<sup>2</sup>/24 hours.

The use of G-CSF (5µg/kg/day, subcutaneously) will start 24 hours after the end of chemotherapy (course B), and will be stopped the day prior to commencing the next course with an interval of at least 24 hours between the last G-CSF injection and the start of chemotherapy, i.e. days 12 to 18, days 32 to 38, days 52 to 58 and days 72-76 (or until harvest).

**COURSE C**  
**Start on days 20 and 60**

Course C (days)	1	2
<b>Vincristine</b>	<input type="checkbox"/>	
<b>Etoposide</b>	<input type="checkbox"/>	<input type="checkbox"/>
<b>Cyclophosphamide</b>	<input type="checkbox"/>	<input type="checkbox"/>
<b>Hyperhydration</b>	<input type="checkbox"/> ➤	<input type="checkbox"/> ➤

DRUG	Time	Dose	Administration
<b>DAY 1</b>			
<b>VINCRIStINE</b>	H0	1.5 mg/m <sup>2</sup> (max dose 2 mg)	As a single iv bolus or over 1 hour according to local policies
<b>ETOPOSIDE</b>	H1	175 mg/m <sup>2</sup>	Infused over 4 hours iv in 0.9% saline
<b>MESNA</b>	H5	200 mg/m <sup>2</sup>	Short infusion iv

<b>CYCLOPHOSPHAMIDE</b>	H5	1050 mg/m <sup>2</sup>	Over 1 hour
<b>HYPERHYDRATION + MESNA</b>	H5	125 ml/m <sup>2</sup> /h (hydration) + 1.2 g/m <sup>2</sup> /24 h (mesna)	Infused over 24 hours: 1.2 g/m <sup>2</sup> / 24 hours mesna 1.5 l/m <sup>2</sup> /24 hours of 0.9% sodium chloride 1.5 l/m <sup>2</sup> /24 hours of 5% glucose + 60 mmol/m <sup>2</sup> /24 hours of potassium chloride
<b>DAY 2</b>			
<b>ETOPOSIDE</b>	H0	175 mg/m <sup>2</sup>	Infused over 4 hours iv in 0.9% saline
<b>CYCLOPHOSPHAMIDE</b>	H4	1050 mg/m <sup>2</sup>	Over 1 hour
<b>HYPERHYDRATION + MESNA</b>	H4	40ml/m <sup>2</sup>	Infused over 24 hours: 1.2 g/m <sup>2</sup> / 24 hours mesna 1.5 l/m <sup>2</sup> /24 hours of 0.9% sodium chloride 1.5 l/m <sup>2</sup> /24 hours of 5% glucose + 60 mmol/m <sup>2</sup> /24 hours of potassium chloride

The use of G-CSF (5µg/kg/day, subcutaneously) during RAPID-COJEC induction will start 48 hours after the end of chemotherapy (course B), and will be stopped the day prior to commencing the next course with an interval of at least 24 hours between the last G-CSF injection and the start of chemotherapy, i.e. days 23 to 28, and days 63 to 68.

### 5.2.2 TVD cycles

If metastatic complete response is not achieved after RAPID COJEC, **2 additional cycles of TVD** are administered in order to increase the number of patients eligible to HDC.

- **Topotecan** To be administered i.v. in the morning, as a 30 minute infusion in saline 100 ml/m<sup>2</sup> at dose of 1.5 mg/m<sup>2</sup>/day for 5 consecutive days (days 1 to 5).
- **Vincristine** To be administered as a 48-hour continuous infusion at a dose of 1 mg/m<sup>2</sup>/day in 50 ml/m<sup>2</sup>/day 0.9% saline (maximum dose 1mg/day), starting one hour after the final topotecan infusion (days 5 and 6).
- **Doxorubicin** To be administered simultaneously with vincristine as a 48-hour continuous infusion at a dose of 22.5 mg/m<sup>2</sup>/day in 50 ml/m<sup>2</sup>/day of 0.9% saline solution (days 5 and 6).

Anti-emetic therapy should be given according to clinical conditions and institutional policies.

G-CSF, 5 µg/kg/day s.c, should be started 72 hours after conclusion of vincristine and doxorubicin infusion and continued until neutrophil recovery (ANC > 1.0 International Units).

---

A second cycle will be administered at the same dose 21-28 days from the start of the first cycle, provided that:

- a) haematological recovery has occurred (ANC >1.0 International Units; platelet count > 100,000/ $\mu$ L),
- b) there is no evidence of progressive disease, and
- c) no non-haematological toxicity greater than grade 1.

Also, the patient should have been off G-CSF for at least 48 hours. A limited disease evaluation after the first cycle of TVD should include ultrasound of primary tumour. Full tumour re-evaluation must be performed after two cycles of TVD to evaluate if the patient has achieved sufficient response to be eligible for HDC.

### 5.3 Peripheral blood stem cells harvest

Pediatric apheresis procedure should be performed by an accredited stem cell transplantation (SCT) programs and conducted by an experienced pediatric team. (See Appendix 8)

Timing of peripheral blood stem cells (PBSC) harvest is summarized in Figure 5.

*Figure 5: Timing of PBSC Harvest*

Patients will undergo harvest of PBSC following the end of the induction. Harvest may be scheduled either after the last chemotherapy cycle (G-CSF 5  $\mu$ g/kg/day until harvest, to be increased to 10  $\mu$ g/kg/day if needed) or out of steady state mobilization (G-CSF 10  $\mu$ g/kg/day until harvest), preferably prior to surgery.

**The aim is to obtain a total harvest of at least  $3 \times 10^6$ /kg CD34+ cells, to be stored in at least 2 separate bags.**

CD34+ positive selection or other purging techniques are not recommended.

Harvest should be performed following stimulation with G-CSF. In case of mobilization failure with G-CSF, the use of plerixafor is allowed according to local practice.



### 5.4 Consolidation therapy regimen and ASCR

Patients with localized disease may proceed to consolidation following the front-line induction provided that there is no evidence of progression and the other eligibility criteria are met.

Patients with metastatic disease at diagnosis may proceed to consolidation after the front-line induction (RAPID COJEC +/- TVD) provided that a sufficient metastatic response has been achieved and the other eligibility criteria are met (see section 2.2).

For patients with metastatic disease not fulfilling the response criteria after induction, **an Expert's advice is highly recommended to define the best approach for such patients.**

Table 2: Consolidation therapy with Bu-Mel schedule

HD (days)	Bu-Mel	- 6	- 5	- 4	- 3	- 2	- 1	Day 0
<b>DRUG</b>	<b>DOSE</b>							
<b>Busulfan</b>	< 9kg: 1.0 mg/kg/dose 9 kg to < 16 kg : 1.2 mg/kg/dose 16 kg to 23 kg : 1.1 mg/kg/dose >23 kg to 34 kg: 0.95 mg/kg/dose >34 kg: 0.8 mg/kg/dose <b>Infusion IV over 2 hours</b> <b>Administration every 6 hours</b> <b>for a total of 16 doses</b>	•	•	•	•	•		
<b>Melphalan</b>	140 mg/m <sup>2</sup> /dose IV short infusion (15'), <b>at least</b> 24 h after the last busulfan dose						▲	
<b>Hydration</b>	3L/m <sup>2</sup> /day = 125 ml/m <sup>2</sup> /h	Continuous until Day 0 (24 h after Melphalan), then 1.5 ml/m <sup>2</sup> /day						
<b>Clonazepam</b>	0.025 – 0.1 mg/kg/day Total dose i.v as continuous infusion or divided in 3 oral doses/day	Continuous infusion from 12 hours before the first dose of Bu until Day +0 If the child is excessively drowsy then reduce dose						
<b>PBSC rescue</b>	Minimum 3X10 <sup>6</sup> /kg CD34+ i.v, <b>at least 24 hours</b> after the last dose of Melphalan							◆

#### 5.4.1 Busulfan

##### Drug Delivery

Busilvex<sup>®</sup> (iv busulfan) is commercially available throughout the European Union.

**Preparation and administration (Table 2 and 3):**

Busilvex® must be diluted prior to administration (see Appendix 9 “Drug Information”). A final concentration of approximately 0.5 mg/ml busulfan should be achieved. Busilvex® should be administered over 2 hours, by intravenous infusion via central venous catheter. Busilvex® should not be given by rapid intravenous, bolus or peripheral injection.

A total of 16 infusions should be administered every 6 hours, starting at day -6 up to day -2.

Table 3: Busulfan dosage guidelines

Actual body Weight (kg)	Busilvex® dose (mg/kg)
<9	1.0
9 to < 16	1.2
16 to 23	1.1
>23 to 34	0.95
>34	0.8

**Precautions**

All patients should be pre-medicated with anticonvulsant drugs to prevent seizures reported with the use of high-dose busulfan. It is recommended to administer anticonvulsants, starting 12 hours prior to Busilvex® up to 24 h after the last dose of Busilvex®.

**5.4.2 Melphalan**

The total dose of melphalan is 140 mg/m<sup>2</sup>/day. It should be administered **at least 24 hours after the last Busulfan dose**. No dose reduction is indicated based on body weight (i.e. 140 mg/m<sup>2</sup>/day even for children < 12 kg body weight).

**Drug Delivery**

Melphalan is commercially available throughout the European Union.

**Preparation**

Melphalan for intravenous administration, 50 mg vials.

Melphalan injection solution has limited stability and should be prepared immediately before use.

Melphalan is reconstituted at room temperature, from the lyophilised powder with 10 ml of the solvent diluent provided, by agitating until complete dissolution. The resultant solution contains 5 mg in 1 ml anhydrous Melphalan.

---

**Administration**

Either give undiluted or further diluted in normal saline to a maximum concentration of 0.4mg/ml. Short IV infusion through the central venous catheter over 10 to 15 minutes. Melphalan should be given within an hour of reconstitution. If this time is exceeded, a new batch of melphalan must be prepared. The diluent contains propylene glycol, which has been reported to cause hypotension and arrhythmias when infused intravenously in large doses. Care should be taken to prevent skin contact or inhalation of aerosolised particles of drug.

**5.4.3 Supportive care during Busulfan-Melphalan**

- During busulfan treatment, no systematic anti-emetic agent is needed. Anti-emetics should be given i.v. approximately 30 minutes prior to the melphalan injection and again scheduled post-melphalan, for a minimum of 24 hours after the last melphalan dose. Anti-emetic therapy may be administered according to institutional policy.
- Adequate hydration is crucial prior to and following melphalan administration due to bladder irritation from high urine concentrations of the drug. Minimal urine output immediately prior to and 24 hours following melphalan administration should be more than 90 ml/m<sup>2</sup>/h. To achieve this urine output, give i.v. hydration at 125 ml/m<sup>2</sup>/h.
- All patients should be pre-medicated with anticonvulsants (i.e. clonazepam) to prevent Busulfan related seizures. It is recommended to administer anticonvulsants starting 12 h prior to Busilvex® up to 24 h after the last dose of Busilvex®.
- **G-CSF 5µg/kg/day IV will be given daily beginning on Day +5 after ASCR.** G-CSF will continue until a stable increase of ANC > 1.0 x 10<sup>9</sup>/l.
- Prophylactic antifungal treatment with ketoconazole, itraconazole or fluconazole should not be used, because of the increased risk of SOS with these drugs in association with busulfan. For proven fungal infection or prolonged febrile neutropenia, amphotericin should be used.
- Antibiotics and antivirals should be given in line with the institutional policy whenever indicated but any prophylactic use is not recommended in view of side effects and potential drug interactions. SOS may occur with Bu-Mel therapy. Prophylaxis for SOS might be performed according to institutional policy. Careful observation of patients during Bu-Mel phase is required.

**5.5 Autologous Stem Cell Rescue (ASCR)**

Please note the stem cells should not be re-infused until **at least 24 hours after the end of Melphalan® infusion.**

**Dosage**

A minimum of 3x10<sup>6</sup> CD34+ stem cells/kg (optimum 5 x 10<sup>6</sup>/kg) must be available for each individual stem cell rescue.

**Premedication/Monitoring**

- Discontinue all other IV fluids where possible and replace them with 0.9% sodium chloride 4 hours prior to and after the stem cell infusion
- Fifteen minutes prior to the stem cell infusion, premedication with antihistamines might be performed according to local policies
- Ambubag, diphenhydramine and epinephrine should be available at bedside
- Place patient on cardiac monitor during infusion and for 1-2 hours following completion
- Discontinue all other IV fluids when possible during stem cell infusion to avoid volume overload
- Hydrate for at least 24 hours post stem cell infusion with 1500 ml/m<sup>2</sup>/day total IV fluids

**Administration**

Stem cells will be infused intravenously on Day 0, at least 24 hours after the end of Thiotepea or Melphalan administration, and within 60-90 minutes of thawing.

**5.6 Surgery**

The aim of surgery in HR-NBL is to achieve complete excision of the tumour with minimal morbidity to improve local control. **There is no place for surgery before induction chemotherapy other than biopsy**, since the risks of operation are higher and the outcome is not better.

The surgery will be performed after the end of induction, ideally after peripheral stem cell harvest.

If complications are expected that may postpone the following treatment, like:

- encasement of celiac axis AND/OR
- encasement of superior mesenteric artery AND/OR
- encasement of both renal pedicles.

surgery may be further postponed (HD Bu-Mel, based on physician decision).

The risk of removal a kidney is not a sufficient reason to postpone surgery until after HDC although everything must be done to save the kidney during surgery.

If induction chemotherapy has been so effective that there is no or minimal residual tumour, surgery may have no benefit.

Tumours where surgery is postponed or deemed not necessary should be discussed first with the **national/international Expert**. After a first surgery, if imaging shows a resectable residual disease, more than minimal residual tumour volume (see below), additional surgery should be considered.

---

### 5.6.1 Definition of Procedures

#### **Biopsy**

Biopsy should be the first procedure on all tumours.

Sufficient tissue must be obtained, ideally from two different areas of the tumour, to allow histological diagnosis and biological studies. In particular, it is essential that sufficient material is obtained for the accurate determination of *MYC* status.

Multiple (at least 4) needle core biopsies with a minimal suggested size of 14 G can provide sufficient tissue for diagnostic studies. If it does not appear sufficient or the tumour is inaccessible with a percutaneous approach, minimally invasive surgery may have advantage on an open approach. Optimum treatment is critically dependent on correct tissue handling. The tissue must not be fixed. Fresh tissue should be delivered to the pathologist immediately, when possible under sterile conditions.

#### **Complete excision**

Complete excision is defined as the removal of all visible tumours, including the removal of abnormal lymph nodes. Microscopic residual will be the most frequent situation.

#### **Excision with minimal residual tumour (MRT)**

Surgeon estimates the volume of residual tumour after surgery as less than 5 cm<sup>3</sup> (5 millilitres), which will be compared with the post-operative imaging.

#### **Incomplete excision (macroscopic residual tumour)**

Surgeon estimates the volume of residual tumour after surgery as more than 5 cm<sup>3</sup> (5 millilitres), which will be compared with the post-operative imaging.

### 5.6.2 Definition of Major Surgical Complications

- Death within 30 days of operation, or obviously related to the operation at any time
- Serious haemorrhage > 30% blood volume
- Serious vascular injury leading to loss of tissue viability
- Any spinal cord injury
- Serious peripheral nerve injury leading to loss of function
- Any organ failure
- Any other surgical complication that delays HDC more than 4 weeks after surgery and radiotherapy more than 90 days after ASCR

Please report any of the above complications as severe adverse event (SAE) within 24 hours of the investigator being aware to the National and International Data Centre.

---

### 5.6.3 Aspects of Surgical Procedures

#### **Surgery of the primary tumour**

The aim of surgery is to remove completely the primary tumour. All suspicious tissue should be excised. Resection should be attempted during or after completion of induction chemotherapy according to the induction regimen, unless there is tumour progression or imaging suggests that complete excision is likely to be associated with a significant risk of death or serious mutilation. In those circumstances, the option of further chemotherapy or alternative therapy should be discussed with **national/international Expert**. Vascular encasement is not a contra-indication to surgery, as this is often present, but it could influence the timing of surgery.

#### **Intraspinal extension**

If feasible, the extraspinal mass should be removed provided that the intraspinal disease is occupying less than 1/3<sup>rd</sup> of the spinal canal. Macroscopic disease may be left in the intervertebral foramina, in order to avoid deep dissection that may damage the spinal cord. If intraspinal disease is occupying more than 1/3<sup>rd</sup> of the spinal canal, the surgical strategy must be discussed with the **national/international Expert** with a formal neurosurgical opinion. If neurosurgical resection of the intraspinal component is indicated, it should be performed before the extraspinal component resection. Preoperative imaging could be performed for the identification of the Adamkevitz artery in lower left thoracic tumours.

#### **Nephrectomy**

Nephrectomy should be avoided whenever possible. Elective nephrectomy is discussed as part of the surgical planning if the kidney is part of the tumour mass to ensure adequate clearance, even before HDC. If on preoperative imaging there is evidence of ureteric obstruction and/or significant renal vessel encasement causing renal compromise, formal assessment of renal function in the form of DMSA (dimercaptosuccinic acid) scan should be considered, and the surgeon should make sure that vessels of the contralateral kidney are free from tumour.

Although radiation will impair renal function, this effect is not manifest for three to five years after treatment. This is a rationale to avoid nephrectomy whenever this is possible. This requires a meticulous dissection of the renal vessels preferably with magnifying loupes and the maintenance of an efficient perfusion of the kidney during this procedure. Papaverine may be used to avoid artery spasm.

#### **Tumour incision**

Incision of the tumour is permissible because this aids excision.

#### **Tumour relation with great vessels**

---

In order to gain further information on the accuracy of the pre-operative imaging, the intra-operative findings should be described in detail. Particular attention should be given to the technical difficulties encountered when the tumour is in contact with the vessels. The new international operative CRF will help to harmonize the collection of this information.

### **Risk factors related to tumour localisation**

Presence or absence of IDRFs is not relevant regarding surgical indication in high-risk patients, but might have an impact on surgical timing (see 5.6).

### **Clips**

Any residual unresectable tumour will be marked with MRI-compatible clips in order to facilitate the management of radiotherapy.

## **5.7 Radiotherapy**

All patients will receive 21.6 Gy-radiotherapy to the primary tumour site after HDC/ASCR. Metastatic sites should not be systematically irradiated.

Careful planning of the radiotherapy volume and dose is needed with consideration given to response, local status after surgery to the primary tumour and neighbouring organs. Some patients may be considered unsuitable for radiotherapy by reason of the site of the primary tumour and the volume which would require irradiation.

Discussion about administration of radiotherapy should include consideration of referral to a center with more extensive experience or more appropriate techniques.

In the last few years radiotherapy delivery technologies have advanced significantly with the development of intensity modulated radiation therapy (IMRT), and in particular dynamic rotational treatments or arc therapy – IMAT. IMAT equipment is supplied by a number of manufacturers under their own trade names including RapidArc™ (Varian), VMAT™ (Elekta) and TomoTherapy™ (Accuray). These offer the scope for treating irregularly shaped target volumes homogeneously, with much greater sparing of adjacent non-target normal tissues from the high dose irradiated volume, although there is greater exposure of normal tissues to low dose irradiation.

Proton therapy can also be considered as an alternative, highly conformal technique.

### **5.7.1 Timing of radiotherapy**

Local radiotherapy will be given after HDC/ASCR and prior to the maintenance treatment. After HD Bu-Mel, the interval between ASCR and radiotherapy must be greater than 60 days, due to the risk of busulfan-enhanced radiotoxicity and ideally not greater than 90 days. However, if more than 90 days are needed to allow haematological recovery, radiotherapy should still be given.

---

Irradiation of persistent metastatic sites is not recommended.

### 5.7.2 Planning and Dose of Radiotherapy

#### CT Planning

Radiotherapy planning should be based on preoperative imaging. Diagnostic contrast-enhanced CT and/or MRI scans performed at this time are required. Postoperatively, the surgical and pathological reports will also be taken into account, as will the postoperative imaging (MRI or CT and MIBG, performed after HDC). For the planning CT scan, it is recommended to position patients in a supine position with arms up if possible. Use of individualized immobilisation devices is recommended. A treatment planning CT with the patient in the treatment position is required. Centres should follow their local planning protocol, but slice thickness  $\leq 3$ mm would be expected. Intravenous contrast should be used unless clinically contraindicated, but a non-enhanced scan may also be required for proton dosimetry. General anaesthesia may be required for younger children. Motion management with 4D-CT is not specifically required but is allowed when it is standard practice in the department.

#### Volume

A virtual GTV1 should be defined on the planning CT-scan based on preoperative imaging. This will include the post-chemotherapy primary tumour and any immediately adjacent persistently enlarged lymph nodes. This GTV1 will be trimmed where, following surgery, uninvolved normal organs such as liver or kidney, which were previously displaced, have returned to their normal position.

The modified virtual GTV1 should be expanded to form a CTV1 by adding a margin which will normally be 0.5 cm. This margin of expansion may include adjacent soft tissues where there is a risk of subclinical tumour infiltration, but need not include barriers to spread such as bone. It should also include all areas of microscopic disease as indicated from the surgical report and the pathological examination.

The planning target volume 1 (PTV1) takes into account uncertainties of positioning and possible organ movement. The margin from CTV1 to PTV1 should be based on departmental audit of movement. Usually it will be 0.5 to 1.0 cm. The PTV1 should be encompassed by the 95 % isodose. The dose within the PTV1 should be between 95 and 107 %. 3D-conformal photon radiotherapy, IMRT, IMAT, electrons or proton beam therapy are allowed, whatever gives the more favorable dose distribution.

For patients with macroscopic residual disease treated in the SIOPEN HR-NBL2 trial and randomized in the boost dose arm, the GTV to be boosted (GTVb) will be defined on the imaging available at the time of radiotherapy. No additional margin to create a CTVb is necessary, so CTVb = GTVb. The margin from CTVb to PTVb should be based on departmental audit of movement. Usually it will be 0.5 to 1.0 cm. The PTVb should be encompassed by the 95 % isodose. The dose within the PTVb should be



---

between 95 and 107 %. 3D-conformal photon radiotherapy, IMRT, IMAT, electrons or proton beam therapy are allowed, whatever gives the more favorable dose distribution.

**Dose**

Doses will be specified according to the International Commission on Radiation Units and measurements (ICRU) recommendations. Patients should receive 21.6 Gy in 12 fractions of 1.8 Gy over not more than 17 days. If a single-phase technique to treat the PTV to 21.6 Gy would result in unacceptable irradiation of normal tissues, it is acceptable to use a two-phase technique with a volume reduction for phase 2.

**Fractionation**

Conventional 1.8 Gy per fraction, 5 fractions per week. All fields will be treated daily. Unavoidable interruptions to treatment should be compensated for, for example by treatment at weekends or by delivering two fractions a day with a six-hour inter-fraction interval, aiming to complete treatment within the same overall treatment time.

**Energy**

High energy photons from a linear accelerator or protons.

**Normal Tissue Tolerance**

Normal tissues within or adjacent to the treated volume may be dose limiting. Doses to normal tissue will be kept as low as reasonably achievable consistent with adequate treatment of the PTV and homogeneous treatment of vertebrae. The following recommendations should be considered.

*Liver*

The dose to the whole liver should not exceed 19 Gy. 21.6 Gy is acceptable for 50 % of the liver volume. Care must be taken if liver function has been compromised by chemotherapy toxicity.

*Spinal cord*

A dose of 21.6 is acceptable for any length of spinal cord. However, as there may be sensitization of the spinal cord after busulfan, it is wise to keep the spinal cord dose as low as reasonably achievable and not higher than 30 Gy.

*Kidney*

The tolerance of normal kidneys is 15 Gy. In patients treated for neuroblastoma renal function may be impaired by a number of factors including chemotherapy and surgery. It may be helpful to have an up to date assessment of renal function including GFR and DMSA scan. It is acceptable to treat one kidney to 21.6 Gy to treat the PTV1 to the prescribed dose providing the opposite kidney function is good.

*Bone*

---

There will be an inevitable effect on the epiphyses of vertebrae within the field of irradiation. Care should be given to maintain the symmetry by irradiation of the whole vertebra to around 21.6 Gy.

#### *Lungs*

Care must be taken to minimise the volume of lung irradiated because of a possible interaction with Busulfan. For example, a V12 of 50 % of total lung volume and a V15 of 25 % of total lung volume should not normally be exceeded, and in some circumstances where tolerance may be impaired a lower dose may be prudent.

#### *Heart*

If it is necessary to include all or part of the heart in the irradiated volume, care should be taken to minimise the dose, particularly when cardiotoxic chemotherapy i.e. doxorubicin has been used.

#### *Other sites*

Normal tissue tolerance is unlikely to be exceeded.

### 5.8 Maintenance phase

Following recovery from major HDC-related toxicities, patients should proceed with radiotherapy (starting between day 60 and day 90) and maintenance therapy if complete re-staging shows no evidence of progression.

Maintenance treatment, starting with one cycle of 13-cis-RA, and then followed by 5 cycles of dinutuximab beta and 13-cis-RA (Figure 6; Figure 7) should start as soon as the criteria are met, **ideally no later than day 120 post ASCR**.

In case of active infection, treatment should be delayed.

**It is acknowledged in the product information sheet for retinoic acid that peanut and soya protein may be used as excipients of this medication. It is advisable to watch carefully any child with known peanut and soya allergy whilst on this treatment.**

In order to achieve timely delivery of dinutuximab beta, ordering should take place at least two weeks prior to the start of immunotherapy.

All patients should receive dinutuximab beta according to the long-term infusion (LTI) schedule.

**If, for any reason, patient cannot receive immunotherapy with dinutuximab beta, the recommended maintenance treatment will be 6 cycles of oral 13-cis-RA.**

*Figure 6. Maintenance phase overview*

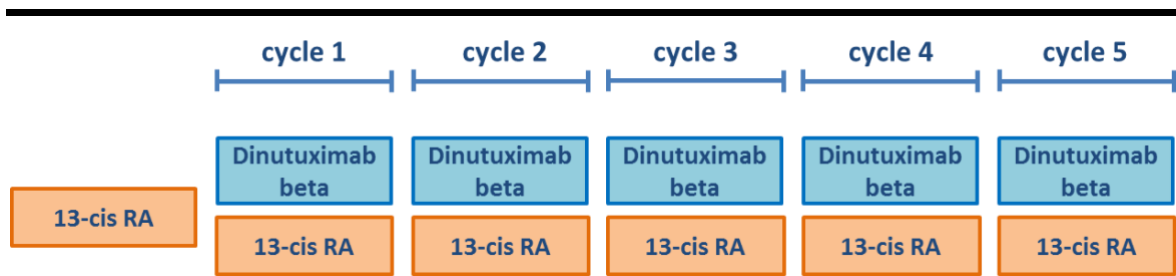
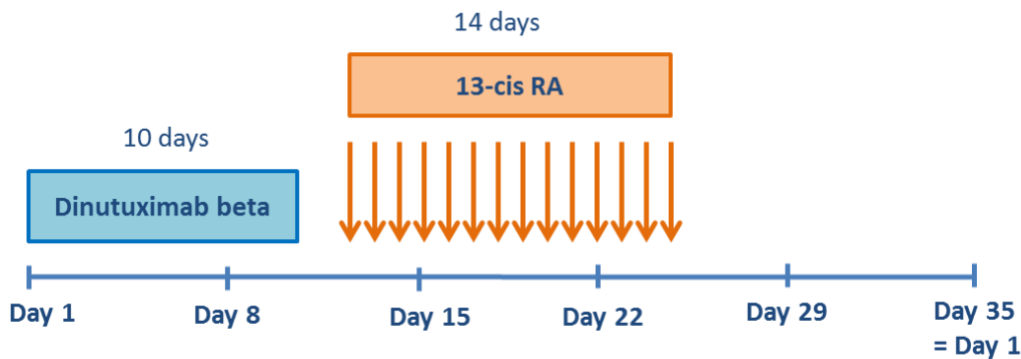


Figure 7. Maintenance cycle overview



### 5.8.1 Treatment schedule

Each complete dinutuximab beta + 13-cis-RA cycle will last 35 days.

#### 13-cis-RA

Patients will receive six cycles of 13-cis-RA.

The first cycle will be given prior to the first immunotherapy cycle at least one week after the end of radiotherapy.

The other five cycles will start **24 hours** after the completion of the dinutuximab beta continuous infusion.

- Each cycle consists of 160 mg/m<sup>2</sup>/day 13-cis-RA divided equally given orally twice a day for 14 days
- Patients unable to swallow 13-cis-RA capsules should receive a dose of 200mg/m<sup>2</sup>/day

#### Suggested Supportive Care for 13-cis-RA

- Topical vitamin E should be applied to the lips twice a day during 13-cis-RA therapy if cheilitis develops.
- Patients should avoid direct sun exposure while on 13-cis-RA.
- Patients should avoid exposure to vitamin A products during 13-cis-RA therapy.

#### Criteria prior to each Cycle of 13-cis-RA

- Total bilirubin  $\leq$  1.5 x normal, and ALT  $\leq$  5 x normal.
- SOS, if present, should be stable or improving.
- Skin toxicity  $\leq$  grade 1

- 
- Kidney toxicity  $\leq$  grade 1
  - Serum triglycerides  $<$  500 mg/dL
  - No haematuria and/or proteinuria on urinalysis
  - Serum calcium  $<$  11.6 mg/dL =  $<$  2.89 mmol/l

### Dinutuximab beta

Patients will receive five cycles of dinutuximab beta given every 5 weeks. Patients will receive dinutuximab beta continuously (LTI) over 10 days within each cycle.

Each dose is calculated based on the body surface area (BSA) or body weight as follows:

- Patients  $>$ 12 kg are dosed based on the BSA: 10 mg/m<sup>2</sup>/day
- Patients  $\leq$  12 kg are dosed according to their body weight: 0.33 mg/kg/day

Dinutuximab beta will be given according to the following administration schedule:

- The dinutuximab beta daily dose will be given intravenously as a continuous infusion over 24 hours over 10 consecutive days (**Day1 - Day10**)
- Start at least 7 days after the end of the previous 13-cis-RA cycle

Administration of dinutuximab beta should be started in an inpatient setting. In this setting, antibody will be delivered by daily infusions in syringes or infusion bags using standard infusion pumps.

If the therapy is well tolerated (oral/transdermal supportive care only) the patient may be discharged to a local outpatient setting. In this case, continuous infusion will continue in the outpatient setting. For this purpose “elastomeric infusion systems” may be used.

## 6. ASSESSMENTS

### 6.1 Disease assessment at diagnosis and during the treatment

Disease assessment will be performed according to the Revised International Neuroblastoma Criteria for Diagnosis, Staging and Response to Treatment (Table 4) [Park JR, JCO 2017; Burchill S, Cancer 2017]

*Table 4. Schedule of the disease evaluations throughout the trial*

Study steps	Study entry	Post Rapid Cojec +/- post 2 TVD	Post Bu-Mel, prior to RTx	Before maintenance	End of treatment
<sup>123</sup> I-mIBG scan (or FDG PET for MIBG negative cases)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> <sup>2</sup>	<input type="checkbox"/>
Primary tumour imaging (MRI or CT) <sup>a</sup>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> <sup>2</sup>	<input type="checkbox"/>
Primary tumour imaging (ultrasound) <sup>a</sup>	<input type="checkbox"/>				<input type="checkbox"/>
Cerebral imaging (MRI or CT) <sup>a</sup>	<input type="checkbox"/>	<input type="checkbox"/> <sup>1</sup>	<input type="checkbox"/> <sup>1</sup>		
Bilateral BM (trephine biopsy)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> <sup>2</sup>	<input type="checkbox"/>
Bilateral BM (aspirates)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> <sup>2</sup>	<input type="checkbox"/>
Pathology <sup>b</sup>	<input type="checkbox"/>				
Urinary Catecholamines	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>

<sup>a</sup> Imaging of the primary tumour and cerebral imaging: by MRI or CT scan as judged appropriate by the patient’s physician

<sup>b</sup> INPC classification and MYC status

<sup>1</sup> only for patients with brain metastases or major skull lesions at diagnosis

<sup>2</sup> to be repeated only if ≥ 8 weeks since last evaluation

## 6.2 Toxicity assessment during treatment

### 6.2.1 Overview of toxicity evaluation for RAPID COJEC induction before each course

Toxicity Evaluation for Rapid-Cojec Induction									
Course	A	B	C	B	A	B	C	B	End of induction
Cycle Number	1	2	3	4	5	6	7	8	
Physical examination	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Blood pressure	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Full blood counts	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Renal/liver function, electrolytes with Ca	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Tubular function		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
GFR		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>
Audiology		<input type="checkbox"/>				<input type="checkbox"/>			<input type="checkbox"/>

Note: for hearing function assessment, please see Appendix 3.

### 6.2.2 Overview of toxicity evaluation during consolidation phase

Timing	Before HD Bu-Mel	End of consolidation
Physical examination	<input type="checkbox"/>	<input type="checkbox"/>
Blood pressure	<input type="checkbox"/>	<input type="checkbox"/>
Full blood counts	<input type="checkbox"/>	<input type="checkbox"/>
Renal/liver function, electrolytes	<input type="checkbox"/>	<input type="checkbox"/>
Tubular function	<input type="checkbox"/>	<input type="checkbox"/>
GFR	<input type="checkbox"/>	<input type="checkbox"/>
Abdominal and Hepatic Ultrasound	<input type="checkbox"/>	<input type="checkbox"/>
Echocardiogram	<input type="checkbox"/>	<input type="checkbox"/> *
Chest Radiography	<input type="checkbox"/>	<input type="checkbox"/>

\* 1 month, 3 months and 6 months after Day 0 of HD Bu-Mel

### 6.2.3 Overview of toxicity evaluation during maintenance phase

Toxicity Evaluation during Maintenance Phase						
Timing	Before 1 <sup>st</sup> cycle	Before 2 <sup>nd</sup> cycle	Before 3 <sup>rd</sup> cycle	Before 4 <sup>th</sup> cycle	Before 5 <sup>th</sup> cycle	End of treatment
Physical examination	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Blood pressure	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Full blood counts	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Renal/liver	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Chest radiography	<input type="checkbox"/>		<input type="checkbox"/>			<input type="checkbox"/>
ECG	<input type="checkbox"/>		<input type="checkbox"/>			<input type="checkbox"/>
Eyes Assessment	<input type="checkbox"/>		<input type="checkbox"/>			<input type="checkbox"/>

## 7. SUMMARY OF KNOWN ADVERSE EVENTS ASSOCIATED WITH DINUTUXIMAB BETA

### Pain

Neuropathic pain usually occurs at the beginning of the treatment and premedication with analgesics, including intravenous opioids, prior to each infusion of dinutuximab beta is required. A triple therapy, including nonopioid analgesics (according to WHO guidelines), gabapentin and opioids, is recommended for pain treatment.

Nonsteroidal anti-inflammatory drugs (NSAIDs), i.e. ibuprofen or metamizole, should be carefully used due to their potential nephrotoxicity and risk of gastrointestinal bleeding in case of low platelet count.

### *Gabapentin*

The patient should be primed with 10 mg/kg/day, starting 3 days prior to dinutuximab beta infusion. The daily dose of gabapentin is increased to  $2 \times 10$  mg/kg/day orally the next day and to  $3 \times 10$  mg/kg/day orally the day before the onset of dinutuximab beta infusion and thereafter. The maximum single dose of gabapentin is 300 mg. This dosing schedule should be maintained for as long as required by the patient. Oral gabapentin should be tapered off after weaning off intravenous morphine infusion, at the latest after dinutuximab beta infusion therapy has stopped. However, if indicated, gabapentin administration could be maintained between cycles based on physician decision.

### *Opioids*

Treatment with opioids is standard with dinutuximab beta. However, according to patient's tolerance, treatment with only non-opioids drugs could be considered for the last cycles.

The first infusion day and the first course usually require a higher dose than subsequent days and courses.

### Administration:

- Before initiation of a continuous intravenous morphine infusion, a bolus infusion of 0.02 to 0.05 mg/kg/hour morphine should be started 2 hours before dinutuximab beta infusion.
- Subsequently, a dosing rate of 0.03 mg/kg/hour is recommended concomitantly with dinutuximab beta infusion.
- In response to the patient's pain perception, it may be possible to wean off morphine over 5 days by progressively decreasing its dosing rate (i.e. to 0.02 mg/kg/hour, 0.01 mg/kg/hour, 0.005 mg/kg/hour).

After weaning off intravenous morphine, in case of severe neuropathic pain, oral morphine sulphate (0.2 to 0.4 mg/kg every 4 to 6 hours) can be administered on demand. For moderate neuropathic pain, oral tramadol or clonazepam may be administered.

---

### Hypersensitivity reactions

Severe infusion-related reactions, including cytokine release syndrome (CRS), anaphylactic and hypersensitivity reactions, may occur despite the use of premedication. Occurrence of a severe infusion related reaction (including CRS) requires immediate discontinuation of dinutuximab beta therapy and may necessitate emergency treatment.

Cytokine release syndrome frequently manifests itself within minutes to hours of initiating the first infusion and is characterised by systemic symptoms such as fever, hypotension and urticaria.

Anaphylactic reactions may occur as early as within a few minutes of the first infusion with dinutuximab beta and are commonly associated with bronchospasm and urticaria.

Patients should be closely monitored for anaphylaxis and allergic reactions, particularly during the first and second treatment course.

#### *Premedication*

Antihistamine premedication (i.e. diphenhydramine) could be administered orally or intravenously approximately 20 minutes before starting each dinutuximab beta infusion according to physician decision. Antihistamine administration can be repeated every 4 to 6 hours if required during dinutuximab infusion.

#### *Treatment of hypersensitivity reactions*

Antihistamine, epinephrine (adrenaline) and prednisolone for intravenous administration should be immediately available during administration of dinutuximab beta to manage life-threatening allergic reactions. In case of bronchial and/or pulmonary hypersensitivity reaction, inhalation with adrenaline is recommended and should be repeated every 2 hours, according to clinical response.

### Capillary leak syndrome (CLS)

CLS is characterised by a loss of vascular tone and extravasation of plasma proteins and fluid into the extravascular space. CLS usually develops within hours after initiation of treatment, while clinical symptoms (i.e. hypotension, tachycardia) are reported to occur after 2 to 12 hours. Careful monitoring of circulatory and respiratory function is required. CLS should be treated according to institutional policies.

### Eye disorders

Eye disorders may occur as dinutuximab beta binds to optic nerve cells. No dose modification is necessary in the case of an impaired visual accommodation that is correctable with eye glasses, as long as this is judged to be tolerable.

Treatment must be interrupted in patients who experience grade  $\geq 3$  vision toxicity (i.e. subtotal vision loss per toxicity scale). In case of any eye problems, patients should be referred promptly to an ophthalmology specialist.



---

### Peripheral neuropathy

Occasional occurrences of peripheral neuropathy have been reported with dinutuximab beta. Cases of motor or sensory neuropathy lasting more than 4 days must be evaluated and non-inflammatory causes, such as disease progression, infections, metabolic syndromes and concomitant medication, should be excluded.

Treatment should be permanently discontinued in patients experiencing any objective prolonged weakness attributable to dinutuximab beta administration. For patients with grade 2 neuropathy treatment should be interrupted and may be resumed after neurologic symptoms resolve.

### Systemic infections

Patients are likely to be immunocompromised as a result of prior therapies. As they typically have a central venous catheter in situ, they are at risk of developing systemic infection. Patients should have no evidence of systemic infection and any identified infection should be under control before starting therapy. Pneumocystis prophylaxis is recommended.

### Haematologic toxicities

Occurrence of haematologic toxicities has been reported with dinutuximab beta, such as erythropenia, thrombocytopenia or neutropenia. Grade 4 haematologic toxicities improving to at least Grade 2 or baseline values by start of next treatment course do not require dose modification.

### Laboratory abnormalities

Regulatory monitoring of liver function and electrolytes is recommended.

### Interaction with other medicinal products

#### *Corticosteroids*

Due to their immunosuppressive activity, concomitant treatment with corticosteroids is not recommended within 2 weeks prior to the first treatment course until 1 week after the last treatment course with dinutuximab beta, except for life-threatening conditions.

#### *Vaccinations*

Vaccinations should be avoided during administration of dinutuximab beta until 10 weeks after the last treatment course, due to immune stimulation through dinutuximab beta and possible risk for rare neurological toxicities.

#### *Intravenous immunoglobulin*

Concomitant use of intravenous immunoglobulins is not recommended as they may interfere with dinutuximab beta-dependent cellular cytotoxicity.

## 8. DOSE MODIFICATIONS AND DELAYS

### 8.1 Induction

#### RAPID COJEC

*Course A dose modifications:*

- **Body weight > 5 kg but < 12 kg: VINCRISTINE 0.05 mg/kg, CARBOPLATIN 25 mg/kg, ETOPOSIDE (VP16) 5.833 mg/kg.**
- **Body weight ≤ 5 kg: a 1/3 reduction (from the mg/kg dose) is indicated.**
- Hepatotoxicity: Do not alter doses for abnormal transaminases. If Bilirubin 26-50 µmol/l then give 50% dose etoposide, if bilirubin ≥ 51 µmol/l omit etoposide and vincristine. Resume normal doses in subsequent cycle once liver function has improved. No dose modifications to carboplatin required.
- Renal function: no modification is required as long as normal urine output.
- Neurotoxicity: omit vincristine if neurotoxicity grade ≥3. Resume at 66% dose in subsequent cycle if recovered.
- Any other unresolved grade ≥ 3 toxicities – *an Expert's advice is highly recommended to define the best approach for such patients.*

*Course B dose modifications:*

- **Body weight > 5 kg but < 12kg: VINCRISTINE 0.05 mg/kg, CISPLATIN 2.666 mg/kg.**
- **Body weight ≤ 5 kg: a 1/3 reduction (from the mg/kg dose) is indicated.**
- Hepatotoxicity: Do not alter doses for abnormal transaminases. If Bilirubin ≥ 51 µmol/l omit vincristine. Resume normal doses in subsequent cycle once liver function has improved. No dose modifications to cisplatin required.
- Neurotoxicity: omit vincristine if neurotoxicity grade ≥ 3. Resume at 66% dose in subsequent cycle if recovered.
- Renal function: If GFR ≤ 60 ml/min/m<sup>2</sup> then omit cisplatin and discuss with *national/international Expert*
- Ototoxicity: if Boston grade ≥ 4 toxicity discuss with *national/international Expert*
- Any other unresolved grade ≥ 3 toxicities – *an Expert's advice is highly recommended to define the best approach for such patients.*

*Course C dose modifications:*

- **Body weight > 5 kg but < 12kg: VINCRISTINE 0.05 mg/kg, cyclophosphamide 35 mg/kg, ETOPOSIDE 5.8333 mg/kg.**
- **Body weight ≤ 5 kg: a 1/3 reduction (from the mg/kg dose) is indicated.**
- Hepatotoxicity: Do not alter doses for abnormal transaminases. If Bilirubin 26-50 µmol/l then give 50% dose etoposide, if bilirubin ≥ 51 µmol/l omit etoposide and vincristine. Resume normal doses in subsequent cycle once liver function has improved. No dose modifications to cyclophosphamide required.
- Neurotoxicity: omit vincristine if neurotoxicity grade ≥ 3. Resume at 66% dose in subsequent cycle if recovered.
- Haemorrhagic cystitis in previous cycle: increase Mesna dose by 50%.
- Any other unresolved grade ≥ 3 toxicities – *an Expert's advice is highly recommended to define the best approach for such patients.*

## TVD

Infants and children with a body weight below 12kg should be dosed according to their weight in kg instead of their body surface area (m<sup>2</sup>) according to the known formula of 30 kg = 1m<sup>2</sup>.

*Topotecan* 0.05 mg/kg/day

*Vincristine* 0.033 mg/kg/day

*Doxorubicin* 0.75 mg/kg/day

In infants weighing ≤ 5 kg, a further 1/3 dose reduction is advised.

## 8.2 Busulfan-melphalan

### Busulfan

In case of low body weight (< 10 kg) and/or hepatic impairment, *an Expert's advice is highly recommended to define the best approach for such patients.*

### Melphalan

In case of low body weight (< 10 kg) or nephrectomy, as well as in case of renal or hepatic impairment, *an Expert's advice is highly recommended to define the best approach for such patients.*

## 8.3 Maintenance treatment

### Dinutuximab beta

Based on the physician's evaluation of the severity of adverse drug reactions to dinutuximab beta, patients may undergo a dose reduction of 50% or an interruption of the infusion, temporarily or for the

---

entire cycle. As a consequence, either the infusion period is prolonged (for a maximum of 11 days) or, if tolerated by the patient, the infusion rate may be increased up in order to administer the total dose.

Treatment with dinutuximab beta should be permanently discontinued if the following toxicities occur:

- grade 3 or 4 anaphylaxis
- prolonged grade 2 peripheral motor neuropathy
- grade 3 peripheral neuropathy
- grade 3 visual eye toxicity
- grade 4 hyponatremia (< 120 mEq/L) despite appropriate fluid management
- recurrent or grade 4 capillary leak syndrome (requires ventilator support).

The solution should be administered via a peripheral or central intravenous line. Whenever possible, other intravenously co-administered agents should be delivered via a separate infusion line.

Pre-medication with antihistamines might be considered before starting each infusion according to institutional policies.

### **13-cis-RA**

A dose reduction of 25% (to 120 mg/m<sup>2</sup>/day) for subsequent cycles should be made for the occurrence of any grade 3 or 4 toxicity,

EXCLUDING: grade 3 or 4 haematologic, grade 3 hepatic, grade 3 nausea, grade 3 vomiting, or grade 3 fever that recover by the start of the following cycle.

If the same grade 3 or 4 toxicity recurs after a 25% dose reduction, then decrease the dose by another 20% (to 100 mg/m<sup>2</sup>/day). If the same grade 3 or 4 toxicity recurs after two dose reductions, **an Expert's advice is highly recommended** before continuing further therapy.

If the criteria to begin the next cycle are not met by the date the cycle is due to begin, delay the cycle for one week. If the criteria are still not met, treat at 25% dose reduction (120 mg/m<sup>2</sup>/day). An additional dose reduction to 100 mg/m<sup>2</sup>/day should occur if criteria are not met within one week after due date for subsequent cycles.

If serum creatinine increases by > 50% in any cycle, measured GFR should be carried out prior to commencing the next cycle. If GFR is < 50 ml/min/1.73 m<sup>2</sup>, then ask for **an Expert's advice** for dose adjustment.

If patient develops haematuria, proteinuria, and/or hypertension during any cycle of therapy, withhold medication and ask for **an Expert's advice**.

---

For localised cheilitis, apply topical vitamin E to lips for subsequent cycles. If this does not control symptoms sufficiently to allow sufficient oral intake, then decrease dose by 25% to 120 mg/m<sup>2</sup>/day.

If serum triglycerides are > 300 mg/dl when next cycle is due, delay starting therapy for two weeks. If still > 300 mg/dl, then start patient on medical therapy for serum triglyceride reduction and begin cycle at previous 13-cis-RA dosage. If serum triglycerides are < 300 mg/dl by time subsequent cycle is due, then continue at same dosage 13-cis-RA. If triglycerides are still > 300 mg/dl after one cycle on medical therapy, then reduce 13-cis-RA dosage by 25% for subsequent cycles.

## 9. PATIENT FOLLOW UP

After treatment discontinuation, progression-free patients will be followed at least for a time period of 5 years or until death, whichever occurs first.

Recommended follow-up investigations are focused on: a) Evaluation of disease status; b) Assessment of treatment sequelae; c) Facilitating novel research approaches.

A homogeneous approach to disease evaluation ensures that survival and toxicity calculations are comparable for all patients and treating centers. The systematic collection of samples according to standard operating procedures (SOPs) for research is strongly recommended since provides a unique resource for innovative translational research.

The following recommendations provide guidance on the minimum required follow-up data and actions. Individual centers and countries may have additional time points of evaluation and sample collection, according to local practice, national guidelines and research projects, covered by additional consent and ethical approvals.

### 9.1 Timing of patient's evaluation

The schedule for mandatory evaluation is as follows:

- Year 1 after the end of treatment : Every 3 months
- Year 2 and 3 after the end of treatment: Every 4 months
- Year 4 and 5 after the end of treatment: Every 6 months
- Then, patients will enter into long-term follow-up.

The minimum recommended elements that need to be evaluated at each visit are the following:

- History/ Symptoms
- Abnormal findings on clinical examination, Tanner stage
- Height, weight, blood pressure, growth percentile
- Urinary catecholamines (UC)
- Audiology with impedance/tympanogram and pure tone audiogram

---

At 1 year following the end of treatment, in addition the following assessments should be made:

- Full Blood Counts (FBC)
- Biochemistry, including renal/liver function, electrolytes, calculated GFR (BCH)
- Urinary catecholamines (UC)

During subsequent annual visits the evaluation of FBC, BCH, and UC will be guided by symptoms and clinical findings.

#### Primary Tumour Evaluation

- Ultrasound of the primary site will be performed at each visit. In case of a thoracic primary disease, an MRI (preferable) or CT scan is suggested.

#### Metastatic Disease Evaluation

- No mandatory MIBG (or PET) scan is required, regardless of the persistence of bone uptakes at the end of treatment.
- No mandatory BM aspirates and/or BM trephines are required by the protocol, regardless of the persistence of bone marrow disease at the end of treatment. \*
- However, in case of persistent metastatic disease at the end of treatment, a metastatic disease evaluation could be performed at 1 year interval.\*

### **9.2 Long term follow-up assessment**

Ideally, patients will be followed for a long term period of at least 15 years to collect information on long-term toxicities, late relapses and second malignancies.

During the long-term follow-up period, data will be collected from examination performed at routine follow-up visit of the disease and recorded in the medical file of the patient.

All efforts must be undertaken by the study sites to record these data but no additional protocol visits will be required.

#### Hearing (See Appendix 3)

Hearing tests should be done at the end of treatment, one year and five years after the end of treatment. This should include a minimum of impedance/tympanogram and a pure tone audiogram including 8KHz and the reliability of both tests should be stated. When children are young they tire quickly, the high frequencies should always be tested before the lower ones when assessing high-frequency hearing loss. If the impedance/tympanogram is abnormal this indicates glue ear and conductive hearing loss and the audiogram should not be graded for ototoxicity and the test should be repeated after 3 months. Oto-acoustic emissions are not behavioural tests and are therefore not adequate to assess hearing. They are only useful as screening tests. Auditory Evoked Potentials have no place in the behavioural testing of hearing and should not be performed.

---

An additional evaluation should be performed before school entry at the age of 5 to ensure adequate hearing function to support the education of the child.

In the case of pathological test results, appropriate follow-up and intervention according to local standards should be instituted.

#### Cardiac Function

Electrocardiogram and cardiac ultrasound should be done at the end of treatment and at one year after the end of treatment. If no abnormal finding is detected, an evaluation every 5 years is indicated.

In the case of pathological test results, appropriate follow-up and intervention according to local standards should be instituted.

#### Lung Function

Pulmonary function test should be performed 3 years after the end of treatment, or later on depending on patient's compliance. In the case of pathological test results, appropriate follow-up and intervention according to local standards should be instituted.

#### Thyroid Function

Impairment of thyroid function may be a late event. Thyroid evaluation (TSH and thyroid gland ultrasound) is recommended at the end of treatment then once a year.

#### Gonadal Function

Follow-up through puberty and fertility/ovarian function should be carefully evaluated.

In addition to a full-history and clinical evaluation:

- Boys: evaluation of FSH, LH and testosterone level at the age of 10-12 years; a spermiogram whenever possible.
- Girls: gynecological and endocrinological follow-up is of major importance. Evaluation of FSH, LH, estradiol, Anti-Müllerian hormone (optional) + follicular ultrasound at puberty and later on according to each patient's case.

#### Assessment of growth and development

Longitudinal growth assessment with accurate measurements of growth and critical analysis of growth data is essential because of the risk of short stature. Bone age (right wrist X-ray) evaluation is not mandatory.

#### Neurocognitive development

Formal neurocognitive evaluation and psychometrics are not mandatory. Elements like school attendance and function, academic achievement recording, task completion should be part of the history taking with each clinic visit. Formal evaluation will depend on symptoms and findings or local clinical practice and research.

#### Second Malignant Neoplasms (SMNs)

Second Malignant Neoplasm is a very important and serious patient outcome.

Recording of SMNs, with exact location, staging and histology is essential to reflect true patient outcome following diagnosis and treatment of patients with high-risk neuroblastoma.

### 10. REFERENCE LIST

- Abbasi MR, Rifatbegovic F, Brunner C, Ladenstein R, Ambros IM, Ambros PF. Bone marrows from neuroblastoma patients: an excellent source for tumour genome analyses. *Mol Oncol.* 2015;9(3):545-54.
- Ambros PF, Ambros IM, Kerbl R, Luegmayr A, Rumpler S, Ladenstein R, et al. Intratumoural heterogeneity of 1p deletions and MYCN amplification in neuroblastomas. *Med Pediatr Oncol.* 2001;36(1):1-4.
- Amoroso L, Erminio G, Makin G, Pearson ADJ, Brock P, Valteau-Couanet D, et al. Topotecan-Vincristine-Doxorubicin in Stage 4 High-Risk Neuroblastoma Patients Failing to Achieve a Complete Metastatic Response to Rapid COJEC: A SIOPEN Study. *Cancer Res Treat.* 2018;50(1):148-155.
- Asgharzadeh S, Salo JA, Ji L, Oberthuer A, Fischer M, Berthold F, et al. Clinical significance of tumour-associated inflammatory cells in metastatic neuroblastoma. *J Clin Oncol.* 2012;30(28):3525-32.
- Berthold F, Boos J, Burdach S, Erttmann R, Henze G, Hermann J, et al. Myeloablative megatherapy with autologous stem-cell rescue versus oral maintenance chemotherapy as consolidation treatment in patients with high-risk neuroblastoma: a randomised controlled trial. *Lancet Oncol* 2005;6:649-658.
- Burchill SA, Beiske K, Shimada H, Ambros PF, Seeger R, Tytgat GA, et al. Recommendations for the standardization of bone marrow disease assessment and reporting in children with neuroblastoma on behalf of the International Neuroblastoma Response Criteria Bone Marrow Working Group. *Cancer.* 2017;123(7):1095-1105.
- Cheung NK, Kushner BH, LaQuaglia M, Kramer K, Gollamudi S, Heller G, et al. N7: a novel multi-modality therapy of high risk neuroblastoma (NB) in children diagnosed over 1 year of age. *Med Pediatr Oncol.* 2001;36(1):227-30.
- Chicard M, Boyault S, Colmet Daage L, Richer W, Gentien D, Pierron G, et al. Genomic Copy Number Profiling Using Circulating Free Tumour DNA Highlights Heterogeneity in Neuroblastoma. *Clin Cancer Res.* 2016 Nov 15;22(22):5564-5573.



- 
- Chicard M, Colmet-Daage L, Clement N, Danzon A, Bohec M, Bernard V, et al. Whole-Exome Sequencing of Cell-Free DNA Reveals Temporo-spatial Heterogeneity and Identifies Treatment-Resistant Clones in Neuroblastoma. *Clin Cancer Res.* 2018 Feb 15;24(4):939-949.
  - Combaret V, Bergeron C, Noguera R, Iacono I, Puisieux A. Circulating MYCN DNA predicts MYCN-amplification in neuroblastoma. *J Clin Oncol.* 2005;23(34):8919-20;
  - Combaret V, Iacono I, Bellini A, Bréjon S, Bernard V, Marabelle A, et al. Detection of tumour ALK status in neuroblastoma patients using peripheral blood. *Cancer Med.* 2015 Apr;4(4):540-50.
  - Corrias MV, Parodi S, Tchirkov A, Lammens T, Vicha A, Pasqualini C, et al. Event-free survival of infants and toddlers enrolled in the HR-NBL-1/SIOPEN trial is associated with the level of neuroblastoma mRNAs at diagnosis. *Pediatr Blood Cancer.* 2018 Mar 30:e27052. doi: 10.1002/pbc.27052. [Epub ahead of print]
  - Decock A, Ongenaert M, Cannoodt R, Verniers K, De Wilde B, Laureys G, et al. Methyl-CpG-binding domain sequencing reveals a prognostic methylation signature in neuroblastoma. *Oncotarget.* 2016 Jan 12;7(2):1960-72.
  - Eleveld TF, Oldridge DA, Bernard V, Koster J, Daage LC, Diskin SJ, et al. Relapsed neuroblastomas show frequent RAS-MAPK pathway mutations. *Nat Genet.* 2015;47(8):864-71.
  - Federico SM, McCarville MB, Shulkin BL, Sondel PM, Hank JA, Hutson P, et al. A Pilot Trial of Humanized Anti-GD2 Monoclonal Antibody (hu14.18K322A) with Chemotherapy and Natural Killer Cells in Children with Recurrent/Refractory Neuroblastoma. *Clin Cancer Res.* 2017;23(21):6441-6449.
  - Furman WL, Shulkin BL, Federico SM, McCarville MB, Davidoff AM, Krasin MJ, et al. Early response rates and Curie scores at end of induction: An update from a phase II study of an anti-GD2 monoclonal antibody with chemotherapy in newly diagnosed patients with high-risk neuroblastoma. *J Clin Oncol.* 2017;35:15\_suppl, 10534-10534.
  - Hartmann O, Valteau-Couanet D, Benhamou E, et al. Stage IV neuroblastoma in patients over 1 year of age at diagnosis: consolidation of poor responders with combined busulfan, cyclophosphamide and melphalan followed by in vitro mafosfamide-purged autologous bone marrow transplantation. *Eur J Cancer.* 1997;33: 2126-2129.
  - Hartmann O, Valteau-Couanet D, Vassal G, Lapiere V, Brugieres L, Delgado R, et al. Prognostic factors in metastatic neuroblastoma in patients over 1 year of age treated with high-dose chemotherapy and stem cell transplantation: a multivariate analysis in 218 patients treated in a single institution. *Bone marrow transplantation.* 1999;23(8):789-95.
  - George RE, Li S, Medeiros-Nancarrow C, et al. High-risk neuroblastoma treated with tandem autologous peripheral-blood stem cell-supported transplantation: long-term survival update. *J Clin Oncol* 2006;24:2891-2896.

- 
- Granger M, Grupp SA, Kletzel M, et al. Feasibility of a tandem autologous peripheral blood stem cell transplant regimen for high risk neuroblastoma in a cooperative group setting: A Pediatric Oncology Group study: A Report from the Children's Oncology Group. *Pediatr Blood Cancer*. 2012;59:902-907.
  - Grupp SA, Stern JW, Bunin N, et al. Tandem high-dose therapy in rapid sequence for children with high-risk neuroblastoma. *J Clin Oncol* 2000;18:2567-2575.
  - Handgretinger R, Anderson K, Lang P, Dopfer R, Klingebiel T, Schrappe M, et al. A phase I study of human/mouse chimeric antiganglioside GD2 antibody ch14.18 in patients with neuroblastoma. *Eur J Cancer*. 1995;31A(2):261-7.
  - Kohler JA, Ellershaw C, Machin D; Neuroblastoma Working Group of the Children's Cancer and Leukaemia Group. Response to N7 induction chemotherapy in children more than one year of age diagnosed with metastatic neuroblastoma treated in UKCCSG centers. *Pediatr Blood Cancer*. 2007;49(3):234-9.
  - Kletzel M, Katzenstein HM, Haut PR, et al. Treatment of high-risk neuroblastoma with triple-tandem high-dose therapy and stem-cell rescue: results of the Chicago Pilot II Study. *J Clin Oncol* 2002;20:2284-2292.
  - Kreissman SG, Seeger RC, Matthay KK, London WB, Sposto R, Grupp SA, et al. Purged versus non-purged peripheral blood stem-cell transplantation for high-risk neuroblastoma(COG A3973): a randomised phase 3 trial. *Lancet Oncol*. 2013;14(10):999-1008.
  - Kretschmar CS, Kletzel M, Murray K, Thorner P, Joshi V, Marcus R, et al. Response to paclitaxel, topotecan, and topotecan-cyclophosphamide in children with untreated disseminated neuroblastoma treated in an upfront phase II investigational window: a pediatric oncology group study. *J Clin Oncol*. 2004;22(20):4119-26
  - Kushner BH, Kramer K, LaQuaglia MP, Modak S, Yataghene K, Cheung NK. Reduction from seven to five cycles of intensive induction chemotherapy in children with high-risk neuroblastoma. *J Clin Oncol*. 2004;22(24):4888-92.
  - Kushner BH, Kramer K, Modak S, Cheung NK. Sensitivity of surveillance studies for detecting asymptomatic and unsuspected relapse of high-risk neuroblastoma. *J Clin Oncol*. 2009;27(7):1041–1046.
  - Ladenstein R, Poetschger U, Gray J, Valteau-Couanet D, Luksch R, Castel V, et al. Toxicity and outcome of anti-GD<sub>2</sub> antibody ch14.18/CHO in front-line, high-risk patients with neuroblastoma: Final results of the phase III immunotherapy randomisation (HR-NBL1/SIOPEN trial). *J Clin Oncol*. 2016; 34(15\_suppl):10500AB-10500.
  - Ladenstein R, Pötschger U, Pearson ADJ, Brock P, Luksch R, Castel V, et al. Busulfan and melphalan versus carboplatin, etoposide, and melphalan as high-dose chemotherapy for high-risk neuroblastoma (HR-NBL1/SIOPEN): an international, randomised, multi-arm, open-label, phase 3 trial. *Lancet Oncol*. 2017;18(4):500-514.

- 
- Ladenstein R, Valteau-Couanet D, Brock P, Yaniv I, Castel V, Laureys G, et al. Randomized Trial of prophylactic granulocyte colony-stimulating factor during rapid COJEC induction in pediatric patients with high-risk neuroblastoma: the European HR-NBL1/SIOPEN study. *J Clin Oncol.* 2010;28(21):3516-24.
  - Längler A, Christaras A, Abshagen K, Krauth K, Hero B, Berthold F. Topotecan in the treatment of refractory neuroblastoma and other malignant tumours in childhood - a phase-II-study. *Klin Padiatr.* 2002;214(4):153-6.
  - Lode HN, Valteau-Couanet D, Garaventa A, Gray J, Castel V, Yaniv I, et al. Long-term infusion of anti-GD2 antibody ch14.18/CHO in combination with interleukin-2 (IL2) activity and efficacy in high-risk relapsed/refractory neuroblastoma patients. *J Clin Oncol.* 2015; 33(15\_suppl):10080.
  - Matthay KK, Villablanca JG, Seeger RC, Stram DO, Harris RE, Ramsay NK, et al. Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-cis-retinoic acid. Children's Cancer Group. *The New England Journal of Medicine.* 1999;341(16):1165-73.
  - Matthay KK, Reynolds CP, Seeger RC, Shimada H, Adkins ES, Haas-Kogan D, *et al.* Long-term results for children with high-risk neuroblastoma treated on a randomized trial of myeloablative therapy followed by 13-cis-retinoic acid: a Children's Oncology Group study. *J Clin Oncol* 2009; 27:1007-1013; Errata, *J Clin Oncol* 2014; 32:1862-1863.
  - Mody R, Naranjo A, Van Ryn C, Yu AL, London WB, Shulkin BL, et al. Irinotecan-temozolomide with temsirolimus or dinutuximab in children with refractory or relapsed neuroblastoma (COG ANBL1221): an open-label, randomised, phase 2 trial. *Lancet Oncol.* 2017;18(7):946-957.
  - Mujoo K, Cheresch DA, Yang HM, Reisfeld RA. Disialoganglioside GD2 on human neuroblastoma cells: target antigen for monoclonal antibody-mediated cytotoxicity and suppression of tumour growth. *Cancer Res.* 1987;47(4):1098-104.
  - Mora J, Cruz O, Lavarino C, Rios J, Vancells M, Parareda A, et al. Results of induction chemotherapy in children older than 18 months with stage-4 neuroblastoma treated with an adaptive-to-response modified N7 protocol (mN7). *Clin Transl Oncol.* 2015;17(7):521-9.
  - Owens C, Li BK, Thomas KE, Irwin MS. Surveillance imaging and radiation exposure in the detection of relapsed neuroblastoma. *Pediatr Blood Cancer.* 2016;63(10):1786-1793.
  - Pasqualini C, Dufour C, Goma G, Raquin MA, Lapierre V, Valteau-Couanet D. Tandem high-dose chemotherapy with Thiotepa and busulfan-melphalan and autologous stem cell transplantation in very high-risk neuroblastoma patients. *Bone Marrow Transplant.* 2016;51(2):227-31.
  - Park JR, Kreissman SG, London WB, Naranjo A, Cohn SL, Hogarty MD, et al. A phase III randomized clinical trial (RCT) of tandem myeloablative autologous stem cell transplant (ASCT) using peripheral blood stem cell (PBSC) as consolidation therapy for high-risk neuroblastoma (HR-NB): A Children's Oncology Group (COG) study. *J Clin Oncol.* 2016;34:18\_suppl, LBA3-LBA3.

- 
- Park JR, Bagatell R, Cohn SL, Pearson AD, Villablanca JG, Berthold F, et al. Revisions to the International Neuroblastoma Response Criteria: A Consensus Statement From the National Cancer Institute Clinical Trials Planning Meeting. *J Clin Oncol*. 2017;35(22):2580-2587.
  - Park JR, Scott JR, Stewart CF, London WB, Naranjo A, Santana VM, et al. Pilot induction regimen incorporating pharmacokinetically guided topotecan for treatment of newly diagnosed high-risk neuroblastoma: a Children's Oncology Group study. *J Clin Oncol*. 2011;29(33):4351-7.
  - Park JR, Slattery J, Gooley T, Hawkins D, Lindsley K, Villablanca JG, et al. Phase I topotecan preparative regimen for high-risk neuroblastoma, high-grade glioma, and refractory/recurrent pediatric solid tumours. *Med Pediatr Oncol*. 2000;35(6):719-23.
  - Pearson AD, Pinkerton CR, Lewis IJ, Imeson J, Ellershaw C, Machin D, et al. High-dose rapid and standard induction chemotherapy for patients aged over 1 year with stage 4 neuroblastoma: a randomised trial. *Lancet Oncol*. 2008;9(3):247-56.
  - Peifer M, Hertwig F, Roels F, Dreidax D, Gartlgruber M, Menon R, et al. Telomerase activation by genomic rearrangements in high-risk neuroblastoma. *Nature*. 2015;526(7575):700-4.
  - Pritchard J, Cotterill SJ, Germond SM, Imeson J, de Kraker J, Jones DR.. High dose wmelphalan in the treatment of advanced neuroblastoma: results of a randomized trial (ENSG-1) by the European Neuroblastoma Study Group. *Pediatr Blood Cancer*. 2005;44:348-357.
  - Proust-Houdemont S, Pasqualini C, Blanchard P, Dufour C, Benhamou E, Goma G, et al. Busulfan-melphalan in high-risk neuroblastoma: the 30-year experience of a single institution. *Bone Marrow Transplant*. 2016;51:1076-1081.
  - Robbins E. Radiation Risks From Imaging Studies in Children With Cancer. *Pediatr Blood Cancer*. 2008;51(January):453-457.
  - Saarinen-Pihkala UM, Hovi L, Koivusalo A, Jahnukainen K, Karikoski R, Sariola H, Wikström S.. Thiotepa and melphalan based single, tandem, and triple high dose therapy and autologous stem cell transplantation for high risk neuroblastoma. *Pediatr Blood Cancer*. 2012;59:1190-1197.
  - Saleh MN, Khazaeli MB, Wheeler RH, Allen L, Tilden AB, Grizzle W, et al. Phase I trial of the chimeric anti-GD2 monoclonal antibody ch14.18 in patients with malignant melanoma. *Hum Antibodies Hybridomas*. 1992;3(1):19-24.
  - Schramm A, Köster J, Assenov Y, Althoff K, Peifer M, Mahlow E, et al. Mutational dynamics between primary and relapse neuroblastomas. *Nat Genet*. 2015 Aug;47(8):872-7.
  - Seif AE, Naranjo A, Baker DL, Bunin NJ, Kletzel M, Kretschmar CS, et al. A pilot study of tandem high-dose chemotherapy with stem cell rescue as consolidation for high-risk neuroblastoma: Children's Oncology Group study ANBL00P1. *Bone Marrow Transplant*. 2013;48:947-952.
  - Shimada H, Umehara S, Monobe Y, Hachitanda Y, Nakagawa A, Goto S, et al. International neuroblastoma pathology classification for prognostic evaluation of patients with

---

peripheral neuroblastic tumours: a report from the Children's Cancer Group. *Cancer*. 2001 Nov 1;92(9):2451-61.

- Simon T, Hero B, Bongartz R, Schmidt M, Müller RP, Berthold F. Intensified external-beam radiation therapy improves the outcome of stage 4 neuroblastoma in children > 1 year with residual local disease. *Strahlenther Onkol*. 2006;182(7):389-94.

- Simon T, Hero B, Faldum A, Handgretinger R, Schrappe M, Niethammer D, Berthold F. Consolidation treatment with chimeric anti-GD2-antibody ch14.18 in children older than 1 year with metastatic neuroblastoma. *J Clin Oncol*. 2004;22(17):3549-57.

- Valteau-Couanet D, Le Deley MC, Bergeron C, Ducassou S, Michon J, Rubie H, et al. Long-term results of the combination of the N7 induction chemotherapy and the busulfan-melphalan high dose chemotherapy. *Pediatric Blood & Cancer*. 2014;61(6):977-981.

- Valteau-Couanet D, Michon J, Boneu A, Rodary C, Perel Y, Bergeron C, et al. Results of induction chemotherapy in children older than 1 year with a stage 4 neuroblastoma treated with the NB 97 French Society of Pediatric Oncology (SFOP) protocol. *J Clin Oncol*. 2005;23(3):532-40.

- Vermeulen J, De Preter K, Naranjo A, Vercruyse L, Van Roy N, Hellemans J, et al. Predicting outcomes for children with neuroblastoma using a multigene-expression signature: a retrospective SIOPEN/COG/GPOH study. *Lancet Oncol*. 2009 Jul;10(7):663-71.

- Viprey VF, Gregory WM, Corrias MV, Tchirkov A, Swerts K, Vicha A, et al. Neuroblastoma mRNAs predict outcome in children with stage 4 neuroblastoma: a European HR-NBL1/SIOPEN study. *J Clin Oncol*. 2014 Apr 1;32(10):1074-83.

- Yu AL, Gilman AL, Ozkaynak MF, London WB, Kreissman SG, Chen HX, et al. Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. *The New England Journal of Medicine*. 2010;363(14):1324-1334.

- Yu AL, Uttenreuther-Fischer MM, Huang CS, Tsui CC, Gillies SD, Reisfeld RA, Kung FH. Phase I trial of a human-mouse chimeric anti-disialoganglioside monoclonal antibody ch14.18 in patients with refractory neuroblastoma and osteosarcoma. *J Clin Oncol*. 1998;16(6):2169-80.

## APPENDIX 1 - TUMOUR STAGING

### INSS Staging

**Stage 1** Localised tumour with complete gross excision, with or without microscopic residual disease; representative ipsilateral lymph nodes negative for tumour microscopically (nodes attached to and removed with the primary tumour may be positive).

**Stage 2A** Localised tumour with incomplete gross excision; representative ipsilateral non-adherent lymph nodes negative for tumour microscopically.

---

**Stage 2B** Localised tumour with or without complete gross excision, with ipsilateral non-adherent lymph nodes positive for tumour. Enlarged contralateral lymph nodes must be negative microscopically.

**Stage 3** Unresectable unilateral tumour, infiltrating across the midline\*, with or without regional lymph node involvement; **or** localised unilateral tumour with contralateral regional lymph node involvement; **or** midline tumour with bilateral extension by infiltration or lymph node involvement.

**Stage 4** Any primary tumour with dissemination to distant lymph nodes, bone, bone marrow, liver and/or other organs.(except as defined for Stage 4S).

**Stage 4S** Localised primary tumour (as defined for Stage 1, 2A or 2B), with dissemination limited to liver, skin, and/or bone marrow †. (limited to infants < 1 year of age)

#### Notes

Multi-focal primary tumours (e.g. bilateral adrenal primary tumours) should be staged according to the greatest extent of disease, as defined above, and be followed by a subscript "M" (e.g. Stage 3M).

\* The midline is defined as the vertebral column. Tumours originating on one side and crossing the midline must infiltrate to or beyond the opposite side of the vertebral column.

† Marrow involvement in stage 4s should be minimal, i.e. less than 10% nucleated cells on bone marrow biopsy or quantitative assessment of nucleated cells on marrow aspirate. More extensive marrow involvement should be considered stage 4. The MIBG scan (if done) should be negative in the marrow for stage 4s.

#### International Neuroblastoma Risk Group Staging System. [Monclair T, JCO 2009]

##### Stage

- L1** Localised tumour not involving vital structures as defined by the list of image-defined risk factors and confined to one body compartment
- L2** Loco-regional 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. Patients with multifocal primary tumours should be staged according to the greatest extent of disease as defined in the table.

## APPENDIX 2: CRITERIA FOR ADVERSE EVENTS

### NATIONAL CANCER INSTITUTE - COMMON TERMINOLOGY\*

\*Except for Hearing (see Appendix 3)

---

*National Cancer Institute - Common Terminology Criteria for Adverse Events*  
(NCI-CTCAE, Version 5.0)

[https://ctep.cancer.gov/protocoldevelopment/electronic\\_applications/docs/CTCAE\\_v5\\_Quick\\_Reference\\_5x7.pdf](https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf)



Cancer Therapy Evaluation Program

<http://ctep.cancer.gov/>

### **APPENDIX 3: HEARING**

Common Terminology for Toxicity Criteria are not well adapted to high-frequency hearing loss from cisplatin in children. In order to assess and compare hearing loss in clinical trials SIOP agreed an adaptation of the Brock ototoxicity hearing scale at the SIOP AGM in Boston 2010. This was published in 2012 (Brock et al.). All children should be tested with a measure of impedance/tympanogram to exclude glue ear (conductive hearing loss) and then a pure tone audiogram starting with the high frequencies and always including 8KHz. The audiologist should be made aware that they are testing for high-frequency hearing and no audiogram should be accepted for analysis unless it has 8KHz measured. As children tire quickly high-frequencies should be tested first. If 500Kz and 250Hz are not tested this is not a problem. Otoacoustic emission and Auditory Brain Resonances/Evoked potentials should not be used as these do not test behavioural hearing and cannot be graded.

### Brock classification of cisplatin-induced bilateral high-frequency hearing loss

Bilateral hearing loss	Grade	Designation
< 40 dB at all frequencies	0	Minimal
=/> 40 dB at 8,000 Hz only	1	Mild
=/> 40 dB at 4,000 Hz and above	2	Moderate
=/> 40 dB at 2,000 Hz and above	3	Marked
=/> 40 dB at 1,000 Hz and above	4	Severe

The results used are obtained by pure-tone audiometry from the "better" ear

Brock grade 0 is not equivalent to normal hearing

### SIOP Boston classification of cisplatin-induced bilateral high-frequency hearing loss

Bilateral hearing loss	Grade	Designation
≤ 20 dB at all frequencies	0	Normal
≥ 20 dB at 6,000 or 8,000 Hz and above	1	Minimal
≥ 20 dB at 4,000 Hz and above	2	Mild
≥ 20 dB at 2,000 or 3,000 Hz and above	3	Moderate
≥ 40 dB at 2,000 Hz and above <sup>†</sup>	4	Marked

The results used are obtained by pure-tone audiometry, from the "better" ear  
<sup>†</sup> < 40 dB at all lower frequencies.

Designed at the 42<sup>nd</sup> SIOP Annual meeting in Boston 2010 Brock P et al. 2012 in JCO

- Brock PR, Bellman SC, Yeomans EC, Pinkerton CR, Pritchard J. Cisplatin ototoxicity in children: a practical grading system. Med Ped Oncol. 1991;19:295-300.
- Brock PR, Knight KR, Freyer DR, et al. Platinum-induced ototoxicity in children: a consensus review on mechanisms, predisposition, and protection, including a new International Society of Pediatric Oncology Boston ototoxicity scale. J Clin Oncol.2012;30(19):2408-17.



**APPENDIX 4: PERFORMANCE SCALE**

LANSKY Play Performance Scale (patients aged 16 years and below)

100%	Fully active, normal
90%	Minor restrictions in physically strenuous activity
80%	Active, but tires more quickly
70%	Both greater restriction of and less time spent in play activity
60%	Up and around, but minimal active play; keeps busy with quieter activities
50%	Gets dressed but lies around much of the day, no active play, able to participate in all quiet play and activities
40%	Mostly in bed; participates in quiet activities
30%	In bed; needs assistance even for quiet play
20%	Often sleeping; play entirely limited to very passive activities
10%	No play; does not get out of bed
0%	Unresponsive

KARNOFSKY Performance Scale (patients above 16 years)

100%	Normal, no complaints, no evidence of disease
90%	Able to carry on normal activity, minor signs or symptoms of disease
80%	Normal activity with effort, some signs or symptoms of disease
70%	Cares for self. Unable to carry on normal activity or to do active work.
60%	Requires occasional assistance, but is able to care for most of own needs
50%	Requires considerable assistance and frequent medical care
40%	Disabled, requires special care and assistance
30%	Severely disabled, hospitalisation is indicated although death is not imminent
20%	Hospitalisation necessary, very sick, active supportive treatment necessary
10%	Moribund, fatal processes progressing rapidly
0%	Dead

**APPENDIX 5: INTERNATIONAL NEUROBLASTOMA RESPONSE CRITERIA**

Data from both prospective and retrospective trials were used to refine the International Neuroblastoma Response Criteria (INRC)[Park JR, JCO 2017; Burchill S, Cancer 2017]:

- Overall response integrates tumour response in the primary tumour, soft tissue and bone metastases, and bone marrow.
- Primary and metastatic soft tissue sites are assessed using Response Evaluation Criteria in Solid Tumours (RECIST) and <sup>123</sup>I-MIBG scans or [<sup>18</sup>F]fluorodeoxyglucose–positron emission tomography scans if the tumour is MIBG nonavid.
- Bone marrow is assessed by histology or immunohistochemistry and cytology or immunocytology. BM with ≤ 5% tumour involvement will be classified as minimal disease.
- Urinary catecholamine levels are not included in response assessment.
- Overall response will be defined as complete response, partial response, minor response, stable disease, or progressive disease.

**Primary (soft tissue) Tumour Response**

Response	Anatomic + MIBG (FDG-PET+) Imaging
CR	< 10 mm residual soft tissue at primary site AND Complete resolution of MIBG or FDG-PET uptake (for MIBG-nonavid tumors) at primary site
PR	≥ 30% decrease in longest diameter of primary site AND MIBG or FDG-PET uptake at primary site stable, improved, or resolved
PD	> 20% increase in longest diameter taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study) AND Minimum absolute increase of 5 mm in longest dimension‡
SD	Neither sufficient shrinkage for PR nor sufficient increase for PD at the primary site

**Determination of Overall Response**

Response	Criterion
CR	All components meet criteria for CR
PR	PR in at least one component and all other components are either CR, MD* (bone marrow), PR (soft tissue or bone), or NI‡; no component with PD
MR	PR or CR in at least one component but at least one other component with SD; no component with PD
SD	SD in one component with no better than SD or NI‡ in any other component; no component with PD
PD	Any component with PD

**BM metastasis response**

Response	Cytology†/Histology‡
CR	Bone marrow with no tumor infiltration on reassessment, independent of baseline tumor involvement
PD	Any of the following: Bone marrow without tumor infiltration that becomes > 5% tumor infiltration on reassessment OR Bone marrow with tumor infiltration that increases by > two-fold and has > 20% tumor infiltration on reassessment
MD	Any of the following: Bone marrow with ≤ 5% tumor infiltration and remains > 0 to ≤ 5% tumor infiltration on reassessment OR Bone marrow with no tumor infiltration that has ≤ 5% tumor infiltration on reassessment OR Bone marrow with > 20% tumor infiltration that has > 0 to ≤ 5% tumor infiltration on reassessment
SD	Bone marrow with tumor infiltration that remains positive with > 5% tumor infiltration on reassessment but does not meet CR, MD, or PD criteria

**Tumour Response at Metastatic Soft Tissue and Bone Sites**

Response	Anatomic + MIBG (FDG-PET*) Imaging
CR	Resolution of all sites of disease, defined as: Nonprimary target and nontarget lesions measure < 10 mm AND Lymph nodes identified as target lesions decrease to a short axis < 10 mm AND MIBG uptake or FDG-PET uptake (for MIBG-nonavid tumors) of nonprimary lesions resolves completely
PR	≥ 30% decrease in sum of diameters† of nonprimary target lesions compared with baseline AND all of the following: Nontarget lesions may be stable or smaller in size AND No new lesions AND ≥ 50% reduction in MIBG absolute bone score (relative MIBG bone score ≥ 0.1 to ≤ 0.5) or ≥ 50% reduction in number of FDG-PET-avid bone lesions‡§
PD	Any of the following: Any new soft tissue lesion detected by CT/MRI that is also MIBG avid or FDG-PET avid Any new soft tissue lesion seen on anatomic imaging that is biopsied and confirmed to be neuroblastoma or ganglioneuroblastoma Any new bone site that is MIBG avid A new bone site that is FDG-PET avid (for MIBG-nonavid tumors) AND has CT/MRI findings consistent with tumor OR has been confirmed histologically to be neuroblastoma or ganglioneuroblastoma > 20% increase in longest diameter taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study) AND minimum absolute increase of 5 mm in sum of diameters of target soft tissue lesions Relative MIBG score ≥ 1.2§
SD	Neither sufficient shrinkage for PR nor sufficient increase for PD of nonprimary lesions

APPENDIX 6: BONE MARROW SAMPLING

GUIDELINES

Evaluation of the bone marrow (BM) is mandatory.

Bone marrow aspirates and trephines should be obtained from right and left posterior iliac crests from various bone marrow locations, i.e. a total of four samples, **two aspirates and two trephines**.

The bone marrow evaluation takes place at study entry, during induction, prior to consolidation, after HDC, during maintenance and at the end of treatment.

The following guidelines have been developed for the purpose of improving initial staging accuracy, treatment response evaluation, and, ultimately, patient care, by enabling a highly sensitive technique for detection and characterisation of rare neuroblastoma cells or tumour cell associated RNA.[Burchill SA, Cancer 2017]

**Bone Marrow Aspirations**

BM aspirations are necessary for bone marrow smears, immunocytology, or other techniques.

The aspirations from the different sites should not be pooled together unless indicated. Two to four syringes with plugs and 10 to 20 glass slides for the bone marrow smears and one polished cover glass should be prepared.

- Aspiration of half a milliliter (0.5 ml) of BM into the syringe and **immediately** dropped on a glass slide.
- Aspiration of 0.2 - 0.5 ml of BM for 10 smears per side air dried for cytology (i.e. Pappenheim stained, keep at least 5 slides unstained).

The methods for preparation of mononuclear cells (MNC), processing, sending and storage of cytopins, evaluation of immunocytological stainings and reporting of results in the SIOPEN Bone Marrow data bank have been standardised in the SIOPEN Bone Marrow Speciality Committee and described in detail elsewhere.[Burchill SA, Cancer 2017]

---

Immunocytological staining can also be combined with FISH and evaluated using an automated scanning and relocation system (AIPF) (i.e. Metafer4/RCDetect, MetaSystems, Altlussheim, Germany).

### **Bone marrow trephine biopsies**

The bone marrow trephine biopsies must be sampled from two sites, i.e., the right and left posterior iliac crests. Trephine biopsies should contain at least 0.5 cm of marrow (better 1 cm).

It is highly recommended to store material and slides bone marrow samples. This is important to conduct further/future biological and genetic analyses and to allow review and quality assessment studies.

### ***References:***

- Burchill SA, Beiske K, Shimada H, Ambros PF, Seeger R, Tytgat GA, et al. Recommendations for the standardization of bone marrow disease assessment and reporting in children with neuroblastoma on behalf of the International Neuroblastoma Response Criteria Bone Marrow Working Group. *Cancer*. 2017;123(7):1095-1105.

- Viprey VF, Corrias MV, Kagedal B, Oltra S, Swerts K, Vicha A, et al. Standardisation of operating procedures for the detection of minimal disease by QRT-PCR in children with neuroblastoma: quality assurance on behalf of SIOPEN-R-NET. *Eur J Cancer*. 2007;43(2):341-350.

## **APPENDIX 7: NUCLEAR MEDICINE GUIDELINES**

In patients with neuroblastoma, radionuclide imaging is indicated for diagnostic purposes, staging and response evaluation during and after treatment. The complexity of diagnostic procedures, radiation burden and necessity to acquire high quality images entail that imaging should be performed in centers with paediatric expertise.

Indications for radionuclide imaging in neuroblastoma are the following:

- Confirmation of suspected neuroectodermally derived tumours, including neuroblastoma, pheochromocytoma and ganglioneuroma
- Staging of the disease
- Assessment of response evaluation during treatment

**The Gold Standard radionuclide imaging in neuroblastoma is the Meta-iodobenzylguanidine (mIBG) scan with SPECT or SPECT/CT acquisitions.**

### ***META-IODOBENZYLGUANIDINE (mIBG)***

<sup>123</sup>I-mIBG has shown a sensitivity ranging between 88% and 92% and a specificity of 83%-92% [17].

### ***Interfering drugs***

---

Many drugs interfere with the uptake and/or retention of mIBG, particularly tricyclic antidepressants (such as amitriptyline), sympathomimetics and some anti-hypertensives (labetalol, reserpine).

Commonly used medications for asthma and cough containing sympathomimetics may also interfere with mIBG uptake. The length of time required before mIBG can be administered after exposure to an interfering medication varies: normally four biological half-lives are sufficient; however there are exceptions, such as labetalol, which requires a longer withdrawal time.

#### *Thyroid blockade*

Thyroid blockade will be performed according to local policies to prevent thyroid uptake of free radioactive iodide dissociating from the mIBG molecule.

#### *Administered activity*

The minimum <sup>131</sup>I-mIBG recommended injected activity, according to the EANM Pediatric Dosage card, is 35 MBq (0.95 mCi) and the maximum is 78 MBq (2.11 mCi).

Meta-iodobenzylguanidine is injected slowly, over 2 minutes or longer, to avoid reactions (especially hypertension, nausea, vomiting and pallor), and flushed thoroughly with saline. Very rarely a patient may have an anaphylactic reaction to mIBG. Most adverse reactions can be avoided by the slow injection technique. Children at risk for hypertensive episodes should be monitored during and shortly after the mIBG injection. Central lines may be used as long as they are flushed with adequate amount of saline.

#### *Images Acquisition*

Motion artifacts, low-resolution images, low-count statistics should be avoided, in order to obtain images of quality as high as possible. The use of sedation or restraining/distraction techniques should be assessed in each case.

Images are acquired 20-24 hours after <sup>123</sup>I-mIBG injection. Early images (4-6 hours post-injection) are no longer routinely recommended.[Bombardieri E, Eur J Nucl Med Mol Imaging 2010] Additional imaging at 48 hours may be very occasionally considered in an attempt to clarify a subtle finding of low grade uptake in comparison to background. The choice of the collimator that provides the best image quality with <sup>123</sup>I-mIBG scintigraphy should be left to the nuclear medicine department.

Careful positioning of the child is crucial, and every effort should be made to place the child at the shortest distance from the collimator.

Images acquisition should be performed as a whole body acquisition in the anterior and posterior projections (4-5 cm/min), whereas in young infants spot views are preferred because of higher resolution. Spot views of body segments can be acquired with about 500 Kcounts per spot (100 Kcounts for lower limbs). Skull imaging requires four views (anterior, posterior and lateral projections) since

---

possible lesions of the skull base or on the orbital plan may be better appreciated on lateral views. In case of full bladder, a delayed static view of the pelvis, once the child has voided, should be attempted. Scanning with  $^{131}\text{I}$  mIBG is performed at 48 hours after injection, and it can be repeated at 72 hours or later. Images can be acquired as a total body scan (speed 4cm/sec) or spot views of the head, neck, chest, abdomen, pelvis, upper and lower extremities (>150 Kcounts).

SPECT is an integral part of the  $^{123}\text{I}$ -mIBG acquisition and should be routinely utilised where available to clarify the anatomical location of abnormal foci of mIBG uptake. A SPECT acquisition protocol consists of 120 projections, in steps of 3 degrees each, in continuous or step-and-shoot mode, 25-35 sec/step, with a 128 x 128 matrix.

In comparison to SPECT alone, SPECT/CT further improves mIBG uptake localisation and certainty of lesion detection [Fukuoka M, Clin Nucl Med 2011]. There are two possible ways of using the CT component of the SPECT/CT study. It can be acquired with diagnostic quality parameters and intravenous radiological contrast. If this protocol is possible, it has the great advantage of performing two examinations in one session. If the CT component of the SPECT/CT examination is done for anatomical localisation and attenuation correction only, then the child will need a fully diagnostic contrast enhanced CT scan or an MRI scan, with the purpose of providing anatomical details of the primary tumour and its relations with the surrounding structures. In this case the radiation dose from the CT component of the SPECT/CT study should be kept as low as possible and the CT acquisition may be limited to abnormal or equivocal sites of mIBG uptake.

There are several protocols for low dose and ultra-low dose CT acquisitions. A possible low-dose CT acquisition may include a voltage around 80-100 kVp and a tube current of approximately 10-40 mAs. With such a kind of low-dose CT acquisition, and with a CT scan limited to the region of interest, the radiation dose administered to the patient is very low, usually within a range of 0.2 – 0.5 mSv [Gelfand MJ, Q J Nucl Med Mol Imaging 2010].

#### *Interpretation of Scan Findings*

The interpretation of mIBG scan should be performed in conjunction with recent cross sectional imaging modalities. In particular, combination of mIBG imaging and MRI can increase the sensitivity and specificity [Pfluger T, AJR Am J Roentgenol 2003].

When mIBG does not adequately depict the full extent of the disease further imaging with alternative tracers should be considered. [Kroiss A, Eur J Nucl Med Mol Imaging 2011; Melzer HI, Eur J Nucl Med Mol Imaging 2011; Piccardo A, Eur J Nucl Med Mol Imaging 2014] This is an area that requires further evaluation (see relevant section of the guidelines on PET tracers).

#### *False Positive Results*

---

These include atelectasis, pneumonia, physiologic liver heterogeneity and focal nodular hyperplasia in the liver (especially in the left lobe), post-radiotherapy changes in the liver, focal pyelonephritis, vascular malformations, an accessory spleen, renal tract dilatation with stasis of mIBG excreted in the urine, adrenal abscess, foregut duplication cyst, vascular anomaly, haemorrhagic cyst, ovarian torsion, diaphragmatic hepatic hernia, chronic inflammatory focus. A SPECT/CT acquisition, or correlation with morphological imaging modalities, is helpful in providing additional information, thus considerably reducing the number of equivocal reports.

#### *False Negative Results*

Approximately 10% of neuroblastomas demonstrate no mIBG uptake.[Vik TA, Pediatric Blood Cancer] In some cases these lesions show somatostatin analogue uptake or glycolytic activity with FDG PET/CT. Small lesions, especially if with low-grade uptake, may be missed if below the resolution of the gamma camera and because of partial volume effect. Meta-iodobenzylguanidine shows a poor sensitivity in the liver, due to the physiologic hepatic mIBG relatively high and heterogeneous uptake; sensitivity is also limited in the brain. Lung lesions, especially if small, can be missed or inaccurately located if situated in the lower lobe and close to the diaphragm, because of free breathing during the acquisition. The addition of SPECT and possibly SPECT/CT significantly increases the sensitivity of <sup>123</sup>I-mIBG scan, especially in case of lesions adjacent to sites of high mIBG uptake (such as heart, liver, primary tumour).

#### *Scoring system* [Lewington V, Eur J Nucl Med Mol Imaging 2017]

The SIOPEN score divides the skeleton in 12 anatomic segments. The extension score for this method is graded as follows: 0 = no sites per segment; 1 = one discrete site per segment; 2 = two discrete sites per segments; 3 = three discrete lesions; 4 = > 3 discrete foci or a single diffuse lesion involving <50% of the segment; 5 = diffuse involvement of 50-95% of the segment; 6 = diffuse involvement of the entire segment.

#### **The SIOPEN score will be reported at each mIBG evaluation.**

The SIOPEN score will be centrally reviewed at the end of induction and at relapse.

The report of mIBG scan performed before and after HDC, and at the end of treatment expressed in terms of SIOPEN score should be integrated with the description of bone sites of disease, in order to differentiate disease relapse/recurrence from progression.

#### **References:**

- Bombardieri E, Giammarile F, Aktolun C, Baum RP, Bischof Delaloye A, Maffioli L, et al. 131I/123I-metaiodobenzylguanidine (mIBG) scintigraphy: procedure guidelines for tumour imaging. Eur J Nucl Med Mol Imaging 2010;37:2436-46.

- 
- Fukuoka M, Taki J, Mochizuki T, Kinuya S. Comparison of diagnostic value of I-123 MIBG and high-dose I-131 MIBG scintigraphy including incremental value of SPECT/CT over planar image in patients with malignant pheochromocytoma/paraganglioma and neuroblastoma. Clin Nucl Med. 2011 Jan;36(1):1-7.
  - Gelfand MJ. Dose reduction in pediatric hybrid and planar imaging. Q J Nucl Med Mol Imaging. 2010;54(4):379-88.
  - Lewington V, Lambert B, Poetschger U, Sever ZB, Giammarile F, McEwan AJ, et al. <sup>123</sup>I-mIBG scintigraphy in neuroblastoma: development of a SIOPEN semi-quantitative reporting method by an international panel. Eur J Nucl Med Mol Imaging. 2017;44(2):234-241.

## APPENDIX 8: HARVEST GUIDELINES

It is recommended that pediatric apheresis procedure should be performed by an accredited SCT program and experienced pediatric team.

### *Timing*

Patients will undergo harvest of PBSC following the end of the induction. Harvest may be scheduled either after the last chemotherapy cycle or out of steady state mobilization preferable before surgery. The aim is to obtain a total CD34 harvest of at least  $3 \times 10^6$ /kg cells in at least 2 separate bags. In vitro purging of the graft is NOT recommended.

### **General Principles of the Technique**

Although operating procedures differ for the various apheresis systems, certain principles apply to all types of equipment.

- Continuous-flow (CF) systems are preferred for pediatric use because they have smaller extracorporeal volumes (ECV).
- In older children with a body weight greater than 40 kg, the technique is very similar to that used in adults. It is in small children that significant modifications of techniques are required to provide safe and effective procedure.

The two most important factors for safe apheresis procedures in pediatric patients are the maintenance of both a constant extracorporeal volume and an adequate red blood cell mass in the circulation.

### **PBSC Mobilization**

Patients should begin Granulocyte Colony Stimulating Factor (G-CSF) starting one day after completing the cycle of induction chemotherapy. They should continue on G-CSF 5 µg/kg/day while recovering from chemotherapy cycle until the post-nadir ANC > 500-1000/µL, at which point it is discussed to increase the dose of G-CSF to at least 10 µg/kg/day.



---

For steady state mobilization, G-CSF is given daily for 4-5 days in a dose of 10 µg/kg/day. It is critical that G-CSF be given daily until PBSC collection is complete. If the WBC is > 60,000/µL, either hold or decrease G-CSF dose per institutional guidelines.

It is recommended to use circulating CD34 cell counts and to begin the collection when the count is ≥ 20 cells/L.

Infants (< 12 months) should only undergo PBSC harvest in highly experienced centers and transfer for this procedure needs to be considered in time. For children weighing less than 15 kg, it is recommended that the cell separator is primed with packed red cells suspended. Decision should take into consideration the patient's blood count. Alternatively, centers can also use the institutional guidelines.

### ***Catheter Use***

PBSC may be collected using a large bore double lumen central venous catheter that will allow at least 10ml/min inlet flow rate required for apheresis. Many institutions use temporary or tunneled apheresis catheters in neuroblastoma patients. A percutaneous radial artery line may also be placed to facilitate collection. Bleeding risks for patients with thrombocytopenia who have also received substantial volumes of ACD should be addressed during catheter placement and removal.

For continuous flow apheresis, two sites of venous access are required. In patients less than 25 kg use for example the MedComp 8.0 French permanent or temporary catheter as required. For patients greater than 25 kg, the MedComp ≥8.0 French or other central venous lines can be used. Depending on the situation of the peripheral veins, a Hickman catheter could be used in combination with a peripheral venous access, also in very small children. A percutaneous radial artery line may also be placed to facilitate collection.

### **Apheresis Machine**

Apheresis machines equipped with continuous flow centrifugation, such as the Optia are recommended because these devices are better suited for the needs of small children as compared to discontinuous flow machines. Equipment should be operated in compliance with the manufacturer's operating guidelines.

The Standard of Care protocols should be written and available in the Apheresis Unit. The standard operating procedure will be specific for each machine.

### ***Blood Priming***

Priming of the machine prior to collection should be with saline according to manufacturer's directions. ACD-A will be used as Anti-coagulant, Heparin can be added to the ACD-A, according to the institutional decision.

The blood prime will be performed with cross-matched, irradiated, filtered red cells.

---

### ***Procedural Support***

There is evidence in the literature that apheresis procedure can be performed in children with platelet counts below  $20 \times 10^9$  /L. However the risk of bleeding following the administration of large dose of ACD in patients with extreme thrombocytopenia should be kept in mind.

### ***Anticoagulant***

Anticoagulant to be used is Acid Citrate Dextrose Formula - A (ACD-A) in a ratio sufficient to prevent extracorporeal clotting. Heparin can be added to the ACD-A according to the institutional decision.

The inlet AC infusion rate should be 0.8/ml/min or less in order to avoid the need of calcium supplement

*\*NOTE:* Hypocalcemia is a well-recognized side effect of citrate. To prevent hypocalcaemia a prophylactic calcium gluconate infusion or scheduled oral calcium supplementation can be used. If patient becomes symptomatic from hypocalcaemia then give oral calcium or alternatively the rate of the calcium gluconate infusion can be increased.

### **Whole Blood Flow Rate**

The choice of whole blood flow rates should follow local protocols or manufacturers recommendations.

The inlet AC ratio should be 13 – 25 according the institute protocol.

### **Collection Goals**

During each leukapheresis procedure, the volume of whole blood processed should be approximately 240 to 480 ml/kg (4 total blood volumes) depending on the patient's weight and machine use. The total time necessary for the whole apheresis procedure should not exceed 5 h.

Optimally, the stem cell collection should have a targeted goal of  $3 \times 10^6$  CD 34+cells/kg, the cells to be subdivided **into  $\geq 2$  units to provide adequate stem cells for 1 transplant**. The targeted number of cells can usually be obtained in 1-3 collection days.

### **Patient Monitoring**

Patients should be observed continuously during the collection. Vital signs should be obtained every 15 minutes, especially for patients <10Kg.

### **Laboratory Studies**

For patients < 20 kg, a type and cross compatibility test for peripheral red blood cells, or an equivalent test, should be performed one day prior to procedure.

Pre-apheresis and immediately post-apheresis lab values should be obtained: CBC with differential and platelet count, ionised calcium and magnesium.

---

### PBSC Analyses

The following studies are recommended for each PBSC collection:

- 1) Culture for bacterial and fungal contamination,
- 2) Nucleated cell count and differential,
- 3) CD34+ cell enumeration
- 4) Cell viability

### Cryopreservation of PBSC Products

Each collection should be processed and cryopreserved within 18 hours of collection using 5-10% dimethyl sulfoxide (DMSO) final concentration, controlled-rate freezer, and liquid nitrogen storage with appropriate monitoring according to institutional SOP's. Stem cells should be frozen at a final concentration of 0.5 to 4 x 10<sup>8</sup> nucleated cells/ml **in at least 3 bags**. The DMSO concentration in the infused bags should not exceed 1mg/Kg/day.

## APPENDIX 9: DRUG INFORMATION

### *Carboplatin*

The Summary of Product Characteristics of carboplatin can be loaded from the website of each National Health Authority.

### *Cisplatin*

The Summary of Product Characteristics of cisplatin can be loaded from the website of each National Health Authority.

### *Etoposide*

The European Summary of Product Characteristics of the approved formulation of Etoposide can be loaded from the EMA website, [www.ema.europa.eu](http://www.ema.europa.eu), => [Find medicine](#) => Human medicines  
=> Etopophos

(or) by following this link:

[http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Referrals\\_document/Etopophos\\_30/WC500226214.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/Etopophos_30/WC500226214.pdf)

### *Cyclophosphamide*

The Summary of Product Characteristics of cyclophosphamide can be loaded from the website of each National Health Authority.

*Vincristine*

The Summary of Product Characteristics of vincristine can be loaded from the website of each National Health Authority.

*Doxorubicin*

The Summary of Product Characteristics of doxorubicin can be loaded from the website of each National Health Authority.

*Busulfan*

The European Summary of Product Characteristics of the approved formulation of Busulfan BUSILVEX® can be loaded from the EMA website, [www.ema.europa.eu](http://www.ema.europa.eu), => [Find medicine](#) => Human medicines => busulfan (or) BUSILVEX®,  
or by following this link:

[http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000472/human\\_med\\_000681.jsp&mid=WC0b01ac058001d124](http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000472/human_med_000681.jsp&mid=WC0b01ac058001d124)

*Melphalan*

The Summary of Product Characteristics of melphalan can be loaded from the website of each National Health Authority.

*13-cis-RA*

The European Summary of Product Characteristics of the approved formulation of 13-cis-RA can be loaded from the EMA website, [www.ema.europa.eu](http://www.ema.europa.eu), => [Find medicine](#) => Human medicines => retinoids,

or by following this link:

[http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/referrals/Retinoids\\_containing\\_medicinal\\_products/human\\_referral\\_prac\\_000061.jsp&mid=WC0b01ac05805c516f](http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/referrals/Retinoids_containing_medicinal_products/human_referral_prac_000061.jsp&mid=WC0b01ac05805c516f)

*Dinutuximab beta*

The European Summary of Product Characteristics of the approved formulation of Dinutuximab beta can be loaded from the EMA website, [www.ema.europa.eu](http://www.ema.europa.eu) or by following this link:

[http://www.ema.europa.eu/docs/en\\_GB/document\\_library/EPAR\\_-\\_Product\\_Information/human/003918/WC500227724.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/003918/WC500227724.pdf)