Sponsor Protocol N°: 2019/2894

EudraCT : 2019-001068-31

High-Risk Neuroblastoma Study 2 of SIOP-Europa-Neuroblastoma (SIOPEN)

HR-NBL2/SIOPEN

V1.1 dated 19th July 2019

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FOLLOW-UP OF VERSIONS

Version	Date	Description of substantial modifications
1.1	19/07/2019	Initial Version

STUDY COMMITTEE

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This protocol was produced following discussions from the following countries and groups

AGPHO	Austrian Group of Haematology and Oncology
AIEOP	Associazione Italiana Ematologia Oncologia Pediatrica
ANZCHOG	Australia and New Zealand Children's Haematology/Oncology Group
BSPHO	Belgian Society of Pediatric Haematology and Oncology
CRCTU	Cancer Research UK Clinical Trials Unit
DCOG	Dutch Childhood Oncology Group
GPOH	German Society of Pediatric Oncology and Haematology
NCRI CCL CSG	UK National Cancer Research Institute Children's Cancer and Leukaemia Clinical Studies Group
HSPHO	Hellenic Society of Pediatric Haematology and Oncology
ISPHO	Israeli Society of Pediatric Haematology and Oncology
NOPHO	Nordic Society for Pediatric Haematology and Oncology (Norway, Sweden, Denmark, Finland)
PSPOH	Polish Society of Pediatric Oncology and Hematology
SFCE	Société Française des Cancers et Leucémies de l'Enfant et de l'Adolescent
SEHOP	Spanish Society of Pediatric Haematology & Oncology
SFOP	Société Française d'Oncologie Pédiatrique
SIAK	Switzerland

As well as the following countries: Portugal, Ireland, and Serbia

SIGNATURE PAGE

Sponsor Protocol N°: 2019/2894

EudraCT : 2019-001068-31

"HR-NBL2/SIOPEN"

V1.1 dated 19th July 2019

Study site:

I have read and approve this protocol. My signature confirms the agreement that the clinical study will be conducted in accordance with the protocol and all applicable laws and regulations including, but not limited to, European regulations, Guideline for Good Clinical Practice (GCP), the ethical principles that have their origins in the Declaration of Helsinki, applicable privacy laws and applicable study specific procedures, and that all persons assisting with the clinical study are adequately trained about the protocol, all subsequent amendments and the investigational products.

Nothing in this document is intended to limit the authority of a physician to provide emergency medical care under applicable regulations.

This protocol describes the HR-NBL2 trial and provides information about procedures for patients taking part in the HR-NBL2 trial. I will not recommend this protocol as a guide for treatment of patients not taking part in the HR-NBL2 trial and will not provide it to doctors from countries not participating.

Investigator Signature: **Date of Signature (DD MM YYYY)**

Investigator Name and Title (print)

STUDY CONTACTS

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SYNOPSIS – PROTOCOL N° (2019/2894)

EudraCT number	2019-001068-31	Version	V1.1	19/07/2019		
Title	"HR-NBL2: High-risk neuroblastoma study 2.0 of SIOP-Europe-Neuroblastoma/SIOPEN" Randomized, international and multicentric phase 3 study that evaluates and compares 2 treatment strategies in 3 therapeutic phases (induction, high-dose chemotherapy and radiotherapy) for patients with high-risk neuroblastoma.					
Abbreviated title	HR-NBL2	Phase	3			
Sponsor	Gustave Roussy 114 rue Edouard Vaillant 94805 Villejuif Cedex France					
Coordonnating Investigator	Dominique VALTEAU COUANET, MD, PhD Gustave Roussy					
Number of centers	Total	TBD	France	28 centers for inclusion	International	TBD
Indication	Patients with High Risk Neuroblastoma					
Background	In this protocol the term high-risk neuroblastoma refers to children with either: ▪ Stage M disease over the age of 12 months, any *MYCN* status ▪ L2, M or Ms neuroblastoma with *MYCN* amplification, any age High-risk neuroblastoma represents the largest neuroblastoma subgroup. The prognosis of these patients has been progressively improved over the years through an intensified induction regimen, surgery of the primary tumor, high-dose chemotherapy (HDC) followed by autologous stem cell rescue (ASCR), radiotherapy and immunotherapy. As a result of this strategy, the current 3-year event-free survival (EFS) is now around 40% from date of randomization and 55% for those patients who complete all the different parts of the treatment. However, a further improvement in patient outcome is warranted.					
Primary Objective	**R-I:** Comparison of the EFS rate of 2 induction regimens, GPOH and RAPID COJEC, in patients with high-risk neuroblastoma. **R-HDC:** Comparison of the EFS rate of single HDC with busulphan and melphalan (Bu-Mel) versus tandem HDC with Thiotepa followed by Bu-Mel in patients with high-risk neuroblastoma. **R-RTx:** Comparison of the EFS rate of 21.6 Gy radiotherapy to the					

	preoperative tumor bed versus 21.6 Gy radiotherapy and a sequential boost up to 36 Gy to the residual tumor in patients with macroscopic residual disease after HDC and surgery.
Secondary Objectives	1) To describe the EFS and overall survival (OS) from date of randomization of the whole cohort, 2) To describe the effect of RAPID COJEC and GPOH induction regimens on metastatic disease during and after the end of induction, 3) To assess the correlation of the response of metastatic disease during and after induction with survival (EFS and OS), 4) To describe the effect of HDC with Bu-Mel versus Thiotepa + Bu-Mel on progression-free survival (PFS) and OS, 5) To describe and compare the toxicity associated with RAPID COJEC and GPOH induction therapy, 6) To describe and compare the acute and long term toxicities of both HDC arms, 7) To describe the long term toxicities of dinutuximab beta, 8) To investigate the relationship between the quality of surgical resection of the primary tumor, local control and survival, 9) To investigate the impact of the radiotherapy dose on local relapse rate. 10) To collect data on selected circulating biomarkers, biological and genomic features (see Laboratory manual) to determine and compare the effect of these on response to treatment, EFS, incidence of relapse/progression and OS.
Exploratory Objectives	1) To conduct sub-group analyses to study the impact of R-I, R-HDC and R-RTx in subpopulations such as patients with L2-*MYCN* amplified neuroblastoma or patients according to age groups (infants, young children, older children and adolescents), 2) To validate prospectively the new international criteria for response assessment in neuroblastoma, 3) To validate prospectively the new international mIBG scoring methodology, 4) To evaluate the impact of mIBG-positive residual bone disease before HDC, after HDC and at the end of treatment on the risk of bone recurrence, 5) To prospectively study the relative prognostic value of planar vs SPECT-SPECT/CT(fusion) methodology of MIBG imaging, 6) To describe quality of standards of care: time from start of symptoms to histological diagnosis, time from diagnosis till initiation of treatment, proportion of dose reductions or interrupted chemotherapy cycles, time to start radiotherapy, among others.
Methodology	This is an international multicenter, open-label, randomized phase III trial including three sequential randomizations to assess efficacy of induction and consolidation chemotherapies and radiotherapy for patients with high-risk neuroblastoma. The first randomization **(R-I)** will compare the efficacy of two induction chemotherapies (RAPID COJEC and GPOH regimens) in a phase III setting. The primary endpoint will be the 3-year EFS from date of

	randomization . The R-I randomization will be stratified on age, stage, *MYCN* status and countries. The second randomization **(R-HDC)** will compare the efficacy of single HDC with Bu-Mel versus tandem HDC with Thiotepa followed by Bu-Mel. The primary endpoint is 3-year EFS calculated from the date of the R-HDC randomization. The R-HDC randomization will be stratified on the age, stage, *MYCN* status, induction chemotherapy regimen, response to induction phase and countries. The impact of local treatment in this phase III setting will be assessed, according to the presence or not of a macroscopic residual disease after surgery and HDC. In case of macroscopic residual disease, 21.6 Gy radiotherapy to the preoperative tumor bed will be randomized **(R-RTx)** versus the same treatment plus a sequential boost of additional 14.4 Gy to the residual tumor. The primary endpoint of R-RTx is 3-year EFS from the date of the R-RTx randomization. The R-RTx randomization will be stratified on age, stage, *MYCN* status, induction chemotherapy regimen, HDC regimen and countries. In case of no macroscopic residual disease, 21.6 Gy radiotherapy will be delivered to the preoperative tumor bed.
Inclusion Criteria **R-I randomization (RAPID COJEC/GPOH)**	At diagnosis (or up to 21 days after one cycle of chemotherapy for patients with localized neuroblastoma with *MYCN* amplification). R-I eligibility criteria: 1) Established diagnosis of neuroblastoma according to the SIOPEN-modified International Neuroblastoma Risk Group (INRG) criteria, High-risk neuroblastoma defined as: • Stage M neuroblastoma above 365 days of age at diagnosis (no upper age limit) and Ms neuroblastoma 12-18 months old, any *MYCN* status* or • L2, M or Ms neuroblastoma with *MYCN* amplification, any age * *In Germany, patients aged less than 18 months with stage M and without MYCN amplification will not be enrolled in HR-NBL2 trial.* 2) No previous chemotherapy (except one cycle of Etoposide-Carboplatin or**,** in Germany and Nertherlands, one course of the current protocol for low/intermediate risk neuroblastoma). 3) Females of childbearing potential must have a negative serum or urine pregnancy test within 7 days prior to initiation of treatment. Sexually active patients must agree to use acceptable and appropriate contraception while on study drug and for one year after stopping the study drug. Acceptable contraception is defined in CTFG Guidelines “Recommendations related to contraception and pregnancy testing in clinical trials” (Appendix 11). Female patients who are lactating must agree to stop breast-feeding. 4) Written informed consent to enter the R-I randomization from patient or parents/legal representative, patient, and age-appropriate assent. 5) Patient affiliated to a social security regimen or beneficiary of the same according to local requrements.

	6) Patients should be able and willing to comply with study visits and procedures as per protocol. **In case of parents'/patient's refusal to R-I, or renal or liver toxicity, patients can still be enrolled in HR-NBL2 trial with parents'/patient's consent within 3 weeks from the beginning of chemotherapy. Patients will be treated with the standard induction regimen per country and will be potentially eligible for subsequent randomizations.**
---------- **R-HDC randomization (Single HDC Bu-Mel/ Tandem HDC Thiotepa +Bu-Mel)**	Randomization for HDC strategy will be performed at the end of induction after the disease evaluation and after surgery of the primary tumor for those patients who will receive surgery before HDC. R-HDC eligibility criteria: 1) - Stage M neuroblastoma above 365 days of age at diagnosis, any *MYCN* status, ***EXCEPT* patients with stage M or *Ms 12-18 months* old with numerical chromosomal alterations only, and in complete metastatic response at the end of induction: in this case, patients will have surgery but will not be eligible for R-HDC and will not be able to pursue the trial.** OR - L2, M or Ms neuroblastoma with *MYCN* amplification 2) Age < 21 years 3) Complete response (CR) or partial response (PR) at metastatic sites: • Bone disease: MIBG uptake (or FDG-PET uptake for MIBG-nonavid tumors) completely resolved or SIOPEN score ≤ 3 and at least 50% reduction in mIBG score (or ≤ 3 bone lesions and at least 50% reduction in number of FDG-PET-avid bone lesions for MIBG-nonavid tumors). • 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. 4) Acceptable organ function and performance status • Performance status ≥ 50%. • Hematological status: ANC>0.5x10^9/L, platelets > 20x 10^9/L • Cardiac function: Shortening fraction ≥ 28% or ejection fraction ≥ 55% by echocardiogram, no clinical congestive heart failure. Normal pulmonary artery pressure. • Normal chest X-ray and oxygen saturation. • Absence of any toxicity ≥ grade 3. 5) Sufficient collected stem cells available; minimum required: 6 x 106 CD34+ cells/kg body weight stored in 3 separate fractions. 6) Written informed consent, including agreement of patient or parents/legal guardian for minors, to enter the R-HDC randomization. 7) Patient affiliated to a social security regimen or beneficiary of the same according to local requirements.

--- **R-RTx randomization (Local Radiotherapy)**	8) Patients should be able and willing to comply with study visits and procedures as per protocol. **In case of parents'/patient's refusal, or insufficient stem cells, collection for tandem HDC but with a minimum of 3 x 10^6 CD34+ cells/kg body weight, or in case of patients older than 21 years, or liver or renal toxicity, HDC will consist on the standard HD Bu-Mel and will be eligible for subsequent randomization.** An evaluation of the local disease will be performed after HDC and surgery: - In case of **no local macroscopic disease**, all patients will receive 21-Gy radiotherapy to the pre-operative tumor bed - In case of **local macroscopic residual disease**, patients will be eligible to R-RTx if the following criteria are met: 1) No evidence of disease progression after HDC/ASCR. 2) Interval between the last ASCR and radiotherapy start between 60 and 90 days. 3) Performance status greater or equal 50%. 4) Hematological status: ANC >0.5x10^9/L, platelets > 20x10^9/L. 5) Written informed consent, including agreement of patient or parents/legal guardian for minors, to enter the R-RTx randomization. 6) Patient affiliated to a social security regimen or beneficiary of the same according to local requirements. 7) Patients should be able and willing to comply with study visits and procedures as per protocol. **In case of parents'/patient's refusal of the randomization, the patient will receive 21.6 Gy radiotherapy to the pre-operative tumor bed and pursue the next step of the trial.**
Non inclusion Criteria	<u>Non-inclusion criteria specific to the R-I randomization (RAPID COJEC/GPOH) :</u> 1) Urinary outflow obstruction 2) severe arrhythmia, heart failure, previous cardiac infarct, acute inflammatory heart disease 3) severe peripheral neuropathy 4) demyelinating form of Charcot-Marie-Tooth syndrome 5) hearing impairment 6) Concurrent prophylactic use of phenytoin 7) cardiorespiratory disease that contraindicates hyperhydration <u>Non-inclusion criteria common to all randomizations (R-I, R-HDC and R-RTx) :</u> 1) Any negative answer concerning the inclusion criteria of R-I or R-HDC or R-RTx will render the patient ineligible for the corresponding therapy phase randomization. However, these patients may remain on study and be considered to receive standard treatment of the respective therapy phase, and may be potentially eligible for subsequent randomizations. 2) Liver function: Alanine aminotransferase (ALT) > 3.0 x ULN and blood bilirubin > 1.5 x ULN (toxicity ≥ grade 2). In case of toxicity ≥

	grade 2, call national principal investigator study coordinator to discuss the feasibility. 3) Renal function: Creatinine clearance and/or GFR < 60 ml/min/1.73m² (toxicity ≥ grade 2). If GFR < 60ml/min/1.73m², call national principal investigator to discuss.the feasibility. 4) Dyspnea at rest and/or pulse oximetry <95% in air. 5) Any uncontrolled intercurrent illness or infection that in the investigator opinion would impair study participation. 6) Patient under guardianship or deprived of his liberty by a judicial or administrative decision or incapable of giving his consent. 7) Participating in another clinical study with an IMP while on study treatment. 8) Concomittant use with yellow fever vaccine and with live virus or bacterial vaccines. 9) Patient allergic to peanut or soya. 10) Chronic inflammatory bowel disease and/or bowel obstruction. 11) Pregnant or breastfeeding women. 12) Known hypersensitivity to the active substance or to any of the excipients of study drugs known 13) Concomitant use with St John's Wort (Hypericum Perforatum).
Treatment	Patients will receive ▪ Induction chemotherapy - Randomization between RAPID COJEC and GPOH chemotherapy ▪ Surgery of the primary tumor ▪ Consolidation chemotherapy - Randomization between single HD Bu-Mel and tandem HDC consisting in Thiotepa (900mg/m^2) and Bu-Mel, followed by ASCR ▪ External radiotherapy of the primary tumor - Randomization of the dose of radiotherapy (21.6 Gy vs 21.6 Gy + 14.4 Gy boost) in patients with macroscopic residual tumor; - 21.6 Gy radiotherapy to the pre-operative tumor bed in patients with no macroscopic residual tumor ▪ Maintenance treatment with immunotherapy and isotretinoin. The duration of the whole treatment for each participant will be of around 1 year. In this trial, the Investigational Products (IMPs) are : - Cisplatin - Carboplatin - Cyclophosphamide - Dacarbazine - Doxorubicin - Etoposide - Ifosfamide - Thiotepa - Busulfan (in the Thiotepa-BuMel arm)

	- Melphalan (in the Thiotepa-BuMel arm) - Vincristine - Vindesine All the IMPs will be taken from pharmacy hospital stocks.
Primary evaluation criterion	**R-I:** 3-year EFS from date of R-I randomization **R-HDC:** 3-year EFS from date of R-HDC randomization **R-RTx:** 3-year EFS from date of RTx randomization
Secondary evaluation criteria	For the whole population of high-risk neuroblastoma: ▪ 3- and 5-year EFS, PFS and OS calculated from date of randomization For each treatment phase: ▪ 5-year EFS, 3- and 5-year PFS and OS calculated from date of randomization ▪ Cumulative incidence of relapse/progression ▪ Cumulative incidence of treatment related mortality and of disease related mortality ▪ Overall response as per the new INRG response criteria [Park JR, JCO 2017] (including primary tumor after induction), skeletal response on MIBG, bone marrow response, local control ▪ Therapy-related toxicity
Exploratory endpoints	▪ Rate of patients that discontinued therapy Response rates, survival rates and the cumulative incidence of relapse/progressions will be analyzed according to: - Clinical factors: age, stage, metastatic response at the end of induction chemotherapy. - Serological factors at diagnosis: LDH, ferritin. - Biological factors: *MYCN*, ALK and TERT and circulating biomarker status.
Sample size determination	**R-I:** induction regimens RAPID COJEC vs GPOH Assuming a baseline 3-year EFS of 40%, with a sample size of 686 patients (343 in each arm) and a two-sided alpha=5% this trial will have 90% power to demonstrate an improvement of 12% in 3-year EFS, within a recruitment period of 3 years and a minimum follow up of 1.5 years. **R-HDC:** consolidation regimen Bu-Mel vs Thiotepa + Bu-Mel The 3-year EFS in the Bu-Mel arm (with immunotherapy) is estimated to be 55%. This study aims to show an improvement of 12% for the Thiotepa + Bu-Mel arm (3-year EFS of 67%). With a recruitment of 448 patients (224 in each arm) over a period of 3 years and a minimum follow-up of 2 years, the power to show a 12% difference is 80% (two-sided logrank test and α=5%). **R-RTx:** 21.6 Gy radiotherapy vs 21.6 Gy + 14.4 Gy boost in patients with macroscopic residual disease The 3-year EFS of patients with 21.6 Gy radiotherapy is estimated to be 38%. This study aims to show an improvement of 15% for the arm with 21.6 Gy + 14.4 Gy boost (3-year EFS of 53%). With a recruitment of 226 patients (113 in each arm) over a period of 4 years and a minimum follow-up of 4 years, the power to show a 15% difference is 80% (two-sided logrank test and α=5%).

Number of patients	Total :	800	
Duration of the trial	Inclusion		6 years
	Treatment		Around 1 year
	Follow-up		5 years
	Duration of the study		12 years
	Long term Follow-up		Overall Survival

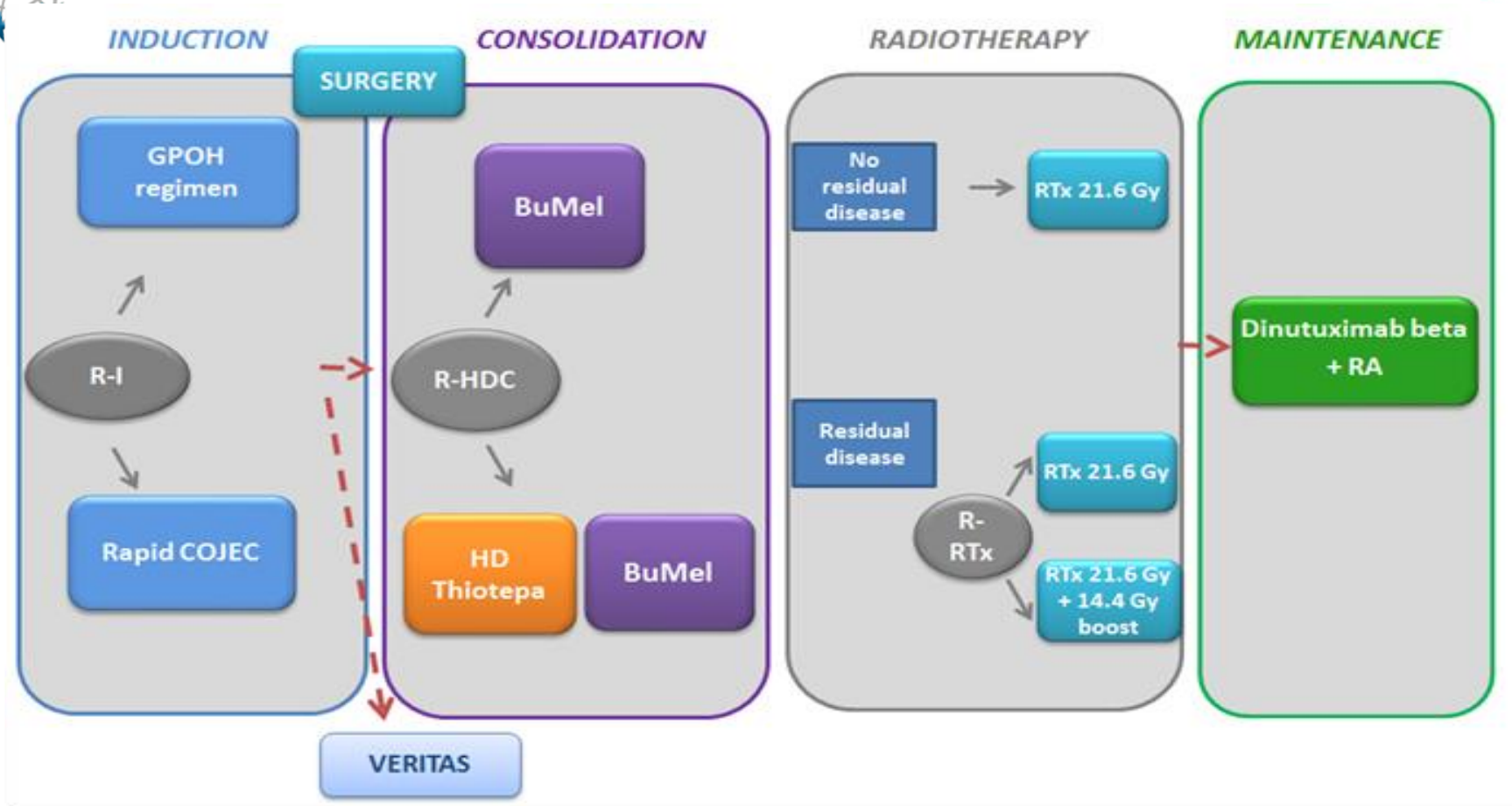

Figure 1: Detailed overall study flowchart

Figure 2 -Flowchart: global overview of the study – ARM RAPID COJEC

Evaluations	Study entry	Induction phase – ARM RAPID COJEC (R-I)									[4]Evaluation End of induction (Day80)	Apheresis (at the end of induction)****	Surgery **	Consolidation phase (R-HDC)		Evaluation end of consolidation	Local treatment phase** (R-RTX)	Evaluation before maintenance	Maintenance phase						End of treatment	Follow up
		A 1	B 2	C 3	B 4	Mid-induction (Day40)	A 5	B 6	C 7	B 8				BuMel	If ARM with Thiotepa [5]				1	2	3	4	5	6		
Eligibility criteria	X																									
Medical history	X																									
Full clinical examination	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	x	x	X	X	X	X	X	
Record AEs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Karnofsky or Lansky	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Pathology[2]	x																									
TREATMENTS																										
Rapid cojec (8 courses)		D0 → D80											X													
Apheresis****												X														
Surgery											When appropriate but before maintenance phase															
BuMel														D-6 to D0 before ASCR												
Thiothepa															D-3 to D0 before ASCR											
ASCR														D7 after Bumel	D4 after Thiotepa											
Radiation																	>D60 and <D90 after last ASCR									
Retinoic acid (6 cycles)																			X	X	X	X	X	X		
Dinutiximab beta (5cycles)																				X	X	X	X	X		
EVALUATIONS																										
456I-mIBG scan (or FDG PET for MIBG negative cases)	x					x					x					x		x[7]			x				x	
Primary tumour imaging (MRI or CT) [1]	x										x					x		x[7]			x				x	
Primary tumour imaging (echography) [1]	x					x																				x	

Refer page 75 87

Evaluations	Study entry	Induction phase – ARM RAPID COJEC (R-I)									[4]Evaluation End of induction (Day80)	Apheresis (at the end of induction)****	Surgery **	Consolidation phase (R-HDC)		Evaluation end of consolidation	Local treatment phase** (R-RTX)	Evaluation before maintenance	Maintenance phase						End of treatment	Follow up
		A 1	B 2	C 3	B 4	Mid-induction (Day40)	A 5	B 6	C 7	B 8				BuMel	If ARM with Thiotepa [5]				1	2	3	4	5	6		
Cerebral imaging (MRI or CT) [1]	x										x [6]					x										
BM (trephine biopsy)	x										x					x		x [7]							x	
BM (aspirates) [a]	x					x					x					x		x [7]							x	Refer page 87
BM MRD testing [3]	x					x					x					x		x [7]							x	
Blood MRD testing [3]	x					x					x					x		x [7]							x	
Urinary catecholamine metabolites	x										x [8]					x [8]									x [8]	
Biochemistry- including renal/liver function, electrolytes, calculated GFR	x	x	x	x	x	x	x	x	x	x	x		x	X	X	x		x	x	x	x	x	x	X	x	
Haematology - Full Blood Counts (FBC)	x	x	x	x	x	X	x	x	x	x	X		x	x	x	x		x	x	x	x	x	x	x	x	
Ferritin; LDH	x																									
Serum Triglycerides																			x	x	x	x	x	x		
Serum pregnancy test within 7 days before 1st administration	x	1/months																								
Echocardiogram***	x					X					x			x		x										
Urine analysis (GFR+tubular function)	x		x		x			x		x	x			x	x		x		x							
ECG																			x		x				x	
HBV and HIV testing	x																									
Auditory function	x		x					x			x															
Chest radiography														x	x	x		x	x		x				x	
Abdominal & hepatic ultrasound														x		x										
Eyes Assessment																			x		x				x	
Pulmonary function														x	x	x		x	x	x	x	x	x	x	x	

[1]Imaging of the primary tumor and cerebral imaging: by MRI or CT scan as judged appropriate by the patient's physician

[2] INPC classification and *MYCN* status

[3] The minimal residual disease (MRD) testing is highly recommended and should be performed as follows:

- in blood a quantitative reverse-transcriptase polymerase chain reaction (RTqPCR)
- in bone marrow by immunocytology (IC) and RTqPCR (See Section 8 for detail on sample collection).

[4]**MRI/CT scan preoperative + MIBG/SPECT scan after surgery (if surgery is feasible), then decide to proceed towards R-HDC/HR-NBL2 or VERITAS trial according to results. If the surgery of the primary tumor seems complicated, MIBG/SPECT scan might be performed before surgery to help the clinical decision**

[5] for patients randomized in the double HDC arm

[6] only for patients with brain metastases or major skull lesions at diagnosis

[7] to be repeated only if ≥ 8 weeks since last evaluation

[8] not mandatory

*In patients randomized to receive a tandem HDC, an interval of at least 8 weeks must be respected between the beginning of the first high dose therapy (Thiotepa) and the start of Bu-Mel. In these patients, Bu-Mel must be started by day 90 after ASCR regardless of haematological recovery unless there is major organ toxicity or progressive disease, in which case the patient will come off trial.

**Timing of surgery changes according to the induction arm: if GPOH: after the 2nd N6 cycle (4th cycle) if Rapid COJEC: after the end of induction, ideally after peripheral stem cell harvest; Surgery may be further postponed (after HDC Thiotepa or after HD Bu-Mel, based on physician decision). 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.

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

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

Figure 3 -Flowchart: global overview of the study – ARM GPOH

Evaluations	Study entry	Induction phase – ARM GPOH (R-I)								[4]Evaluation End of induction (Day126)	Surgery **	Consolidation phase (R-HDC)		Evaluation end of consolidation	Local treatment phase** (R-RTX)	Evaluation before maintenance	Maintenance phase						End of treatment	Follow up
		N5 1	N6 2	After 2sd cycle	N5 3	N6 4	After 4th cycle	N5 5	N6 6			BuMel	If ARM with Thiotepa [5]				1	2	3	4	5	6		
Eligibility criteria	x																							
Medical history	x																							
Full clinical examination	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Blood pressure	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Record AEs	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Karnofsky or Lansky	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Pathology[2]	x																							
TREATMENTS																								
GPOH (6 courses)		D0 → D126									X													
Apheresis****						PBSC collection must be done following cycle 3																		
Surgery								When appropriate but before maintenance phase																
BuMel												D-6 to D0 before ASCR												
Thiothepa													D-3 to D0 before ASCR											
ASCR												D7 after Bumel	D4 after Thiotepa											
Radiation															>D60 and <D90 after last ASCR									
Retinoic acid (6 cycles)																	X	X	X	X	X	X		
Dinutiximab beta (5cycles)																		X	X	X	X	X		
EVALUATIONS																								
456I-mIBG scan (or FDG PET for MIBG negative cases)	x			x			x			x				x		x[7]			x				x	
Primary tumour imaging (MRI or CT) [1]	x						x			x				x		x[7]			x				x	
Primary tumour imaging (echography) [1]	x			x						x													x	

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Evaluations	Study entry	Induction phase – ARM GPOH (R-I)								[4]Evaluation End of induction (Day126)	Surgery **	Consolidation phase (R-HDC)		Evaluation end of consolidation	Local treatment phase** (R-RTX)	Evaluation before maintenance	Maintenance phase						End of treatment	Follow up
		N5 1	N6 2	After 2sd cycle	N5 3	N6 4	After 4th cycle	N5 5	N6 6			BuMel	If ARM with Thiotepa [5]				1	2	3	4	5	6		
Cerebral imaging (MRI or CT) [1]	x									x [6]				x										
BM (trephine biopsy)	x						x			x				x		x [7]							x	
BM (aspirates) [a]	x			x			x			x				x		x [7]							x	
BM MRD testing [3]	x						x			x				x		x [7]							x	
Blood MRD testing [3]	x						x			x				x		x [7]							x	
Urinary catecholamine metabolites	x									x [8]				x [8]									x [8]	
Biochemistry- including renal/liver function, electrolytes, calculated GFR	x	x	x	x	x	x	x	x	x	x	x	X	X	x		x	x	x	x	x	x	X	x	
Haematology - Full Blood Counts (FBC)	x	x	x	x	x	X	x	x	x	X	x	x	x	x		x	x	x	x	x	x	x	x	
Ferritin; LDH	x																							
Serum Triglycerides																	x	x	x	x	x	x		
Serum pregnancy test within 7 days before 1st administration	x	1/months																						
Echocardiogram***	x		x			X			x	x		x		x										
Urine analysis (GFR+tubular function)	x	x	x	x	x	X	x	x	x	x		x	x		x		x							
ECG																	x		x				x	
HBV and HIV testing	x																							
Auditory function	x	x			x			x		x														
Chest radiography												x	x	x		x [7]	x		x				x	
Abdominal & hepatic ultrasound												x		x										
Eyes Assessment																	x		x				x	
Pulmonary function												x	x			x	x	x	x	x	x	x		

[1]Imaging of the primary tumor and cerebral imaging: by MRI or CT scan as judged appropriate by the patient's physician

[2] INPC classification and *MYCN* status

[3] The minimal residual disease (MRD) testing is highly recommended and should be performed as follows:

- in blood a quantitative reverse-transcriptase polymerase chain reaction (RTqPCR)
- in bone marrow by immunocytology (IC) and RTqPCR (See Section 8 for detail on sample collection).

[4]**MRI/CT scan preoperative + MIBG/SPECT scan after surgery (if surgery is feasible), then decide to proceed towards R-HDC/HR-NBL2 or VERITAS trial according to results. If the surgery of the primary tumor seems complicated, MIBG/SPECT scan might be performed before surgery to help the clinical decision**

[5] for patients randomized in the double HDC arm

[6] only for patients with brain metastases or major skull lesions at diagnosis

[7] to be repeated only if ≥ 8 weeks since last evaluation

[8] not mandatory

*In patients randomized to receive a tandem HDC, an interval of at least 8 weeks must be respected between the beginning of the first high dose therapy (Thiotepa) and the start of Bu-Mel. In these patients, Bu-Mel must be started by day 90 after ASCR regardless of haematological recovery unless there is major organ toxicity or progressive disease, in which case the patient will come off trial.

**Timing of surgery changes according to the induction arm: if GPOH: after the 2nd N6 cycle (4th cycle) if Rapid COJEC: after the end of induction, ideally after peripheral stem cell harvest; Surgery may be further postponed (after HDC Thiotepa or after HD Bu-Mel, based on physician decision). 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.

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

**** Patients receiving GPOH as induction will have the BM evaluation following cycle 2 and the PBSC collection following cycle 3 (G-CSF 5 µg/kg/day until harvest) depending on bone marrow disease status.

Table 1: Detailed schedule of the Disease Evaluations throughout the trial

*Table 1.1: Schedule of the disease evaluations throughout the trial – **arm RAPID COJEC***

Study steps	Study entry	Day 40 Rapid Cojec	Post Rapid Cojec[4]	Post-Thio[5]	Post Bu-Mel, prior to RTx	Before maintenance	After 2nd cycle of dinutuximab beta	End of treatment
^{456}I-mIBG scan (or FDG PET for MIBG negative cases)	❒	❒	❒	❒	❒	❒[7]	❒	❒
Primary tumor imaging (MRI or CT)[1]	❒		❒		❒	❒[7]		❒
Primary tumor imaging (ultrasound)[1]	❒	❒		❒			❒	❒
Cerebral imaging (MRI or CT)[1]	❒		❒[6]		❒			
Bilateral BM (trephine biopsy)	❒		❒		❒	❒[7]		❒
Bilateral BM (aspirates)	❒	❒	❒		❒	❒[7]	❒	❒
Pathology[2]	❒							
Urinary Catecholamins	❒		❒[8]		❒[8]			❒[8]
Ferritin, LDH	❒							
Blood MRD testing[3]	❒	❒	❒		❒	❒[7]	❒	❒
BM MRD testing[3]	❒	❒	❒		❒	❒[7]	❒	❒

[1]Imaging of the primary tumor and cerebral imaging: by MRI or CT scan as judged appropriate by the patient's physician

[2] INPC classification and *MYCN* status

[3] The minimal residual disease (MRD) testing is highly recommended and should be performed as follows:

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[4]**MRI/CT scan preoperative + MIBG/SPECT scan after surgery (if surgery is feasible), then decide to proceed towards R-HDC/HR-NBL2 or VERITAS trial according to results. If the surgery of the primary tumor seems complicated, MIBG/SPECT scan might be performed before surgery to help the clinical decision**

[5] for patients randomized in the double HDC arm

[6] only for patients with brain metastases or major skull lesions at diagnosis

[7] to be repeated only if ≥ 8 weeks since last evaluation

[8] not mandatory

*Table 1.2: Schedule of the disease evaluations throughout the trial – **arm GPOH***

Study steps	Study entry	After the 2nd cycle GPOH	After the 4th cycle GPOH[1]	Post GPOH induction	Post-Thio[5]	Post Bu-Mel, prior to RTx	Before maintenance	After 2nd cycle of dinutuximab beta	End of treatment
^{456}I-mIBG scan (or FDG PET for MIBG negative cases)	❒	❒	❒	❒	❒	❒	❒[7]	❒	❒
Primary tumor imaging (MRI or CT)[1]	❒		❒	❒		❒	❒[7]		❒
Primary tumor imaging (ultrasound)[1]	❒	❒		❒	❒	❒		❒	❒
Cerebral imaging (MRI or CT)[1]	❒			❒[6]		❒			
BM (trephine biopsy)	❒		❒	❒		❒	❒[7]		❒
BM (aspirates)	❒	❒	❒	❒		❒	❒[7]	❒	❒
Pathology[2]	❒								
Urinary Catecholamins	❒			❒[8]		❒[8]			❒[8]
Ferritin, LDH	❒								
Blood MRD testing [3]	❒	❒	❒	❒		❒	❒[7]	❒	❒
BM MRD testing[3]	❒	❒	❒	❒		❒	❒[7]	❒	❒

[1] Imaging of the primary tumor and cerebral imaging: by MRI or CT scan as judged appropriate by the patient's physician

[2] INPC classification and *MYCN* status

[3] the minimal residual disease (MRD) testing is highly recommended and should be performed as follows:

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[5] for patients randomized in the double HDC arm

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[7] to be repeated only if ≥ 8 weeks since last evaluation

[8] not mandatory

ABBREVIATIONS USED IN THIS PROTOCOL IN ALPHABETICAL ORDER

AE	Adverse Event
ANC	Absolute Neutrophil Count
ARDS	Acute Respiratory Distress Syndrome
ASCR	Autologous Stem Cell Rescue
BM	Bone Marrow
Bu-Mel	Busulfan and Melphalan HDC regimen
CCSG	Children's Cancer Study Group
CEM	carboplatin, etoposide and melphalan HDC
CI	confidence interval
COG	children's oncology group
CR	complete response
CRF	case report form
CRP	C-reactive protein
CT	computed tomography
CTCAE	common terminology criteria for adverse events
CTV	clinical target volume
CXR	chest x-ray
CYC	cyclophosphamide
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
DTC	disseminated tumor cell
DWIBS-MRI	diffusion-weighted whole-body magnetic resonance imaging with background body signal suppression
EFS	event-free survival
^{18}F-FDG	fludeoxyglucose radiolabelled with fluorine-18
^{18}F-DOPA	dihydroxyphenylalanine radiolabelled with fluorine-18
FISH	fluorescence in situ hybridisation
G-CSF	granulocyte stimulating growth factor
^{68}Ga-DOTA-peptide	DOTA-Phe1-(Tyr3)-octreotide and/or DOTA-(Tyr3)-octreotate radiolabelled with gallium-68
GFR	glomerular filtration rate
GPOH	German Society of Pediatric Oncology and Hematology
GTV	gross tumor volume
Gy	Gray
HaChA	human anti-chimeric antibody
HDC	high-dose chemotherapy
HREC	health research ethics committee
HVA	homovanillic acid
^{123}I-mIBG	mIBG radiolabelled with iodine-123
^{131}I-mIBG	mIBG radiolabelled with iodine-131
IC	immunocytology
ICRU	international commission of radiation units
IMP	investigational medicinal product

INCR	international neuroblastoma response criteria
INSS	international neuroblastoma staging system
IVIG	intravenous immune globulin
KVp	kilovoltage peak
LCWGS	low coverage whole genome sequencing
LDH	lactate dehydrogenase
LI	local irradiation
LTI	long-term continuous infusion
mAs	milliampere-second
MBq	megabecquerel
mCi	millicurie
mIBG	meta-iodobenzylguanidine
MLC	multi-leaf collimator
MNA	*MYCN* amplification
MRD	minimal residual disease
MRI	magnetic resonance imaging
MRI/CT	magnetic resonance imaging or computed tomography
MTD	maximum tolerated dose
NBL	neuroblastoma
OS	overall survival
PBSC	peripheral blood stem cell
PBSCR	peripheral blood stem cell rescue
PCA	patient controlled analgesia
PCP	*Pneumocystis jiroveci* pneumonia
PCR	polymerase chain reaction
PD	progressive disease
PET	positron emission tomography
PET/CT	positron emission tomography with computed tomography
PR	partial remission
PTV	planning target volume
RTqPCR	quantitative reverse transcriptase polymerase chain reaction
13-cis-RA	isotretinoin (13-cis-retinoic acid)
RAPID COJEC	rapid platinum-containing induction schedule (carboplatin, cisplatin, vincristine, etoposide, cyclophosphamide)
RNA	ribonucleic acid
SAE	serious adverse event
SADR	serious adverse drug reaction
SD	stable disease
SIOP	société internationale d'oncologie pédiatrique
SIOPEN	société internationale d'oncologie pédiatrique European Neuroblastoma
SmPC	summary of product characteristics
SNP array	single nucleotide polymorphism array
SPECT	single-photon emission computed tomography
SPECT/CT	single-photon emission computed tomography with computed tomography
SUSAR	suspected unexpected serious adverse reaction
SOS	sinusoidal obstructive syndrome
SMZ	sulfamethoxazol

SPECT	single-photon emission computed tomography
TMP	trimethoprim
TVD	topotecan, vincristine, doxorubicin
VCR	vincristine
VHR-NBL	very high risk neuroblastoma
VMA	vanillyl mandelic acid
VOD	veno-occlusive disease
WBC	white blood cells
WES	whole exome sequencing
WGS	whole genome sequencing

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1 RATIONALE OF THE STUDY

1.1 Overview

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.
In 2013, a randomization (R3) was introduced into the SIOPEN (SIOP-Europe-Neuroblastoma)/HR-NBL1 trial to compare the standard SIOPEN induction regimen RAPID COJEC with the modified N7 regimen, developed in North America. The primary aim of the R3 randomization was to compare metastatic response rates and event-free survival (EFS) of both arms. The early results of this randomization showed no difference in terms of survival and metastatic response rates between the two arms. RAPID COJEC having less acute toxic than the modified N7, this regimen has been selected to be the SIOPEN reference induction regimen. In the German (GPOH) NB2004-HR trial, patients were randomized either for standard induction chemotherapy with six N5-N6 cycles or the experimental induction chemotherapy having two additional topotecan-based cycles (N8-N5-N6 cycles). Final results of the trial are expected by the end of 2018.
In order to define the most effective induction chemotherapy regimen, both RAPID COJEC (SIOPEN) and N5-N6 regimens, considered as the standard practice in different regions, will be evaluated head to head in a randomized trial.
HDC followed by ASCR has improved outcomes in European and North America randomized trials, becoming the standard of care for HR-NBL. Questions regarding the optimal consolidation regimen, its interaction with the induction chemotherapy and the role of tandem regimens remain of major interest. Tandem strategies have been successfully introduced by the Children's Oncology Group (COG) in high-risk patients and are currently under investigation by SIOPEN in very high-risk neuroblastoma (VERITAS trial, NCT03165292). It is now of major importance to study the impact on survival of an intensified HDC based on the standard SIOPEN HD Busulfan-Melphalan (Bu-Mel) strategy for patients with HR-NBL. In order to evaluate the role of tandem HDC in the SIOPEN context, a single HDC with Bu-Mel will be randomized versus tandem HDC with Thiotepa and Bu-Mel, followed by ASCR.
Local treatment is another important step of the treatment of patients with HR-NBL. Surgery has the purpose to remove completely the primary tumor. Local radiotherapy of the preoperative bed at 21 Gy will be performed in patients with no macroscopic residual disease after HDC/ASCR. In case of persistent macroscopic residual disease, the SIOPEN and GPOH standards differ in terms of recommended dose (21 Gy versus 21 Gy plus a boost of 15 Gy, respectively). In these patients, the optimal dose of radiotherapy will be established through a randomization of both strategies.
As maintenance treatment, the standard of care based on the results of previous SIOPEN trials (Long-term infusion-LTI and HR-NBL1 trials) will consist on the use of monoclonal anti-GD2 antibody (dinutuximab beta) in combination with isotretinoin (13-cis-RA).

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.

Induction regimens evaluated by SIOPEN group

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^2 per week) of COJEC was 1.8 fold higher. Therapy in the COJEC arm was administered every 10 days, regardless of hematological recovery, whilst it was delivered every 21 days in the OPEC/OJEC arm, dependent on hematological 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 tumor was undertaken, followed by HDC with single agent Melphalan (180 mg/m2) 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 SIOPEN/HR-NBL1 trial, and was administered to all patients recruited to the trial between 2002 and 2013.

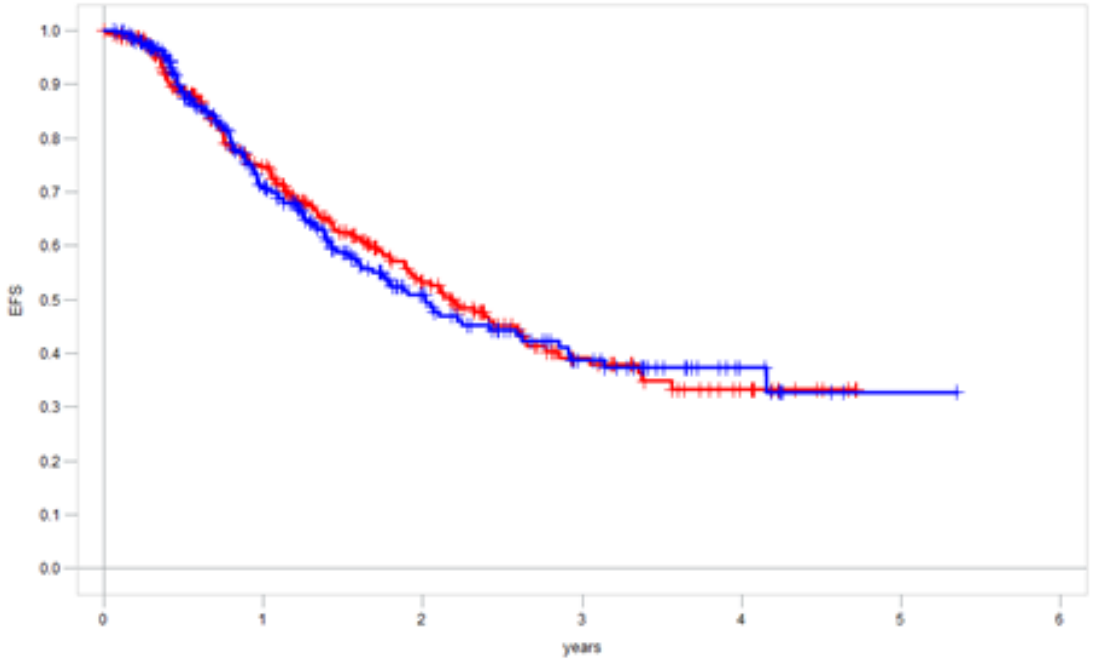
Within the HR-NBL1 trial the addition of granulocyte colony stimulating factor (G-CSF) to COJEC induction was randomized (R0), showing a significantly reduced toxicity profile when G-CSF was used. In this randomized patient cohort, response in the bone marrow compartment was achieved in 70% of patients,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

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^2/day for 5 days, followed by a 48-hour infusion of vincristine, 2 mg/m^2, and doxorubicin, 45 mg/m^2 (TVD). Following two courses of TVD, four (6%) patients had an overall CR, while 23 patients achieved a metastatic CR or a PR with ≤ 3 mIBG skeletal areas and no bone marrow disease and were eligible to receive HDC.[Amoroso L, Canc Res Treat 2018]

In 2013, a new randomization (R3) was introduced into the SIOPEN/HR-NBL1 trial to compare COJEC with the modified N7 induction regimen [Cheung NK, Med Pediatr Oncol 2001; Kushner BH, J Clin Oncol 2004, Mora J, Clin Transl Oncol 2015], developed at Memorial Sloan-Kettering Cancer Center (MSKCC) and adopted by the Children's Oncology Group (COG). This intensive induction chemotherapy regimen included two putatively non cross-resistant drug combinations: high-dose cyclophosphamide plus doxorubicin/vincristine (CAV) and high-dose cisplatin/etoposide (P/E). The original regimen with 7 cycles was modified reducing the number of cycles to 5, with a lower dosage of vincristine (VCR) and using G-CSF. 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 primary aim of the R3 randomization in SIOPEN/HR-NBL 1 was to compare metastatic response rates and EFS of COJEC versus the modified N7 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 4). The 3-

year EFS was 39% ± 4% for COJEC vs 39% ± 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) (Table 2). With RAPID COJEC having less acute toxicity than modified N7, RAPID COJEC (without TVD) has been selected to be the SIOPEN reference induction regimen.

Figure 4: EFS of COJEC vs modified N7 (R3 in SIOPEN/HR-NBL1)



	Patients	Events	1-yrs EFS	2-yrs. EFS	3-yrs. EFS	5-yrs. EFS	p-value
Cojec	302	126	0.75±0.03	0.53±0.04	0.39±0.04	0.33±0.04	0.805
N7	305	122	0.71±0.03	0.51±0.04	0.39±0.04	0.33±0.06	.

Table 2: Toxicity of COJEC vs modified N7 (R3 in SIOPEN/HR-NBL1)

	Cojec			N7			p-value
	n	Grade 3/4		n	Grade 3/4		
Toxicity		n	%		n	%	
General Condition	257	32	12%	270	50	19%	0.055
Haemoglobin	256	234	91%	272	255	94%	0.303
WBC	256	240	94%	272	266	98%	0.020
Granulocytes	255	240	94%	271	262	97%	0.159
Platelets	256	234	91%	272	261	96%	0.031
Infection	260	63	24%	271	96	35%	0.005
Fever	259	12	5%	271	22	8%	0.102
Stomatitis	257	8	3%	269	70	26%	0.000
Nausea/Vomiting	257	17	7%	270	44	16%	0.001
Diarrhea	257	7	3%	270	18	7%	0.033

Toxicity	Cojec n	Cojec Grade 3/4 n	Cojec Grade 3/4 %	N7 n	N7 Grade 3/4 n	N7 Grade 3/4 %	p-value
Skin	257	5	2%	269	7	3%	0.614
Allergy	257	1	0%	269	2	1%	0.590
Cardiac function	250	0	0%	264	0	0%	.
Hypotension	252	2	1%	264	1	0%	0.536
Hypertension	251	26	10%	264	15	6%	0.050
Kidney function	257	0	0%	269	0	0%	.
CNS	256	0	0%	267	4	1%	0.049
Peripheral neurotoxicity	256	2	1%	266	0	0%	0.149
Bilirubin	256	6	2%	269	8	3%	0.654
SGOT/SGPT	256	24	9%	269	36	13%	0.149

Induction regimens evaluated by GPOH group

From the very first GPOH NB79 trial on high-risk neuroblastoma that was initiated in 1979, multiple chemotherapy regimens have been evaluated by the German cooperative group. The NB79 induction chemotherapy consisted of three ACVD cycles (doxorubicin, cyclophosphamide, vincristine and dacarbazine) followed by 5 AC cycles (doxorubicin and cyclophosphamide). In the subsequent NB82 trial, a total number of 10 alternating chemotherapy cycles ACVD and PCVm (cisplatin, cyclophosphamide, and etoposide) was scheduled. In the NB85 trial, the combination of ifosfamide and etoposide was introduced (IVp). It consisted of nine chemotherapy cycles by repeating the sequence IVp, ACVD, PCVm three times. After three chemotherapy cycles the objective response rate was 89% (12% complete response, 77% partial response) but with an increased toxic death rate of 9%. The 5-year EFS rate of the NB85 trial was 14%.[Berthold F, Klin Padiatr 1990; Berthold F, Cancer Lett 2003] In the NB90 trial, short infusions of cytotoxic drugs were substituted by continuous infusions aiming to higher efficacy. Further, the cytotoxic drugs were re-arranged into two different cycles referred to as N1 (cisplatin, etoposide and vindesine) and N2 (ifosfamide, vincristine, dacarbazine and doxorubicin). Among the 230 evaluable patients, the complete and partial remission rate was 31% and 44% after 4 cycles, and 58% and 11% after 8 cycles respectively. The toxic-death rate was 5%. The 5-year EFS rate of all patients treated in NB90 was 27%.[Berthold F, Cancer Lett 2003] The improvement of the outcome of patients with HR-NBL was mostly related to the evolution of induction chemotherapy since only a limited number of patients underwent consolidation by HDC and ASCR. In the NB97 trial, the NB90-induction chemotherapy was modified to decrease toxicity, with lower etoposide dose, shorter doxorubicin infusion time and reduced number of chemotherapy cycles from 8 to 6. The modified chemotherapy cycles were referred to as N5 (cisplatin, etoposide, and vindesine) and N6 (ifosfamide, vincristine, dacarbazine and doxorubicin). The response rate at the end of the NB97 induction chemotherapy was maintained and the toxic-death rate during induction chemotherapy decreased to 0.6%.[Berthold F, Lancet Oncol 2005]

The GPOH NB2004-HR trial was opened between 2004 and 2016. Patients with HR-NBL were either randomized for standard induction chemotherapy identical to the NB97 trial or experimental induction chemotherapy having two additional topotecan-containing cycles (cyclophosphamide, topotecan, and etoposide - N8). Topotecan-containing chemotherapy was chosen because its proved efficacy in previous phase II trials.[Park JR, Med Pediatr Oncol 2000; Längler A, Klin Padiatr 2002; Kretschmar CS, J Clin Oncol 2004] Preliminary data from NB2004-HR trial were extracted for the design of HR-NBL2 trial. In this data cut-off (October 2017), complete metastatic response rate

at the end of induction was 40% and 3-year EFS was 36%. Of note, most of the patients received HD Melphalan-Etoposide-Carboplatin (MEC) and had no immunotherapy.

Induction regimens evaluated in North America

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 regimen had 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 6 cycles of chemotherapy, with cycles 1, 2, 4 and 6 of CAV and cycles 3 and 5 of 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%.

Rationale for the induction randomization

Table 3 summarizes the major induction regimens used by cooperative groups that have been published over the last two decades.

Table 3: Overview of induction regimens

Regimen	Schedule	Survival	Evidence
COG A3973 Kreissman Lancet Oncol (n=486)	alternating CAV + P/E x 6	5-year EFS 38% 5-year OS 50%	Single arm prospective trial
NB97/GPOH Berthold Lancet Oncol	alternating N5 + N6 x 6	3-year EFS 39% 3-year OS 58%	Single arm prospective trial
ENGS5 Pearson Lancet Oncol (n=262)	OPEC/OJEC vs COJEC	5-year EFS 18.2% vs 30.2% (p=0.022)	Randomized trial
CCG3971 Matthay NEJM	5 cycles of CDDP, DOX, VP, CPM	3-year EFS 30 % 3-year OS 45 %	Single arm prospective trial
ANBL0532/COG Park JCO	T/C x 2, P/E, CAV, P/E, CAV	3-year EFS 51 % 3-year OS 68 %	Single arm prospective trial
HR-NBL1/SIOPEN Ladenstein Preliminary results (n=607)	Rapid COJEC vs modified N7 (CAV + P/E x 5)	3-year EFS 39% vs 39% (p=0.805)	Randomized trial
NB2004-HR/GPOH Berthold Preliminary results	N5/N6 x 6 vs N8/N5/N6 x 8	N5/N6 arm: 3-year EFS 36%	Randomized trial

Through these years, the main lessons learnt have been:

- More intensive induction chemotherapy achieves better response rates (time intensity and dose intensity);
- Achievement of metastatic complete response at the end of the induction is one of the most powerful prognostic factor known to date;
- The optimal duration of induction chemotherapy has not yet been established. However, short induction regimens such as COJEC achieve similar response rates compared to others. Prolonging induction with additional courses of chemotherapy has not improved long-term outcomes [Kushner BH, J Clin Oncol 2004; Amoroso L, Cancer Res Treat 2018]
- The role of anthracyclines is still not established [Ladenstein R, Lancet Oncol 2017; Amoroso L, Cancer Res Treat 2018]

In order to develop the most effective induction chemotherapy regimen and improve overall outcome for HR-NBL, it is necessary to evaluate the induction regimens that are used as standard practice in different regions of the world in a randomized trial and ensure standardisation of disease assessment.

In light of the early results of R3/HR-NBL1, the RAPID COJEC induction was considered as standard therapy. COG induction is very similar to modified N7 regimen, the only difference being the 2 topotecan-based cycles, thus not justifying a new randomization with RAPID COJEC. The early results from NB2004-HR German trial show comparable 3-year EFS to RAPID COJEC; however, these results were achieved using MEC conditioning regimen for high dose therapy and without maintenance with anti-GD2 immunotherapy. Therefore, R-I is designed to compare head to head both RAPID COJEC and GPOH induction regimens within the same clinical trial, using the same consolidation (R-HDC) and immunotherapy regimen, in order to identify which induction regimen provides superior outcome.

Potential role of anti-GD2 immunotherapy in induction

Disialoganglioside (GD2) is a high priority target in neuroblastoma. Anti-GD2 monoclonal antibodies, have demonstrated efficacy in the frontline setting (maintenance) and in patients with high-risk refractory/relapsed neuroblastoma [Yu AL, NEJM 2010; Ladenstein R, J Clin Oncol 2016; Lode HN, J Clin Oncol 2015].

Recent data from a phase 2 trial conducted by the COG suggest that dinutuximab (ch14.18 anti-GD2) and granulocyte-macrophage colony stimulating factor (GM-CSF) given concurrently with chemotherapy (irinotecan-temozolomide) in the relapsed setting have greater activity than chemotherapy alone, even in children previously treated with anti-GD2 monoclonal antibodies.[Mody R, Lancet Oncol 2017]. In this study, 9/17 (53%) of patients with relapsed/refractory neuroblastoma experienced CR or PR. The trial has been extended and results to confirm the initial promising activity are awaited.

Additionally, there are single-institution data from concomitant combination of induction chemotherapy with the humanized hu14.18K322A anti-GD2 antibody, GM-CSF and low dose interleukin 2 (IL-2) during frontline treatment in a clinical trial conducted at St Jude [Furman WL, J Clin Oncol 2017; Federico SM, Clin Cancer Res 2017], showing no added toxicity and enhanced responses. Finally, 3F8 anti-GD2 antibody was given following each of the last 3 cycle of the induction chemotherapy in the MSKCC experience: no safety issue was encountered but no data in terms of response rate has been reported.[Kushner BH, J Clin Oncol 2004]

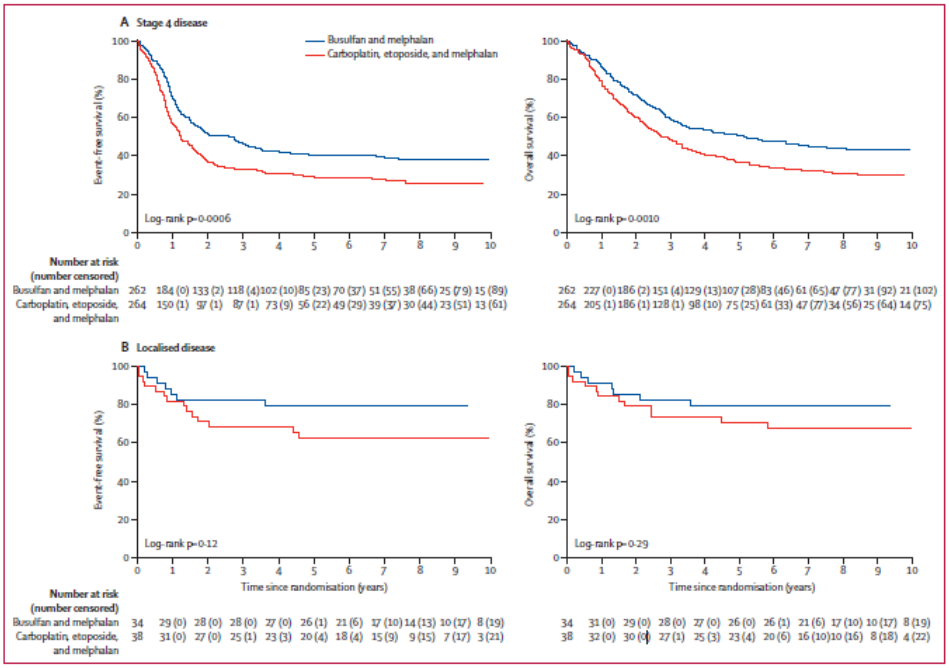
Given the positive signals of activity shown with the concomitant combination of chemotherapy with antiGD2 monoclonal antibodies, SIOPEN is planning to conduct pilot studies to evaluate the feasibility of adding anti-GD2 targeted therapy to conventional induction chemotherapy. If these

studies hold promise, this combination would be taken forward into the frontline setting in a randomizedclinical trial to definitely evaluate the role of anti-GD2 added to induction therapy.

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 5). 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 the improved EFS was associated with the 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 5: Bu-Mel vs CEM in R1/HR-NBL1 (adapted from Ladenstein R, Lancet Oncol 2017)



Questions regarding the optimal consolidation regimen, its interaction with the induction chemotherapy and the role of tandem or multiple regimens remain of major interest on an international level. As HR-NBL remains incurable for more than 50% of patients, further dose intensity may yield further improvements in EFS. To achieve this, tandem HDC approaches have been explored.

Data from past studies in North America support the use of multiple-cycle HDC as consolidation therapy in neuroblastoma.[Kletzel M, J Clin Oncol 2002; Grupp SA, J Clin Oncol 2000; George RE, J Clin Oncol 2006] However, the late effects of TBI-containing regimens prompted COG investigators to evaluate alternative consolidation regimens. Pediatric Oncology Group-POG9047 study enrolled 33 patients treated with 5 cycles of induction chemotherapy and tandem HDC with Thiotepa and cyclophosphamide followed by CEM.[Granger M, Pediatr Blood Cancer 2012] The combination of Thiotepa and cyclophosphamide was chosen as a non-overlapping regimen with CEM. This strategy was found to be feasible and tolerable, the 5-year EFS and OS rates was 27%±8% and 39%±9%, respectively. Twenty-two/33 patients received at least one HDC, these patients having a 5-year EFS and OS from time of first ASCR of 41%±12% and 49%±12% respectively.

These promising results led the COG Neuroblastoma Committee to consider intensification of CEM in a tandem regimen. The pilot COG study ANBL00P1 explored the feasibility of tandem HDC as consolidation for HR-NBL in a cooperative group setting [Seif AE, Bone Marrow Transplant 2013]. The condition regimen included Thiotepa 900mg/m2 (Thio900) over 3 days and cyclophosphamide 1500mg/m2 over 4 days, followed by a “modified CEM” (continuous infusion of carboplatin 1500mg/m2, etoposide 1200mg/m2 over 4 days plus melphalan 180 mg/m2 over 3 days). Overall, 3 patients experienced treatment-related mortality. Twenty-two events occurred in 41 eligible patients enrolled at diagnosis resulting in 2-year EFS from time of date of randomization of 38% ±11%. This study showed that tandem HDC/ASCR with non-overlapping, non-TBI-containing HD regimens is feasible with acceptable toxicity in children with HR-NBL, although small numbers of accrued patients limit the ability to assess efficacy.

Based on these results, the last COG ANBL0532 trial enrolled 652 patients with newly diagnosed HR-NBL who were randomized to single HDC with CEM versus tandem HDC as in ANBL00P1. The study reported a 3-year EFS of 61% with tandem HDC and 48% with single HDC (p=0.008). The 3-year OS was similar, 74% and 69%, respectively. The benefit of tandem HDC was preserved in patients who also received GD2-directed immunotherapy, with a 3-year EFS of 74% vs 56% (p=0.003).[Park JR, J Clin Oncol 2016] Both regimens were well tolerated, with no additional non-hematological toxicities for tandem HDC compared to single HDC. In this study, patients who were benefiting from the intensified strategy could not been identified by tumor burden at diagnosis, metastatic response after the induction or tumor biology. In the future COG protocol, this tandem HDC consisting in Thiotepa-Cyclophosphamide and modified CEM will be considered as the standard of care, and will be randomized with alternative intensified consolidation regimens.

In Europe, the feasibility of Thiotepa- and melphalan- based single, tandem and triple HDC for patients with HR-NBL has been recently evaluated in a single institution trial, with a 5-year EFS of 73% for tandem HDC.[Saarinen-Pihkala UM, Pediatrc Blood Cancer 2012]

Data from a single institution cohort of patients with HR-NBL treated with HD Bu-containing regimens reported a lack of benefit from the addition of HD cyclophosphamide [Hartmann O, Bone Marrow Transplant 1999]; moreover the risk of SOS seemed to be increased by the association of Cyclophosphamide and Bu-Mel. Based on these results, a tandem HDC strategy has been explored in a single institution pilot trial for patients with very high-risk (VHR) neuroblastoma given the insufficient metastatic response after induction chemotherapy.[Pasqualini C, Bone Marrow Transplant 2016] The study enrolled 26 patients who received HD Thio900 over 3 days and Bu-Mel, both followed by ASCR. This consolidation strategy was shown to be feasible, with manageable toxicities. The 3-year EFS and OS of 37% and 69% appeared very encouraging compared with previously reported data in this specific population.[Hartmann O, Eur J Cancer 1997] In the SIOPEN/VHR-NBL protocol (VERITAS, NCT03165292), patients with an insufficient response to induction chemotherapy are randomized to receive either tandem HDC comprising Thio900 and Bu-Mel courses, or ^{131}-mIBG and Bu-Mel, followed by ASCR.

It is now of major importance to study the impact on survival of an intensified HDC based on the European HD Bu-Mel strategy for patients with “standard HR” neuroblastoma. In order to evaluate the role of tandem HDC in the SIOPEN context, in the HR-NBL2/SIOPEN trial children with HR-NBL will receive either single HD Bu-Mel or tandem HDC with Thio900 and Bu-Mel, followed by ASCR (R-HDC).

Table 3 summarizes the randomized trials on HDC in neuroblastoma that have been published over the last two decades.

Table 4: Randomized trials on HDC in neuroblastoma

	Treatments	**Survival**	**Selected arm**
CCG **Matthay** **NEJM 1999** (n= 379)	HD CEM + TBI vs continuation chemotherapy	3-year EFS 34 ± 4 % vs 22 ± 4 % from randomization, p = 0.034 No significant difference in OS	HD CEM + TBI
GPOH **Berthold** **Lancet Oncol 2005** (n= 295)	HD MEC vs maintenance chemotherapy	3-year EFS 47% [95% CI 38–55] *vs* 31% [95% CI 23–39]; hazard ratio 1.404 [95% CI 1·048–1·881], p=0.0221 No significant difference in OS	HD MEC

ENSG1 **Pritchard** **PBC 2005** (n=90)	HD Mel vs no further treatment	5-year EFS 38% [95% CI 21-54%) vs 27% [95% CI 12-42%]; p=0.08 In stage IV, > 1 year (n=48): 33% versus 17% (p = 0.01)	HD Mel
Cochrane **Yalçin** **Cochrane Database Syst Rev 2015** (n=739)	Metanalysis HDC/ASCR vs conventional chemotherapy or no further treatment	HR 0.78, 95% CI 0.67 to 0.90	Significant statistical difference in EFS in favor of HDC/ASCR
COG **Park** **JCO 2016** (n=355)	HD CEM vs HD Cyclo-Thio + modified CEM	3-year EFS 48.4 ± 3.8% vs 61.4 ± 3.7 % from randomization, p = 0.0081 No significant difference in OS	Cyclo-Thio + modified CEM
SIOPEN **Ladenstein** **Lancet Oncol 2017** (n=598)	HD Bu-Mel vs HD CEM	3-year EFS rate 50% [95% CI 45-56%] vs 38% [95% CI 32-43; p=0.0005]	HD Bu-Mel

1.4 Surgery

This study aims to achieve complete primary tumor excision, ideally prior to HDC, to improve local control. Surgical issues are discussed in detail in section 5.5.

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. As there are some important uncertainties and controversies surrounding the best use of radiotherapy, there is a need for clinical trials to produce high-level evidence to optimize its use in order to improve the current unsatisfactory outcomes.

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% ± 3% in patients with macroscopic residual tumour, and 15% ± 1% in patients with complete resection. In these two groups, the 5-year EFS was 38% ± 3% and 49% ± 2%, respectively.

In Germany, it was recommended practice to administer 36 to 40 Gy radiotherapy only in patients > 1 year old with MIBG-positive residual primary tumour.[Simon T, Strahlenther Onkol. 2006] Preliminary GPOH data on 301 patients showed a 5-year cumulative incidence of local relapse (“local only” + “combined”) of 33% without radiotherapy (43% in patients with residual tumour, n=43/100; 24% in patients without residual tumour, n=38/157).

In the USA the current strategy according to COG protocol ANBL0532 is to give 21.6 Gy to the primary tumour bed in all patients, followed by a boost up to 36 Gy in the case of any residual

disease greater than $1cm^3$. Up to five metastatic sites with persistent MIBG-positivity before the HDC are also treated with 21.6 Gy.
Based on these data, and given the poor prognosis of the HR population, investigation of escalation of the local radiotherapy treatment is highly desirable. The SIOPEN/HR-NBL2 trial offers an excellent opportunity to provide the evidence of the impact of the dose of radiotherapy on survival. For this reason, radiotherapy at the preoperative tumor site will be performed for all patients. In addition we want to address the question whether dose escalation beyond 21.6 Gy would translate into better outcomes in terms of survival for patients with residual disease. This randomisation (21.6 Gy vs 21.6 Gy to the preoperative tumor bed + 14.4 Gy boost to the residual tumor) will determine whether patients with macroscopic residual disease after HDC/ASCR and surgery do better with a higher radiotherapy dose.
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, GPOH and the COG have 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/m2/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%±4% for dinutuximab vs 57%±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±5% vs 46±5% and OS 86±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^2/cycle as 5 daily 8 hour infusions) alone or in combination with IL-2 (6 x 10^6 IU/m^2 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^2/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 10^6 IU/m^2/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.

MARKETING AUTORISATION for dinutuximab beta
The European Medicines Agency marketing authorization for dinutuximab beta was given on 8th of May 2017 for the following indication:
“Treatment of high-risk neuroblastoma in patients aged 12 months and above, who have previously received induction chemotherapy and achieved at least a partial response, followed by myeloablative therapy and stem cell transplantation.”
The recommended total dose is 100 mg/m^2/cycle for 5 cycles, each lasting 35 days. There are two possible methods of administration:

- 10 day continuous infusion (total of 240 hours) at 10 mg/m^2/day
- 5 infusions of 20 mg/m^2/day over 8 hours, days 1-5

Based on previous SIOPEN data, SIOPEN currently recommends that dinutuximab beta be administered using the LTI schedule <u>without</u> co-administration of IL-2 (10 day continuous infusion at 10 mg/m^2/day), for a total of 5 cycles.
Maintenance treatment is completed by oral 13-cis-RA for a total of 6 cycles.
Future amendments to the recommended maintenance strategy may be necessary following the evaluation of SIOPEN HR-NBL1/R4 results and long-term dinutuximab beta toxicity reports.

1.7 Biology investigations

Neuroblastoma can demonstrate varying levels of genomic instability and harbor a wide variety of numerical and structural genetic abnormalities reflecting the heterogeneous clinical and biological behavior of the disease.
Homogeneous amplification of the *MYCN* oncogene is present in ~25% of all neuroblastomas. The *MYCN* status is routinely used in clinical practice for treatment stratification, and a *MYCN* amplified

tumor should be considered as high risk, regardless of stage and age (apart from INRG-L1 + INSS1 tumors.
Other recurrent structural chromosomal alterations commonly associated with advanced stage of disease and poor outcome include deletion at chromosome arms 1p, 3p, 4p and 11q, and gain of 1q, 2p or 17q. A subgroup of stage M patients with extremely bad outcome with current therapy regimens is identified by the presence of two or more of the following: 1q, 17p, 19q, *ATRX* deletion and/or telomerase reverse transcriptase (*TERT*) aberrations.[Peifer M, Nature 2015]
Activating point mutations of *ALK* (~10% of cases) as well as gene amplification have also been described, making *ALK* a promising target for molecular therapy in neuroblastoma treatment.
Several deep-sequencing studies on neuroblastoma consistently reported a low frequency of recurrent mutations. More recent studies in relapsed neuroblastoma have suggested that clonal evolution is common and results in the acquisition of targetable somatic aberrations in known oncogenic pathways (temporal heterogeneity). Early evidence suggests that activation of the MAPK pathway and other signaling pathways inducing epithelial-mesenchymal transition (EMT) processes might contribute to treatment failure and might be promising targets for molecular targeted treatment approaches.[Eleveld TF, Nat Genet 2015; Schramm A, Nat Genet 2015] Spatial heterogeneity might also exist either as intra-tumoral heterogeneity or as heterogeneity between a primary tumor and its metastatic sites.[Ambros PF, Med Pediatr Oncol. 2001; Abbasi MR, Mol Oncol 2015] The development of liquid biopsies for the study of circulating tumor DNA (ctDNA) in the cell free DNA (cfDNA) fraction, RNA and micro-RNAs (miRNA) represents a powerful tool to enable sequential analysis of tumor cells and tumor heterogeneity.[Combaret V, J Clin Oncol 2005; Viprey VF, J Clin Oncol 2014; Combaret V, Cancer Med 2015; Chicard M, Clin Cancer Res 2016; Chicard M, Clin Cancer Res 2018; Corrias MV, Pediatr Blood Cancer 2018] Clinically informative biomarkers that can be detected in blood are attractive to guide treatment decisions for children with neuroblastoma in real-time, and are suitable for monitoring children as collection of blood is minimally invasive and cost effective.
A large number of studies have focused on the analysis of differential expression patterns in neuroblastoma, seeking to define different prognostic groups and to potentially identify new therapeutic targets. A retrospective SIOPEN/COG/GPOH study has identified a multi-gene expressqion signature, which will have to be prospectively validated.[Vermeulen J, Lancet Oncol 2009] The expression of micro-RNAs, noncoding RNA molecules, is also highly variable in neuroblastoma and may be used to predict patientoutcome. The level of gene expression may depend on epigenetic modifiers, recent studies have sought to identify promoter methylation patterns which might identify patient subgroups.[Decock A, Oncotarget 2016], and tumor microenvironment. However, these different expression signatures have not been tested in a prospective setting, nor have they been compared in the same patient population. Thus, the place for prospective implementation of expression profiling in treatment strategies of HR-NBL remains to be determined (See Section 8).

1.8 Follow-up

A homogeneous approach for the assessment of disease status and toxicity will allow to evaluate the impact of the different treatment arms. A common algorithm for follow-up will also provide a platform for research projects.
There are several approaches among different groups concerning the type and intensity of mandatory follow-up studies. *Owens et al* recently displayed that, among 50/183 patients who experienced relapse, 37 had symptomatic and/or evaluable disease with X-ray (XR), ultrasound

(US), or urinary catecholamines (UC). MIBG scans identified 8 additional recurrences and cross-sectional imaging (CT or MRI) was only required to identify 5 more cases.[Owens C, Pediatr Blood Cancer 2016] *Kushner et al* reported that patients whose monitoring included ^{123}I-MIBG scan were significantly less likely to have an extensive bone and bone marrow disease at relapse, and that they had a significantly longer survival from relapse ($p < .001$) and from diagnosis ($p = .002$).[Kushner BH, J Clin Oncol 2009] However, these results from a single institution were obtained with an intense follow-up schedule characterized by high radiation exposure.

The previous HR-NBL1/SIOPEN trial [Ladenstein R, Lancet Oncol 2017] recommended imaging of the primary site (US or CT as appropriate) every 3 months for the first year, every 6 months for the second and third year and yearly thereafter. Metastatic disease assessment with mIBG scan was suggested if this was positive at the end of treatment every 3 months until negative (or progression). If stable over a year, the mIBG scan was repeated yearly. In case of residual BM disease at the end of treatment, BM evaluation was suggested every 3 months until negative or progression. For asymptomatic patients in complete remission at the end of treatment, no routine surveillance with mIBG scan or BM evaluation was recommended.

The GPOH NB2004-HR trial recommended clinical evaluation, UC and US/Chest X-ray every 6 weeks during the first year, every 3 months from the second to the fifth year and every 6 months thereafter. MRI imaging was suggested every 3 months during the first year, and every 6 months thereafter only in case of abnormal findings. BM evaluation and mIBG scans were recommended only in case of persistant bone marrow and bone disease at the end of treatment evaluation, respectively. No routine mIBG scans and BM evaluation were advised for asymptomatic patients in complete remission.

All these recommendations mostly reflect customary practice rather than evidence-based follow-up strategies. Moreover, there is rising concern about radiation-induced morbidities in pediatric patients with solid tumors.[Robbins E, Pediatr Blood Cancer 2008]

Taking all this into account, the HR-NBL2/SIOPEN study will include a follow-up schedule with a selected number and type of mandatory evaluations. Individual centers and countries may have additional examinations as well as data/sample collection, according to local practice, national guidelines and research projects covered by additional consent and ethical approvals (See Section 6.3).

2 STUDY OBJECTIVES

2.1 Primary objective

- **R-I:**
  Comparison of the EFS rate from date of randomization of 2 induction regimens, GPOH and RAPID COJEC, in patients with high-risk neuroblastoma
- **R-HDC:**
  Comparison of the EFS rate from date of randomization of single HDC with Bu-Mel versus tandem HDC with Thiotepa followed by Bu-Mel in patients with high-risk neuroblastoma
- **R-RTx:**
  Comparison of the EFS rate from date of randomization of 21.6 Gy radiotherapy to the preoperative tumor bed versus 21.6 Gy radiotherapy and a sequential boost of an additional 14.4 Gy to the residual tumor in patients with macroscopic residual disease with high-risk neuroblastoma after HDC and surgery.

2.2 Secondary objectives

1) To describe the EFS and overall survival (OS) from date of randomization of the whole cohort,
2) To describe the effect of RAPID COJEC and GPOH induction regimens on metastatic disease during and after the end of induction,
3) To assess the correlation of the response of metastatic disease during and after induction with survival (EFS and OS),
4) To describe the effect of HDC with Bu-Mel versus Thiotepa + Bu-Mel on progression-free survival (PFS) and OS,
5) To describe and compare the toxicity associated with RAPID COJEC and GPOH induction therapy,
6) To describe and compare the acute and long term toxicities of both HDC arms,
7) To describe the long term toxicities of dinutuximab beta,
8) To investigate the relationship between the quality of surgical resection of the primary tumor, local control and survival,
9) To investigate the impact of the radiotherapy dose on local relapse rate,
10) To collect data on selected circulating biomarkers, biological and genomic features (see Laboratory Manual) and compare the effect of these on response to treatment, EFS, incidence of relapse/progression and OS.

2.3 Exploratory objectives

1) To conduct sub-group analyses to study the impact of R-I, R-HDC and R-RTx in subpopulations such as patients with L2-*MYCN* amplified neuroblastoma or patients according to age groups (infants, young children, older children and adolescents),
2) To validate prospectively the new international criteria for response assessement in neuroblastoma [Park JR, JCO 2017; Burchill SA, Cancer 2017]
3) To validate prospectively the new international mIBG scoring methodology,
4) To evaluate the impact of mIBG-positive residual bone disease before HDC, after HDC and at the end of treatment on the risk of bone recurrence,
5) To prospectively study the relative prognostic value of planar vs SPECT-SPECT/CT(fusion) methodology of MIBG imaging,
6) To describe quality of standards of care: time from start of symptoms to histological diagnosis, time from diagnosis till initiation of treatment, proportion of dose reductions or interrupted chemotherapy cycles, time to start radiotherapy, among others.

3 METHODOLOGY

This is an international open-label, randomized, multicenter phase III trial including three sequential randomizations to assess efficacy of induction and consolidation chemotherapies, as well as radiotherapy, for patients with high-risk neuroblastoma.

The first randomization (**R-I**) will compare the efficacy of two induction chemotherapies (RAPID COJEC and GPOH regimens) in a phase III setting. The primary endpoint will be the 3-year EFS from date of randomization diagnosis. The R-I randomization will be stratified on age, stage, MYCN status and countries.

The second randomization (**R-HDC**) will compare the efficacy of single HDC with Bu-Mel versus tandem HDC with Thiotepa followed by Bu-Mel. The primary endpoint is 3-year EFS calculated from the date of the R-HDC randomization. The R-HDC randomization will be stratified on the age, stage, MYCN status, induction chemotherapy regimen, response to induction phase and countries.
The impact of local treatment in this phase III setting will be assessed, according to the presence or not of a macroscopic residual disease after surgery and HDC.
In case of macroscopic residual disease, 21.6 Gy radiotherapy to the preoperative tumor bed will be randomized (**R-RTx**) versus the same treatment plus a sequential boost of additional 14.4 Gy to the residual tumor. The primary endpoint of R-RTx is 3-year EFS from the date of the R-RTx randomization. The R-RTx randomization will be stratified on age, stage, MYCN status, induction chemotherapy regimen, HDC regimen and countries.
In case of no macroscopic residual disease, 21.6 Gy radiotherapy will be delivered to the preoperative tumor bed.

3.1 Measures to minimize bias

- Stratified randomizations
  By introducing a deliberate element of chance into the assignement of treatments to subjects in the trial, the randomization produces treatments groups with similar distribution of pronostic factors known and unknown. The randomization provides sound statistical basis for the evaluation of the treatments effects based on the prospectively collected data. The randomization will be stratified on age, MYCN status, stage, and country. Moreover, in order to control for interactions between each treatment phase on the EFS, the randomizations for the allocation of the consolidation and radiotherapy treatment group will be stratified by the previous received treatment and by response to previous treatments. This allows the assessement of the consolidation and radiotherapy treatment effect on the EFS regardless of the effect of the previous treatment.
- Central review of MIBG scans and bone marrow evaluation.
- Central review of tumor imaging before radiotherapy and of radiotherapy plans (QUARTET platform).

4 PATIENT SELECTION AND WITHDRAWAL

The following eligibility criteria are designed to select patients for whom protocol treatment is considered appropriate. All relevant medical and non-medical conditions should be taken into consideration when deciding whether this protocol is suitable for a particular patient.
Deviations from eligibility criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or patient safety.

4.1 Eligibility criteria

4.1.1 Eligibility criteria for R-I

Enrollment in HR-NBL2 and randomization for induction strategy will be performed at diagnosis (or up to 21 days after one cycle of chemotherapy for patients with localized neuroblastoma with *MYCN* amplification).

1) 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) and Ms neuroblastoma 12-18 months, any *MYCN* status*
  or
- L2, M or Ms neuroblastoma with *MYCN* amplification, any age

** In Germany, patients aged less than 18 months with stage M and without MYCN amplification will not be enrolled in HR-NBL2 trial.*

2) No previous chemotherapy (except one cycle of Etoposide-Carboplatin or, in Germany and Nertherlands, one cycle of the current protocol for low/intermediate risk neuroblastoma).
3) Females of childbearing potential must have a negative serum or urine pregnancy test within 7 days prior to initiation of treatment. Female patients who are lactating must agree to stop breast-feeding. Females and males with partners of childbearing potential (i.e. not post-menopausal or surgically sterilised) must use adequate methods of contraception Acceptable contraception are listed in Appendix 11 to prevent pregnancy or abstain* from heterosexual activity for the duration of the trial and for at least 12 months following treatment discontinuation.

**Abstinence must be in line with the preferred and usual lifestyle of the subject. Periodic abstinence (such as calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.*

4) Written informed consent to enter the R-I randomization from patient or parents/legal representative, patient, and age-appropriate assent.
5) Patient affiliated to a social security regimen or beneficiary of the same according to local requirements.
6) Patients should be able and willing to comply with study visits and procedures as per protocol.

In case of parents'/patient's refusal to R-I, or renal or liver toxicity, patients can still be enrolled in HR-NBL2 trial with parents'/patient's consent within 3 weeks from the beginning of chemotherapy. Patients will be treated with the standard induction regimen per country and will be potentially eligible for subsequent randomizations.

4.1.2 Eligibility criteria for R-HDC randomization

Randomization for HDC strategy will be performed at the end of induction after the disease evaluation and after surgery of the primary tumor for those patients who will receive surgery before HDC.

R-HDC eligibility criteria:

1) - Stage M neuroblastoma above 365 days of age at diagnosis, any *MYCN* status,
   ***EXCEPT* patients with stage M or Ms 12-18 months old with numerical chromosomal alterations only, and in complete metastatic response at the end of induction. In this case, patients will have surgery but will not be eligible for R-HDC and will not be able to pursue the trial.**
   Or
   - L2, M or Ms neuroblastoma with *MYCN* amplification.

1) Age < 21 years.
2) Complete response (CR) or partial response (PR) at metastatic sites:
   - Bone disease: MIBG uptake (or FDG-PET uptake for MIBG-nonavid tumors) completely resolved or SIOPEN score ≤ 3 and at least 50% reduction in mIBG score (or ≤ 3 bone lesions and at least 50% reduction in number of FDG-PET-avid bone lesions for MIBG-nonavid tumors).
   - 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 ≥ 50%
- Hematological status: ANC >0.5 x 109/L, platelets > 20 x 109/L
- Cardiac function: Shortening fraction ≥ 28% or ejection fraction ≥ 55% by echocardiogram, no clinical congestive heart failure. Normal pulmonary artery pressure.
- Normal chest X-ray and oxygen saturation.
- Absence of any toxicity ≥ grade 3.

3) Sufficient collected stem cells available; minimum required: 6 x 10^6 CD34+ cells/kg body weight stored in 3 separate fractions.
4) Written informed consent, including agreement of patient or parents/legal guardian for minors, to enter the R-HDC randomization.
5) Patient affiliated to a social security regimen or beneficiary of the same according to local requirements.
6) Patients should be able and willing to comply with study visits and procedures as per protocol.

In case of parents'/patient's refusal, or insufficient stem cells collection for tandem HDC but with a minimum of 3 x 10^6 collected CD34+ cells/kg body weight, or in case of patients older than 21 years, or liver or renal toxicity, HDC will consist on the standard HD Bu-Mel and will be eligible for subsequent randomization.

Patients with insufficient metastatic response at the end of induction ("refractory disease") should be enrolled in the SIOPEN very-high risk neuroblastoma trial (VERITAS, NCT03165292).

NOTE: *In countries in which VERITAS trial is not available, it is strongly recommended to refer those patients to countries in which the trial is open. In case of family or patient's refusal or other reasons, the Coordinating Investigator's advice is highly recommended to define the best approach for such patients. The patient should continue to be followed in the HRNBL2 study and data about subsequent treatment modalities collected.*

4.1.3 Eligibility criteria for R-RTx randomization

An evaluation of the local disease will be performed after HDC/ASCR and surgery.

- In case of **no local macroscopic disease**, all patients will receive 21.6 Gy radiotherapy to the pre-operative tumor bed.
- In case of **local macroscopic residual disease**, patients will be eligible to R-RTx if the following criteria are met:

1) No evidence of disease progression after HDC/ASCR.
2) Interval between the last ASCR and radiotherapy start between 60 and 90 days.
3) Performance status ≥ 50%.
4) Hematological status: ANC >0.5 x 10^9/L, platelets > 20 x 10^9/L.
5) Written informed consent, including agreement of patient or parents/legal guardian for minors, to enter the R-RTX randomization.
6) Patient affiliated to a social security regimen or beneficiary of the same according to local requirements.
7) Patients should be able and willing to comply with study visits and procesudres as per protocol.

In case of parents'/patient's refusal of the randomization, the patient will receive 21.6 Gy radiotherapy to the pre-operative tumor bed and pursue the next step of the trial.

4.1.4 *Recommended criteria to enter maintenance treatment*

- No progressive disease
- Performance status ≥ 50%
- Maintenance (starting with 13-cis-RA) must start no later than day 120 post ASCR
- Hematological status: ANC >0.5 x 10^9/L, platelets > 20 x 10^9/L and haemoglobin > 7.0 g/dL
- Acceptable organ function:
  - Cardiac function: Shortening fraction ≥ 28% or ejection fraction ≥ 55% by echocardiogram, no clinical congestive heart failure.
  - Normal chest X-ray and oxygen saturation
  - Absence of any toxicity ≥ grade 3

4.2 Non inclusion criteria

Non-inclusion criteria specific to the R-I randomization (RAPID COJEC/GPOH) :

1) Urinary outflow obstruction
2) Severe arrhythmia, heart failure, previous cardiac infarct, acute inflammatory heart disease
3) Severe peripheral neuropathy

4) Demyelinating form of Charcot-Marie-Tooth syndrome
5) Hearing impairment
6) Concurrent prophylactic use of phenytoin
7) Cardiorespiratory disease that contraindicates hyperhydration

Non-inclusion criteria common to all randomizations (R-I, R-HDC and R-RTx) :

1) Any negative answer concerning the inclusion criteria of R-I or R-HDC or R-RTx will render the patient ineligible for the corresponding therapy phase. However, those patients may be kept on study and be considered to receive standard treatment of the respective therapy phase, and may be potentially eligible for subsequent randomizations.
2) Liver function: Alanine aminotransferase (ALT) > 3.0 x ULN and blood bilirubin > 1.5 x ULN (toxicity ≥ grade 2). In case of toxicity ≥ grade 2, call national principal investigator study coordinator to discuss the feasibility.
3) Renal function: Creatinine clearance and/or GFR < 60 ml/min/1.73m² (toxicity ≥ grade 2). If GFR < 60ml/min/1.73m², call national principal investigator to discuss.the feasibility.
4) Dyspnea at rest and/or pulse oximetry <95% in air.
5) Any uncontrolled intercurrent illness or infection that in the investigator opinion would impair study participation.
6) Patient under guardianship or deprived of his liberty by a judicial or administrative decision or incapable of giving his consent.
7) Participating in another clinical study with an IMP while on study treatment.
8) Concomitant use with yellow fever vaccine and with live virus and bacterial vaccines.
9) Patient allergic to peanut or soya.
10) Chronic inflammatory bowel disease and/or bowel obstruction.
11) Pregnant or breastfeeding women.
12) Known hypersensitivity to the active substance or to any of the excipients of study drugs.
13) Concomitant use with St John's Wort (Hypericum Perforatum).

4.3 Withdrawal criteria

4.3.1 Withdrawal criteria from study treatment

A patient will not receive any further study treatment if any of the following occurs:

- Progressive disease
- Request from the patient/parents/legal guardian not to receive further study treatment
- Withdrawal of consent or lost to follow up
- Adverse events or any condition incompatible with continuation of the study treatment according to investigator's judgement
- Any medical event requiring administration of an unauthorized concomitant treatment (i.e. any other anticancer treatment or investigational agent)
- Pregnancy or intent to become pregnant
- Subject non-compliance to study procedures and/or treatment that in the investigator and/or sponsor judgement warrants withdrawal
- Study terminated by Sponsor

Patients who are withdrawn for other reason than withdrawal of consent will have a withdrawal visit including end of treatment visit procedures and follow-up visits but will still be followed up for the trial primary and secondary endpoints.

4.3.2 Withdrawal criteria from the trial

Reasons for withdrawal from the trial (study treatment and follow-up) may include:

- Lost to follow-up
- Withdrawal of consent
- Death

Lost to follow-up

If a patient does not return for a scheduled visit, every effort should be made to contact him/her. In any circumstance, every effort should be made to document the patient outcome and all attemps should be documented in the corresponding medical file.

The investigator should inquire about the reason for withdrawal, ask the patient to have a final visit, to evaluate any unresolved adverse events. The early termination final visit should include all assessments listed for the “End of Treatment” visit.

Withdrawal of consent

If the patient/parents/legal guardian withdraws his/her consent for the study, no further study specific evaluations should be performed, and no additional data will be collected. The sponsor may retain and continue to use any data and samples collected before such refusal except in case of patient opposition. Any opposition should be transmitted by the investigator to the sponsor without undue delay.

Patients/parents/legal guardian who withdraws from the study before receiving study drug will be considered as a screening failure, will be replaced and will not be included in the safety or efficacy assessments.

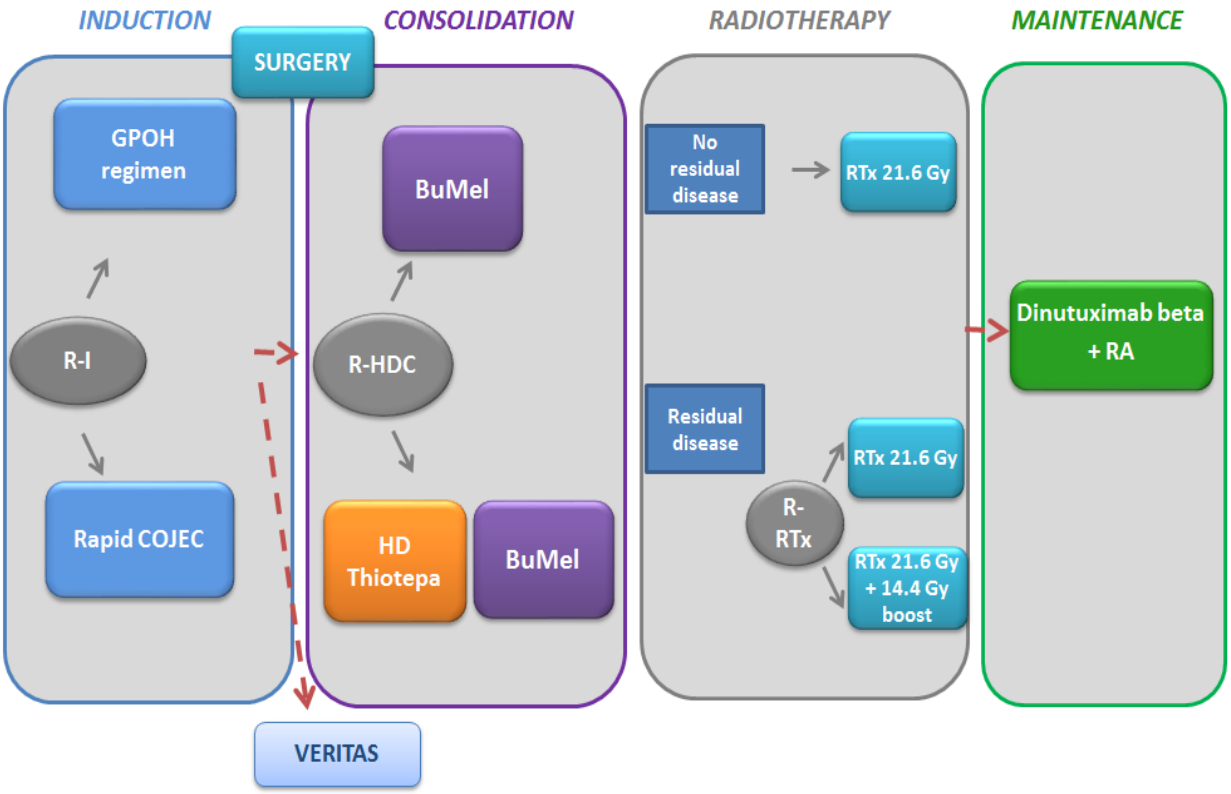
5 TREATMENTS

5.1 General plan: treatment overview

HR-NBL2/SIOPEN trial consists in 4 main treatment phases (Figure 6):

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

Figure 6 - Treatment overview



Induction phase

Patients will be randomized (R-I) to RAPID COJEC or GPOH induction regimen.
Patients that are initially diagnosed with localised disease that start one course of Carboplatin-Etoposide as per the LINES clinical trial recommendation (or, in Germany and Nertherlands, one course of the current protocol for low/intermediate risk neuroblastoma) and are subsequently identified as *MYCN* amplified will also be allowed to enter the trial and all its randomizations. For these patients, the Carboplatin-Etoposide course will replace the first induction course regardless of the arm they are randomized to.

During or following induction phase according to the allocated chemotherapy regimen, autologous stem cell harvest (ASCH) will be performed and complete excision of the primary tumor will be attempted.
At the end of induction, if an adequate metastatic response is achieved (see section 4.1.2), patients may continue the treatment on HR-NBL2 study with the consolidation phase.

Of note:

- Patients 12-18 months old with stage M non-*MYCN* amplified, and with numerical chromosomal alterations only, are thought to have a better prognosis and will stop treatment after induction therapy and surgery to the primary tumor in case of complete remission. In case of no complete remission, call principal national investigator.
- Patients with insufficient metastatic response at the end of induction ("refractory disease") should be enrolled in the SIOPEN very-high risk neuroblastoma trial (VERITAS) in order to receive a more intensive treatment due to their worse outcome.

In countries in which VERITAS trial is not available, it is strongly recommended to refer those patients to countries in which the trial is open. In case of family or patient's refusal or other reasons, the Coordinating Investigator's advice is highly recommended to define the best approach for such patients. The patient should continue to be followed in the HR-NBL2 study and data about subsequent treatment modalities collected.

Consolidation phase

In the consolidation phase, patients will be randomized (R-HDC) to single high-dose Bu-Mel or tandem high-dose Thiotepa and Bu-Mel followed by ASCR. Patients randomized for tandem HDC and without disease progression after the first HDC (Thiotepa) will proceed to high-dose Bu-Mel.

Local treatment phase

Surgery of the primary tumor will be performed after the 4th cycle (GPOH induction) or at the end of induction chemotherapy (RAPID COJEC), according to the allocated induction regimen. If specific surgical complications are expected, surgery may be further postponed until the end of induction (GPOH induction) or after HDC/ASCR (both inductions).

Radiotherapy of the primary tumor site will be performed after HD Bu-Mel chemotherapy and before maintenance treatment. In case of persistent macroscopic residual primary tumor after HDC and surgery, the dose of radiotherapy on the tumor bed will be randomized (R-RTx) between 21.6 Gy to the preoperative tumor extension and 21.6 Gy plus a boost of 14.4 Gy on the residual disease. In case of no macroscopic residual tumor, 21.6 Gy radiotherapy will be performed at the preoperative tumor bed.

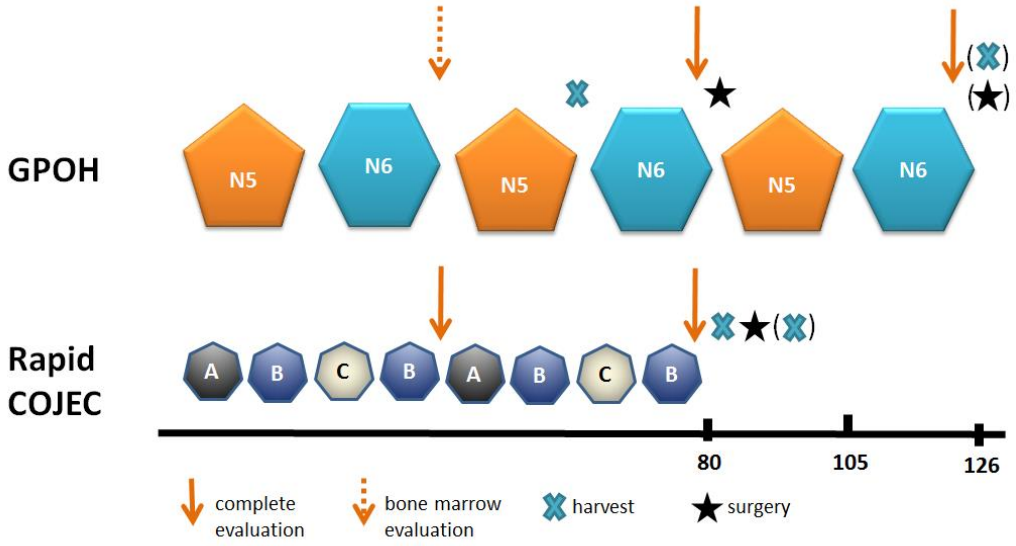
Maintenance phase

Maintenance phase will consist on 6 cycles of 13-cis-RA and 5 cycles of dinutuximab beta.

5.2 Induction chemotherapy

Figure 7 depicts the schema of the two induction chemotherapy regimens, RAPID COJEC and GPOH, proposed for this randomization, including the time points for surgery and stem cell harvest.

Figure 7: Induction chemotherapy regimens schema



For the purpose of this trial, RAPID COJEC and GPOH are investigational regimens Thus Cisplatin, Carboplatin, Cyclophosphamide, Dacarbazine, Doxorubicin, Etoposide, Ifosfamide, Thiotepa, Busulfan-Melphalan (when randomized with Thiotepa), Vincristine and Vindesine are IMPs. All the IMPs will be taken from pharmacy hospital stocks.

Patients that are initially diagnosed with localised disease that start one course of Carboplatin-Etoposide as per the LINES clinical trial recommendation or the GPOH NB2015 LR trial and are subsequently identified as *MYCN* amplified patients will also be allowed to enter the trial and all its randomizations. For these patients, the chemotherapy course will replace the first course of the selected induction.

5.2.1 RAPID COJEC chemotherapy induction

Table 5: 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		→		→		→		→
CYCLOPHOSPHAMIDE			↓↓				↓↓	
G-CSF (days of administration)	**3→8**	**12→18**	**23→28**	**32→38**	**43→48**	**52→58**	**63→68**	**72→76 or until harvest**

↓ One administration/day

→ Continuous administration

Three different courses **(A, B, C)** are given every 10 days regardless of neutrophil or platelet counts, except in case of uncontrolled infection.
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 5) after the end of chemotherapy and until the ANC is > 0.5 x 10^9/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	●	
Carboplatin	●	
Etoposide	●	●

DRUG	Time	Dose	Administration
DAY 1			
VINCRISTINE	H0	1.5 mg/m^2 (max dose 2 mg)	As a single iv bolus or over 1 hour according to local policies
CARBOPLATIN	H1	750 mg/m^2	Infused over 60 minutes iv in 5% destrose
ETOPOSIDE	H2	175 mg/m^2	Infused over 4 hours iv in 0.9% saline
DAY 2			
ETOPOSIDE	H0	175 mg/m^2	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.

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 – discuss with national coordinator.

COURSE B
Start on days 10 - 30 - 50 – 70

Course B (days)	1	2
Vincristine	●	
Cisplatin	●➔	
Hyperhydration	●➔	●➔

➔ Continuous administration

DRUG	Time	Dose	Administration
VINCRISTINE	H0	1.5 mg/m^2 (max dose 2 mg)	As a single iv bolus or over 1 hour according to local policies
PRE-HYDRATION	H1	200 ml/m²/h	Infused over 3 hours before cisplatin: 0.9% sodium chloride with 10 mmol/l potassium chloride
MANNITOL 20%	H1	40ml/m²	Short infusion iv
MANNITOL 20%	H3.5	40ml/m²	Short infusion iv
HYDRATION During cisplatin	H4	125 ml/m²/h	Infused over 24 hours in parallel with cisplatin: 1.5 l/m²/24h of 0.9% sodium chloride 1.5 l/m²/24h of 5% glucose, 30 mmol/m²/ 24h of potassium chloride, 2.5 mmol/m²/24h of calcium gluconate,10 mmol/m²/24h of magnesium sulphate
CISPLATIN	H4	80 mg/m²/24h	Over 24 hours in 0.9% sodium chloride alongside the hydration
POST-HYDRATION	H28 - H52	125 ml/m²/h	1.5 l/m²/ 24 hours of 0.9% sodium chloride 1.5 l/m²/24 hours of 5% glucose, 60 mmol/ m²/24h of potassium chloride, 2.5 mmol/ m² calcium gluconate, 10 mmol /m²/24h of magnesium sulphate
MANNITOL 20%	If needed	40ml/m²	If diuresis falls below 400 ml/m²/6 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/m²/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^2/24 hours.

The use of G-CSF (5µg/kg/day, subcutaneously) during RAPID-COJEC induction 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).

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/m2 then omit cisplatin and discuss with national coordinator.
- Ototoxicity: if Boston grade ≥ 4 toxicity discuss with national coordinator.
- Any other unresolved grade ≥ 3 toxicities – discuss with national coordinator.

COURSE C
Start on days 20 and 60

Course C (days)	1	2
Vincristine	●	
Etoposide	●	●
Cyclophosphamide	●	●
Hyperhydration	●➔	●➔

➔ Continuous administration

DRUG	Time	Dose	Administration
DAY 1			
VINCRISTINE	H0	1.5 mg/m^2 (max dose 2 mg)	As a single iv bolus or over 1 hour according to local policies
ETOPOSIDE	H1	175 mg/m²	Infused over 4 hours iv in 0.9% saline
MESNA	H5	200 mg/m²	Short infusion iv
CYCLOPHOSPHAMIDE	H5	1050 mg/m²	Over 1 hour

HYPERHYDRATION + MESNA	H5	125 ml/m²/h (hydration) + 1.2 g/m^2/24 h (mesna)	Infused over 24 hours: 1.2 g/m²/ 24 hours mesna 1.5 l/m²/24 hours of 0.9% sodium chloride 1.5 l/m²/24 hours of 5% glucose + 60 mmol/m²/24 hours of potassium chloride
DAY 2			
ETOPOSIDE	H0	175 mg/m²	Infused over 4 hours iv in 0.9% saline
CYCLOPHOSPHAMIDE	H4	1050 mg/m²	Over 1 hour
HYPERHYDRATION + MESNA	H4	40ml/m²	Infused over 24 hours: 1.2 g/m²/ 24 hours mesna 1.5 l/m²/24 hours of 0.9% sodium chloride 1.5 l/m²/24 hours of 5% glucose + 60 mmol/m²/24 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.

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 – discuss with national coordinator.

5.2.2 GPOH induction chemotherapy

Table 6: GPOH induction overview

Cycle (n°)	1	2	3	4	5	6
Course	**N5**	**N6**	**N5**	**N6**	**N5**	**N6**
Days (approx.)	**1**	**21**	**42**	**63**	**84**	**105**
VINDESINE	↓		↓		↓	
CISPLATIN	→ → → →		→ → → →		→ → → →	
ETOPOSIDE	→ → → →		→ → → →		→ → → →	
VINCRISTINE		↓ ↓		↓ ↓		↓ ↓
DACARBAZINE		↓ ↓ ↓ ↓ ↓		↓ ↓ ↓ ↓ ↓		↓ ↓ ↓ ↓ ↓
IFOSFAMIDE		→ → → → →		→ → → → →		→ → → → →
DOXORUBICIN		↓↓		↓↓		↓↓
G-CSF (5µg/kg/d), until ANC > 500/mmc or until harvest	**Day 7 →**	**Day 9 →**	**Day 7 → Harvest**	**Day 9 →**	**Day 7 →**	**Day 9 →**

↓ One administration/day

→ Continuous administration

Two different courses **(N5, N6)** are given every 21 days and according to haematological recovery.

Requirements to start each N5 and N6 cycle:

- ANC ≥ 0.5 x 10^9 *without G-CSF for at least 48 hours*
- Platelets ≥ 50 x 10^9/L and rising, without platelets transfusion (except patients with extensive bone marrow involvement)
- No active infection
- Creatinine clearance and/or cystatin-C-clearance ≥60ml/minx1.73m² (toxicity grade < 2)
- For N5 cycle : Boston grade < 4 toxicity ; if ≥ 4 toxicity then substitute cisplatin with carboplatin (see dose modification)
- For N6 cycle: no cardiomyopathy grade ≥ 3 (cardiac ultrasound)

G-CSF:

The use of G-CSF (5µg/kg/day, subcutaneously) during GPOH induction will start 24-72 hours (according to the course; see Table 6) after the end of chemotherapy and until the ANC is > 0.5 x 10^9/L. There should be an interval of at least 48 hours between the last G-CSF injection and the start of the next course of chemotherapy.

N5 CYCLE
Start on days 1 - 42 - 84 (approximately)

N5 cycle (days)	1	2	3	4	5
DRUG					
Vindesine	●				
Cisplatin	●➔	●➔	●➔	●➔	
Etoposide	●➔	●➔	●➔	●➔	
Hydration	●➔	●➔	●➔	●➔	●➔

➔ Continuous administration

DRUG	Time	Dose	Administration
DAY 1			
VINDESINE	H0	3 mg/m^2/day (max dose 6 mg)	Infused over 1 hour in NaCl 0,9%
HYDRATION Continuous over 120 hours: Starting one hour prior to chemotherapy, until 24h after the end of cisplatin	H0 continuous over 120 hours	125 ml/m²/h	Infused over 120 hours: 1.5 l/m²/24 hours of 0.9% sodium chloride, 1.5 l/m²/24 hours of 5% glucose, 30 mmol/m²/24 hours of potassium chloride, 2.5 mmol/ m²/24 hours of calcium gluconate, 10 mmol /m²/24 hours of magnesium sulphate
MANNITOL	H1 continuous over 96 hours	1 g/kg/day (max 1,5g/kg/day)	Infused over 24 hours in parallel with cisplatin
CISPLATIN	H1 continuous over 96 hours	40 mg/m²/day	Infused over 96 hours in 0.9% sodium chloride alongside the hydration

ETOPOSIDE	H1 continuous over 96 hours	100 mg/m²/day	Infused over 96 hours in 0.9% sodium chloride alongside the hydration
DAYS 2 - 4			
CISPLATIN	continous	40 mg/m²/day	Continous in 0.9% sodium chloride alongside the hydration
ETOPOSIDE	continous	100 mg/m²/day	Continous in 0.9% sodium chloride alongside the hydration
HYDRATION	continuous	125 ml/m²/hr	Continous 1.5 l/m²/ 24 hours of 0.9% sodium chloride, 1.5 l/m²/24 hours of 5% glucose, 60 mmol/ m²/24 hours of potassium chloride, 2.5 mmol/ m² calcium gluconate, 10 mmol /m²/24hrs of magnesium sulphate
MANNITOL	continuous until the end of cisplatin	1 g/kg/day (max 1,5g/kgxday)	Continous in parallel with cisplatin during 96 hours
DAY 5			
HYDRATION	continuous until 24h after the end of cisplatin	125 ml/m²/hr	1.5 l/m²/ 24 hours of 0.9% sodium chloride, 1.5 l/m²/24 hours of 5% glucose, 60 mmol/ m²/24 hours of potassium chloride, 2.5 mmol/ m² calcium gluconate, 10 mmol /m²/24hrs of magnesium sulphate

After N5 cycle, G-CSF administration (5µg/kg/day, subcutaneously) will start at **DAY 7** and will be continued until the ANC is > 0.5 x 10^9/L (or until harvest).
There should be an interval of at least 48 hours between the last G-CSF injection and the start of the next course of chemotherapy.

Dose modification:

- **Body weight > 5 kg but < 12kg: Cisplatin: 1.3 mg/kg/day; Etoposide: 4.2 mg/kg/day; Vindesine: 0.1 mg/kg/day.**
- **Body weight ≤ 5 kg: 1/3 reduction (from the mg/kg dose) is indicated.**

- Delayed count recovery:
  - if recovery from previous N5 cycle ≥ 28 days or grade 4 toxicity then reduce doses of etoposide to 80% of full dose. Full dose of cisplatin and vindesine.
- Renal function: If GFR ≤ 60 ml/min/m^2 then substitute cisplatin with carboplatin (160 mg/m^2/day for 4 days as continuous infusion over 96 hours). Full dose etoposide.
- Ototoxicity: if Boston grade ≥ 4 toxicity then substitute cisplatin with carboplatin (160mg/m^2/day for 4 days). Full dose etoposide.
- Any other unresolved grade ≥ 3 toxicities – discuss with national coordinator.

N6 CYCLE
Start on days 21 - 63 - 105

N6 cycle	1	2	3	4	5	6	7	8
DRUG								
Vincristine	●							●
Dacarbazine	●	●	●	●	●			
Ifosfamide	●➔	●➔	●➔	●➔	●➔			
Doxorubicin						●	●	
Hydration	●➔	●➔	●➔	●➔	●➔	●➔		

➔ Continuous administration

DRUG	Time	Dose	Administration
DAY 1			
VINCRISTINE	H0	1.5 mg/m^2/day (max 2 mg)	As a single iv bolus or over 1 hour according to local policies
HYDRATION Starting 1 hour prior to ifosfamide	H0 continuous over 144 hours	125 ml/m²/h	Infused over 24 hours: Dextrose 5%/Sodium Chloride 0.45% with Potassium 20mmol/L
MESNA	H0 continuous over 144 hours	900 mg/m²/day	Infused over 24 hours *May be given in hydration*
DACARBAZINE	H1	200 mg/m^2/day	Infused over 1 hour *STOP Ifosfamide during dacarbazine*

IFOSFAMIDE	H2 continuous over 115 hrs	1500 mg/m^2/day	Infused **over 23 hours** in sodium chloride 0.9% during 5 days ***STOP Ifosfamide during dacarbazine***
DAYS 2 - 5			
DACARBAZINE	H1	200 mg/m^2/day	Infused over 1 hour *STOP Ifosfamide during infusion*
HYDRATION	continous	125 ml/m²/h	Infused continuously Dextrose 5%/Sodium Chloride 0.45% with Potassium 20mmol/L
MESNA	continous	900 mg/m²/day	Infused continuously May be given in hydration
IFOSFAMIDE	continous	1500 mg/m^2/day	Over **23 hours** in sodium chloride 0.9% ***STOP Ifosfamide during dacarbazine***
DAY 6			
HYDRATION	continous until 24 hours after the end of ifosfamide	125 ml/m²/h	Infused continuously: Dextrose 5%/Sodium Chloride 0.45% with Potassium 20mmol/L
MESNA	continous until 24 hours after the end of ifosfamide	900 mg/m²/day	Infused continuously May be given in hydration
DOXORUBICIN	H0	30 mg/m²/dose	Infused over 4 hours in sodium chloride 0.9%
DAY 7			
DOXORUBICIN	H0	30 mg/m²/dose	Infused over 4 hours in sodium chloride 0.9%
DAY 8			
VINCRISTINE	H0	1.5 mg/m^2/day (max 2 mg)	As a single iv bolus or over 1 hour according to local policies

After N6 cycle, G-CSF administration (5µg/kg/day, subcutaneously) will start at **DAY 9** and will be continued until the ANC is > 0.5 x 10^9/L.
There should be an interval of at least 48 hours between the last G-CSF injection and the start of the next course of chemotherapy.

Dose modification :

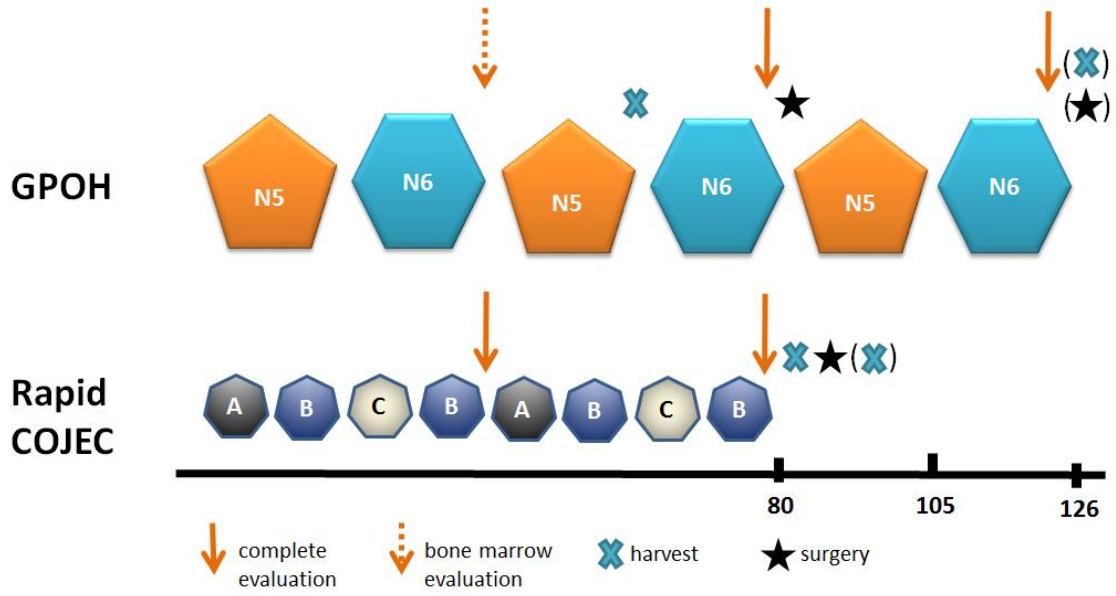
- **Body weight > 5 kg but < 12kg: Vincristine 0.05 mg/kg/dose, dacarbazine 6.7 mg/kg/dose, ifosfamide 50 mg/kg/dose, doxorubicin 1 mg/kg/dose. Mesna 30 mg/kg/day.**
- **Body weight ≤ 5 kg: 1/3 reduction (from the mg/kg dose) is indicated.**

- Delayed count recovery:
  - if recovery from previous N6 cycle ≥ 28 days or grade 4 toxicity: reduce dose of ifosfamide to 1000 mg/m^2 (33.3 mg/kg for infants < 12 kg). Full doses of other drugs.
  - if recovery from previous N6 cycle ≥ 28 days or grade 4 toxicity despite reducing ifosfamide to 1000 mg/m^2 (33.3 mg/kg for infants < 12 kg): omit dacarbazine in subsequent cylcles and discuss with national coordinator.
- Renal function: If GFR ≤ 60 ml/min/m^2 then substitute ifosfamide with cyclophosphamide 300 mg/m²/day continuous infusion days 1-5 and discuss with national coordinator
- Ifosfamide induced encephalopathy grade ≥3: Application of methylene blue or substitution of ifosfamide by cyclophosphamide 300 mg/m²/day continuous infusion days 1-5.
- Any other unresolved grade ≥ 3 toxicities - discuss with national coordinator

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 validated by the national co-sponsor. (See Appendix 7)
Timing of peripheral blood stem cells (PBSC) harvest is specific to each induction schedule (Figure 8).

Figure 8: Timing of PBSC Harvest



Except in case of documented or suspected disease progression, PBSC harvest should be performed in all patients since PBSC will be needed in both HR-NBL2 and VERITAS consolidation.

Patients receiving **GPOH** as induction will have the BM evaluation following cycle 2 and the PBSC collection following cycle 3 (G-CSF 5 µg/kg/day until harvest) depending on bone marrow disease status. It is highly recommendend to collect bone marrow aspirate and harvest into PAXgene^{TM} blood RNA tubes for RTqPCR to establish best practice. Documentation of clearance of tumor cells from the bone marrow (CR or minimal disease as per the INRG RC Burchill, Cancer 2017) is required for early collection. If medical condition prohibits safe apheresis, it is appropriate to delay PBSC mobilization and harvest after subsequent induction course or at the end of induction therapy.
Patients receiving **COJEC** as induction will undergo harvest of PBSC following the end of the induction. Harvest may be scheduled either after the last chemotherapy cycle (G-CSF 5 µg/kg/day until harvest, to be increased to 10 µg/kg/day if needed) or out of steady state mobilization (G-CSF 10 µg/kg/day until harvest), preferably prior to surgery.
The aim is to obtain a total harvest of at least 6 x 10^6/kg CD34+ cells, to be stored in at least 3 separate bags (i.e. 3 x10^6/kg CD34+: in 1 bag for the first rescue; 1.5 x10^6/kg CD34+: in each of the 2 other bags for the 2nd rescue). In case of single HDC, all the bags will be administered. In case of tandem HDC, one bag will be used as the first rescue and the other two bags as the second rescue.
CD34+ positive selection or other purging techniques are not recommendend.
Harvest should be performed following stimulation with G-CSF. In case of mobilisation 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 R-HDC randomization 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 R-HDC randomization after the front-line induction provided that a sufficient metastatic response has been achieved and the other eligibility criteria are met (see section 4.1.2).
In the case of Thiotepa/Bu-Mel randomization, Thiotepa, Busulfan and Melphalan are investigational medicinal products (IMPs) in this trial and will not be supplied by the sponsor.
In the case of Bu-Mel randomization, Busulfan and Melphalan are not investigational medicinal products (IMPs) in this trial because they should be considered standard high-dose chemotherapy in children with high-risk neuroblastoma [Lancet Oncology 2017, HRNBL1/SIOPEN].

Patients with metastatic disease not fulfilling the response criteria after induction should be included in SIOPEN/VERITAS protocol, except in case of disease progression, in order to receive a more intensive treatment due to their worse outcome (refractory disease).
In countries in which VERITAS trial is not available, it is strongly recommended to refer those patients to countries in which the trial is open. In case of family or patient's refusal or other reasons, the Coordinating Investigator's advice is highly recommended to define the best approach for such patients. The patient should continue to be followed in the HRNBL2 study and data about subsequent treatment modalities collected.

5.4.1 High-dose Thiotepa

Patient is eligible for the HD Thiotepa if the following safety criteria are fulfilled:

1) Performance status ≥ 50%
2) Liver function: toxicity < grade 2
3) Renal function: toxicity < grade 2
4) Cardiac function: Shortening fraction ≥ 28% or ejection fraction ≥ 55% by echocardiogram
5) Normal chest X-ray and oxygen saturation
6) Absence of any ≥ grade 3 toxicity
7) Pulmonary function: children should have no dyspnoea at rest, and a pulse oximetry > 94% on room air. In case of pulmonary dysfunction history, pulmonary function tests should be performed, in order to check the eligibility criteria: FEV1 and FVC > 60% of the predicted by the pulmonary function tests (PFTs).

Figure 9: Flowchart of the consolidation therapy with high-dose Thiotepa

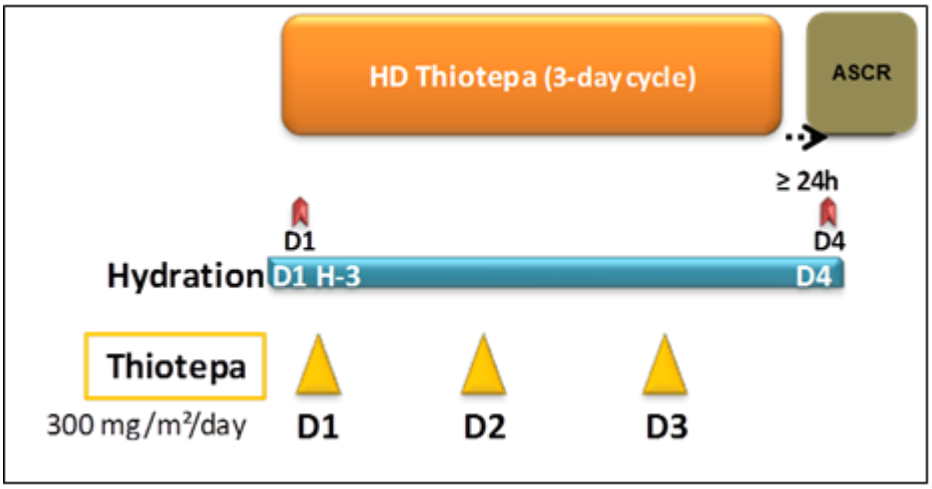


Table 7: Consolidation therapy with high-dose Thiotepa

HD Thiotepa (days)		**- 3**	**- 2**	**- 1**	**Day 0**
DRUG	**DOSE**				
Thiotepa	300 mg/m^2/day over 2 hours	•	•	•	
Hydration	3L/m^2/day = 125 ml/m^2/h	Continuous until Day 0 (24 h after last Thiotepa), then 1.5 ml/m^2/day			
ASCR	Minimum 3x10^6/kg/CD34+cells i.v. **At least** 24 hours after the last dose of Thiotepa				◆

Thiotepa is delivered at the dose of 300 mg/m²/day, once a day, for 3 consecutive days, i.e., 900 mg/m² overall (Table 7).

Dose modification of Thiotepa:
No dose modification of Thiotepa is indicated.

Drug delivery:
Thiotepa is commercially available throughout the European Union.

Preparation:
Thiotepa is reconstituted at room temperature from the lyophilised powder with 10 ml of water for injection and agitated until complete dissolution. The resultant solution contains 10 mg in 1 ml anhydrous Thiotepa.

Administration:
Thiotepa is diluted in normal glucose 5% to a maximum concentration of 5 mg/ml. In children, if the dose is lower than 250 mg, an appropriate volume of sodium chloride 9 mg/ml (0.9%) solution for injection may be used in order to obtain a final Thiotepa concentration at 1 mg/ml. The Thiotepa solution should be administered as a *two-hour IV* infusion through the central venous catheter.

Common side effects and recommended supportive care:
- The most frequently adverse events reported in the different conditioning treatments including Thiotepa are: cytopenia, infections, gastrointestinal disorders, mucosal inflammation and neurological disorders.
- Anti-emetics should be given i.v. approximately 30 minutes prior to the Thiotepa injection and again scheduled post-Thiotepa, for a minimum of 24 hours after the last Thiotepa dose. Anti-emetic therapy may be administered according to institutional policy. *Aprepitant should be avoided due to potential interaction*.
- At least three hours prior to Thiotepa administration, start the hydration with a polyionic solution for infusion at a rate of 125 ml/m^2/h. Continue 24 hours after the end of the Thiotepa day 3 infusion, i.e., until day 0, then continue hydration at 1.5 ml/m^2/day.
- **G-CSF 5µg/kg/day IV will be given daily beginning on Day+5 after ASCR**. G-CSF will be continued until a stable increase of ANC > 1.0 x 10^9/l.
- Prophylactic antifungal treatment with ketoconazole, itraconazole or fluconazole is not recommended. For proven fungal infection or prolonged febrile neutropenia, antifungal treatment avoiding the azole antifungals will be administered according to institutional policy.
- Antibiotics and antivirals should be given in line with the institutional policy whenever indicated but any prophylactic use should be prudent in view of side effects and drug interactions.
- Concomitant use with phenytoin, fosphenytoin and aprepitant should be avoided.

5.4.2 High-Dose Bu-Mel

The Bu-Mel therapy is the second course of the intensified consolidation chemotherapy, and is the same regardless of the R-HDC arm.
In patients randomized to receive a tandem HDC, an interval of at least 8 weeks must be respected between the beginning of the first high dose therapy (Thiotepa) and the start of Bu-Mel. In these patients, Bu-Mel must be started by day 90 after ASCR regardless of haematological recovery unless there is major organ toxicity or progressive disease, in which case the patient will come off trial.

Patient is eligible for the HD Bu-Mel if the following safety criteria are fulfilled:

- Performance status ≥ 50%
- Liver function: toxicity < grade 2
- Renal function: toxicity < grade 2
- Cardiac function: Shortening fraction ≥ 28% or ejection fraction ≥ 55% by echocardiogram
- Normal chest X-ray and oxygen saturation
- Absence of any ≥ grade 3 toxicity
- Pulmonary function: normal chest X-ray and normal oxygen saturation

Figure 10: Flowchart of the consolidation therapy with Bu-Mel

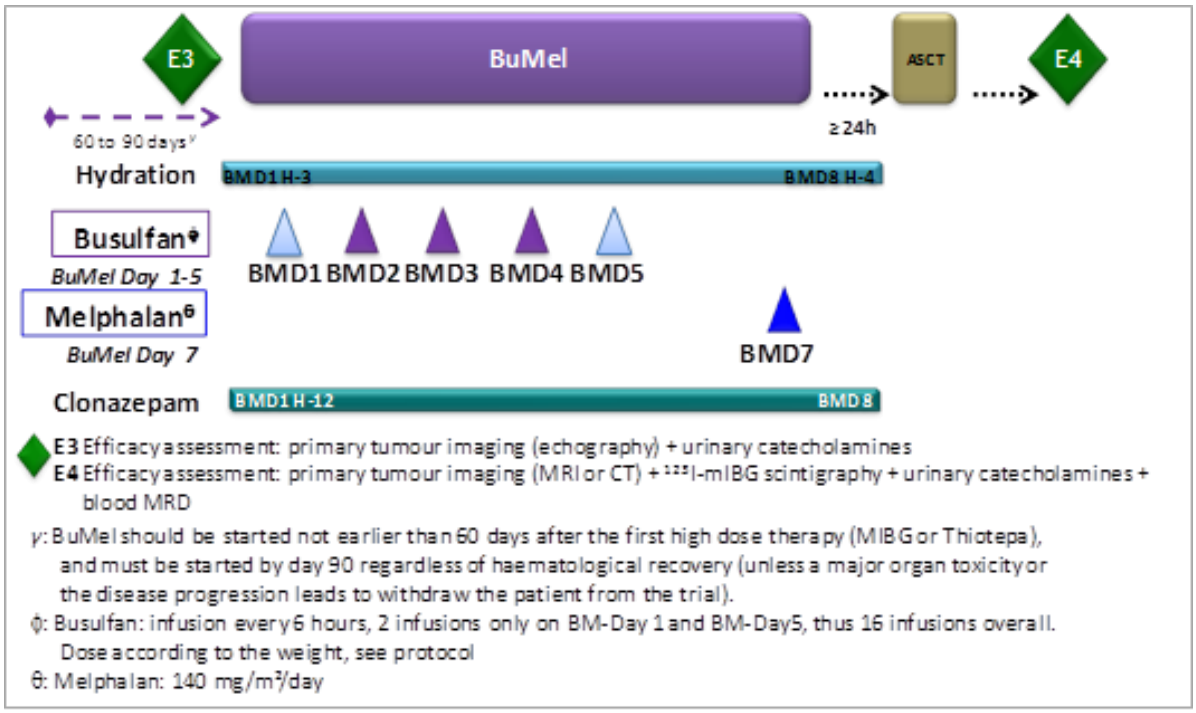


Table 8: Consolidation therapy with Bu-Mel schedule

HD Bu-Mel (days)		**- 6**	**- 5**	**- 4**	**- 3**	**- 2**	**- 1**	**Day 0**
DRUG	**DOSE**							
Busulfan	< 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 **Infusion IV over 2 hours** **Administration every 6 hours for a total of 16 doses**	•	•	•	•	•		
Melphalan	140 mg/m^2/dose IV short infusion (15'), **at least** 24 h after the last busulfan dose						▲	
Hydration	3L/m^2/day = 125 ml/m^2/h	Continuous until Day 0 (24 h after Melphalan), then 1.5 ml/m^2/day						
Clonazepam	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 Busulfan until Day +0 If the child is excessively drowsy then reduce dose						
PBSC rescue	Minimum 3X10^6/kg CD34+ i.v, **at least** 24 hours after the last dose of Melphalan							◆

Drug Delivery:
Busilvex® (iv busulfan) is commercially available throughout the European Union.

Preparation and administration (Table 8 and 9):
Busilvex® must be diluted prior to administration (see Appendix 8 "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 9: 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

Dose modification:
In case of low body weight (< 10 kg), PK evaluation should be discussed for Bu adaptation. In such case, contact the study PI for dose adaptation and/or busulfan pharmacokinetic evaluation.
Patients with real or hepatic impairment are not eligible for R-HDC. Contact the national coordinator for the management of the consolidation phase with Bu-Mel.

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®.

I.V. MELPHALAN

The total dose of melphalan is 140 mg/m²/day. It should be administer **at least 24 hour after the last Busulfan dose.** No dose reduction is indicated based on body weight (i.e. 140 mg/m²/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.

Dose modification:
In case of low body weight (< 10 kg) or nephrectomy, PK evaluation should be discussed for Mel adaptation. In such case, contact the study PI for dose adaptation and/or Mel pharmacokinetic evaluation.
Patients with real or hepatic impairment are not eligible for R-HDC. Contact the national coordinator for the management of the consolidation phase with Bu-Mel.

Common side effects and recommended supportive care:

- 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/m²/h. To achieve this urine output, give i.v. hydration at 125 ml/m²/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 10^9/l.
- Prophylactic antifungal treatment with ketoconazole, itraconazole or fluconazole should be avoided, because of the increased risk of SOS with these drugs in association with busulfan. For proven fungal infection or prolonged febrile neutropenia, amphotericin would 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.

5.4.3 Autologous Stem Cell Rescue (ASCR)

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

Dosage:
A minimum of 3x10^6 CD34+ stem cells/kg (optimum 5 x 10^6/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/m²/day total IV fluids

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

5.5 Surgery

The aim of surgery in HR-NBL is to achieve complete excision of the tumor 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.
This study will collect data on the surgical procedure, particularly on the completeness of excision through a new CRF validated by the SIOPEN and COG groups. This will allow a larger inclusion of patients to study the impact of surgery on HR-NBL outcome and surgical related questions. Verification of post-operative residue by postoperative imaging (CT scan/MRI) is mandatory and will be performed after HDC, before radiotherapy.
The operative CRF should be used for each surgical procedure, including biopsies.

5.5.1 Timing

Timing of surgery changes according to the induction arm:

- GPOH: after the 2nd N6 cycle (4th cycle)
- Rapid COJEC: 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 (after HDC Thiotepa or after 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 tumor, surgery may have no benefit.
Tumors where surgery is postponed or deemed not necessary should be discussed first with the national coordinator. After a first surgery, if imaging shows a resectable residual disease, more than minimal residual tumor volume (see below), additional surgery should be considered. Where possible tumor should also be taken at the time of relapse.

5.5.2 Definition of Procedures

Biopsy
Biopsy should be the first procedure on all tumors.
Sufficient tissue must be obtained, ideally from two different areas of the tumor, to allow histological diagnosis and biological studies. In particular, it is essential that sufficient material is obtained for the accurate determination of *MYCN* 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 tumor 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 tumors, including the removal of abnormal lymph nodes. Microscopic residual will be the most frequent situation.

Excision with minimal residual tumor (MRT)
Surgeon estimates the volume of residual tumor after surgery as less than 5 cm^3 (5 millilitres), which will be compared with the post-operative imaging.

Incomplete excision (macroscopic residual tumor)
Surgeon estimates the volume of residual tumor after surgery as more than 5 cm^3 (5 millilitres), which will be compared with the post-operative imaging.

5.5.3 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 Sponsor.

5.5.4 Aspects of Surgical Procedures

Surgery of the primary tumor
The aim of surgery is to remove completely the primary tumor. 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 tumor 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 the national coordinator. 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 1/3rd 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 1/3rd of the spinal canal, the surgical strategy must be discussed with the national coordinator 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 tumors.

Nephrectomy
Nephrectomy should be avoided whenever possible. Elective nephrectomy is discussed as part of the surgical planning if the kidney is part of the tumor 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 tumor.
Although radiation will impair renal function, this effect is not manifest for three to five years after treatment. This is a rational 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.

Tumor incision
Incision of the tumor is permissible because this aids excision.

Tumor 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 tumor is in contact with the vessels. The new international operative CRF will help to harmonize the collection of this information.

Risk factors related to tumor 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 5.6.1).

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

5.6 Radiotherapy

All patients will receive radiotherapy to the primary tumour site after HDC/ASCR. Patients will be stratified by whether or not there is residual macroscopic disease left after surgery. Following incomplete excision, patients will be randomized to receive either a standard dose (21.6 Gy) to the tumour bed, or that plus an additional 14.4 Gy subsequent boost to the residual disease.
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 tumor and neighbouring organs. Some patients may be considered unsuitable for radiotherapy by reason of the site of primary tumor and the volume which would require irradiation. Discuss with the current Radiotherapy Panel in case of doubts. Discussion about administration of radiotherapy should include consideration of referral to a centre 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.

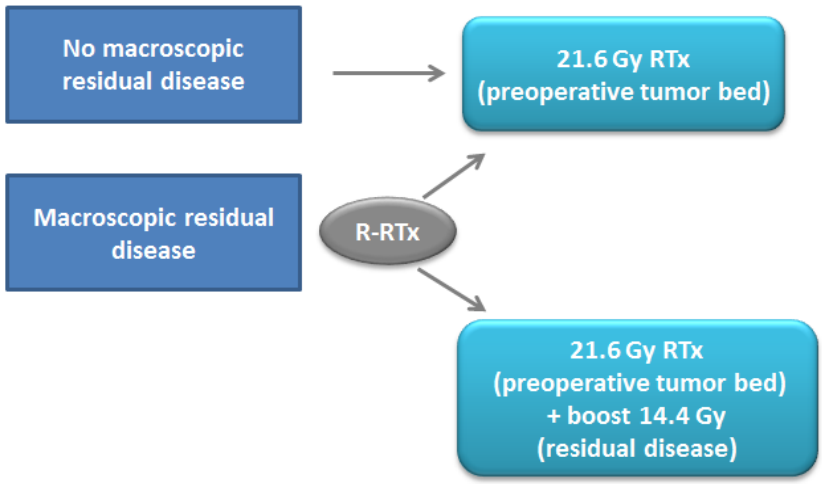
5.6.1 Timing of radiotherapy

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.

5.6.2 Radiotherapy administration

Patients will be stratified by whether or not there is macroscopic residual disease present on the local evaluation demonstrated on postoperative cross sectional imaging (MRI or CT if no MRI available) and MIBG (preferably SPECT-CT), performed after HDC or 2-3 weeks after surgery whenever possible (Figure 11).

Figure 11: Radiotherapy overview



No macroscopic residual disease
The patient is considered to have **no macroscopic residual disease at time of radiotherapy** if, cumulatively:

- the MRI (or CT if no MRI available) shows no definite residual tumour **and**
- the MIBG scan shows no residual tumour **and**
- the surgical report mentions a complete resection

In case of NO MACROSCOPIC RESIDUAL DISEASE, radiotherapy to a dose of 21.6 Gy in 12 fractions of 1.8 Gy to a volume covering the pre-operative extent of the tumour will be administered.

Macroscopic residual disease

The patient is considered to have **macroscopic residual disease at time of radiotherapy** if:

- the MRI (or CT if no MRI available) shows definite residual tumour and/or
- the MIBG scan shows residual tumour and/or
- the surgical report mentions residual tumour

If the surgical report mentions residual macroscopic unresectable tumour, it is mandatory that the surgeon describes the location clearly in the surgical report, and, if practicable, marks this with MRI-compatible clips.

In case of MACROSCOPIC RESIDUAL DISEASE, patients will be randomized (R-RTx) to:

1: Radiotherapy to the entire preoperative tumor bed (obviously including macroscopic residual tumor) to a dose of 21.6 Gy in 12 fractions of 1.8 Gy, or

2: Radiotherapy to the entire preoperative tumor bed (obviously including macroscopic residual tumor) to a dose of 21.6 Gy in 12 fractions of 1.8 Gy and a sequential boost of additional 14.4 Gy in 8 fractions of 1.8 Gy to the residual tumor.

In the event of a very big target volume or very young children a more protracted schedule may be proposed by the quality assurance panel.

Patients will only be randomized after residual disease status has been confirmed at a multidisciplinary meeting and the patient has been evaluated by the radiotherapy team.

If, for any reason, the patient cannot or refuses to be randomized for R-RTx, standard 21.6 Gy radiotherapy will be performed to the entire preoperative tumor bed.

5.6.3 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 ≤3mm 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 tumor 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 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 without macroscopic residual disease and randomized to the treatment arm 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.
In patients with macroscopic residual disease and randomized to the boost arm, an additional 14.4 Gy in 8 fractions of 1.8 Gy will be given.

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 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 or 36 Gy 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 in patients randomized to the boost.

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 or higher if necessary 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.

5.6.4 Quality control

Radiotherapy plans should be reviewed prior to commencement of treatment in order to correct potential deviations before treatment. To facilitate this, it is recommended that proposed radiotherapy plans (in DICOM-RT format) and the diagnostic imaging from which the target volume has been defined should be uploaded at least one week before the planned start of radiotherapy on the QUARTET platform (at http://www.eortc.org/tools/ RTQA upload). A more detailed manual is added in attachment to this protocol (See Radiotherapy Manual).
Regarding treatment positioning verification, the following recommendations are considered as a minimum standard. For conventional radiotherapy KV OBI first 3 days, then weekly, for IMAT/IMRT daily KV OBI and weekly CBCT, or according to local policy whatever is the most stringent.
Following completion of treatment, data of the treatment actually given should be uploaded onto the database to allow further review by the Radiotherapy Panel.

5.7 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 12; Figure 13) should start as soon as the criteria are met, **ideally no later than day 120 post ASCR**, if complete re-staging shows no evidence of progression.
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.
Dinutuximab beta and oral 13-cis-RA are not investigational medicinal products (IMPs) in this trial and will not be supplied by the sponsor.
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 12: Maintenance phase overview

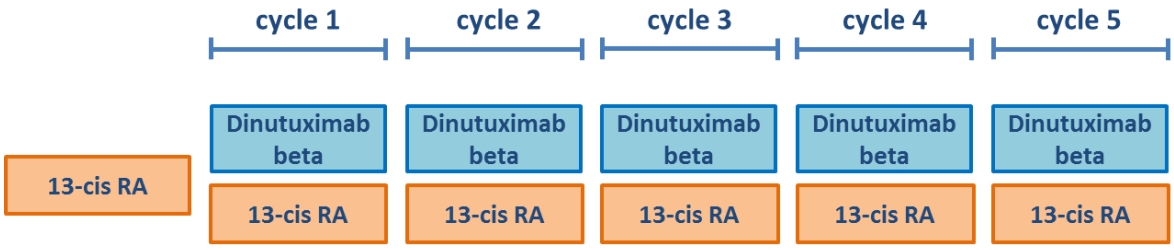
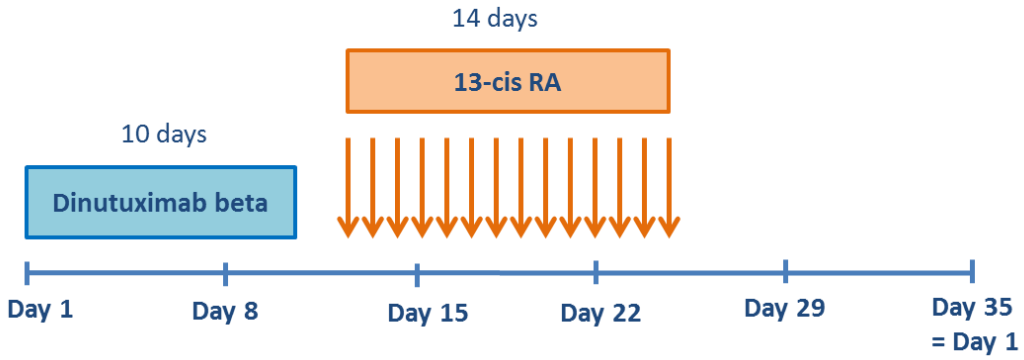


Figure 13: Maintenance cycle overview



5.7.1 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/m²/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^2/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 ≤ 1.5 x normal, and ALT ≤ 5 x normal.
- SOS, if present, should be stable or improving.
- Skin toxicity ≤ grade 1
- Kidney toxicity ≤ grade 1
- Serum triglycerides < 500 mg/dL
- No haematuria and/or proteinuria on urinalysis
- Serum calcium < 11.6 mg/dL = < 2.89 mmol/l
- **Pulmonary function: perform a functional pulmonary test if pulmonary dysfunction was experienced and/or if clinically indicated**

Dose Modifications for 13-cis-RA

A dose reduction of 25% (to 120 mg/m²/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/m²/day). If the same grade 3 or 4 toxicity recurs after two dose reductions, then discuss with national co-ordinator 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/m²/day). An additional dose reduction to 100 mg/m²/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 m², then call the study co-ordinator for dose adjustment.
If patient develops haematuria, proteinuria, and/or hypertension during any cycle of therapy, withhold medication and contact study co-ordinator.

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/m²/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.

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^2/day
- Patients ≤ 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.

Dose modification of 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, temporarly or for the entire cycle. As a consequence, either the infusion period is prolonged (<u>for a maximum of 11 days</u>) 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.

Special warnings and supportive care measures

<u>Pain</u>

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 2×10 mg/kg/day orally the next day and to 3×10 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 maintened 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.

<u>Hypersensitivity reactions</u>
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 ≥ 3 vision toxicity (i.e. subtotal vision loss per toxicity scale). In case of any eye problems, patients should be referred promptly to an ophtalmology 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 (motor with or without sensory), 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. Pneumocysits prophylaxis is recommendend.

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.

6 ASSESSMENT OF EXTENT OF DISEASE, RESPONSE AND TOXICITY

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 10; Table 11) [Park JR, JCO 2017; Burchill S, Cancer 2017]

*Table 10: Schedule of the disease evaluations throughout the trial – **arm RAPID COJEC***

Study steps	**Study entry**	**Day 40 Rapid Cojec**	**Post Rapid Cojec[4]**	**Post-Thio[5]**	**Post Bu-Mel, prior to RTx**	**Before maintenance**	**After 2nd cycle of dinutuximab beta**	**End of treatment**
^{456}I-mIBG scan (or FDG PET for MIBG negative cases)	❒	❒	❒	❒	❒	❒[7]	❒	❒
Primary tumor imaging (MRI or CT)[1]	❒		❒		❒	❒[7]		❒
Primary tumor imaging (ultrasound)[1]	❒	❒		❒			❒	❒
Cerebral imaging (MRI or CT)[1]	❒		❒[6]		❒			
Bilateral BM (trephine biopsy)	❒		❒		❒	❒[7]		❒
Bilateral BM (aspirates)	❒	❒	❒		❒	❒[7]	❒	❒
Pathology[2]	❒							
Urinary Catecholamins	❒		❒[8]		❒[8]			❒[8]
Ferritin, LDH	❒							
Blood MRD testing[3]	❒	❒	❒		❒	❒[7]	❒	❒
BM MRD testing[3]	❒	❒	❒		❒	❒[7]	❒	❒

[1]Imaging of the primary tumor and cerebral imaging: by MRI or CT scan as judged appropriate by the patient's physician

[2] INPC classification and *MYCN* status

[3] The minimal residual disease (MRD) testing is highly recommended and should be performed as follows:

- in blood a quantitative reverse-transcriptase polymerase chain reaction (RTqPCR)
- in bone marrow by immunocytology (IC) and RTqPCR (See Section 8 for detail on sample collection).

[4]**MRI/CT scan preoperative + MIBG/SPECT scan after surgery (if surgery is feasible), then decide to proceed towards R-HDC/HR-NBL2 or VERITAS trial according to results. If the surgery of the primary tumor seems complicated, MIBG/SPECT scan might be performed before surgery to help the clinical decision**

[5] for patients randomized in the double HDC arm

[6] only for patients with brain metastases or major skull lesions at diagnosis

[7] to be repeated only if ≥ 8 weeks since last evaluation

[8] not mandatory

*Table 11: Schedule of the disease evaluations throughout the trial – **arm GPOH***

Study steps	**Study entry**	**After the 2nd cycle GPOH**	**After the 4th cycle GPOH[1]**	**Post GPOH induction**	**Post-Thio[5]**	**Post Bu-Mel, prior to RTx**	**Before maintenance**	**After 2nd cycle of dinutuximab beta**	**End of treatment**
^{456}I-mIBG scan (or FDG PET for MIBG negative cases)	❒	❒	❒	❒	❒	❒	❒[7]	❒	❒
Primary tumor imaging (MRI or CT)[1]	❒		❒	❒		❒	❒[7]		❒
Primary tumor imaging (ultrasound)[1]	❒	❒		❒	❒	❒		❒	❒
Cerebral imaging (MRI or CT)[1]	❒			❒[6]		❒			
BM (trephine biopsy)	❒		❒	❒		❒	❒[7]		❒
BM (aspirates)	❒	❒	❒	❒		❒	❒[7]	❒	❒
Pathology[2]	❒								
Urinary Catecholamins	❒			❒[8]		❒[8]			❒[8]
Ferritin, LDH	❒								
Blood MRD testing [3]	❒	❒	❒	❒		❒	❒[7]	❒	❒
BM MRD testing[3]	❒	❒	❒	❒		❒	❒[7]	❒	❒

[1] Imaging of the primary tumor and cerebral imaging: by MRI or CT scan as judged appropriate by the patient's physician
[2] INPC classification and *MYCN* status
[3] the minimal residual disease (MRD) testing is highly recommended and should be performed as follows:
- in blood a quantitative reverse-transcriptase polymerase chain reaction (RTqPCR)
- in bone marrow by immunocytology (IC) and RTqPCR. (See Section 8 for detail on sample collection).

[4] **MRI/CT scan preoperative + MIBG/SPECT scan after surgery (if surgery is feasible), then decide to procede towards R-HDC/HR-NBL2 or VERITAS trial according to results. If the surgery of the primary tumor seems complicated, MIBG/SPECT scan might be performed before surgery to help the clinical decision**
[5] for patients randomized in the double HDC arm
[6] only for patients with brain metastases or major skull lesions at diagnosis
[7] to be repeated only if ≥ 8 weeks since last evaluation
[8] not mandatory

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	●	●	●	●	●	●	●	●	●
Blood pressure	●	●	●	●	●	●	●	●	●
Full blood counts	●	●	●	●	●	●	●	●	●
Renal/liver function, electrolytes with Ca and Mg	●	●	●	●	●	●	●	●	●
Tubular function		●		●		●		●	●
GFR		●		●		●		●	●
Audiology		●				●			●

Note: for hearing function assessment, please see Appendix 2.

6.2.2 Overview of toxicity evaluation for GPOH induction

Toxicity Evaluation for GPOH Induction							
Course	**N5**	**N6**	**N5**	**N6**	**N5**	**N6**	**End of induction**
Cycle Number	**1**	**2**	**3**	**4**	**5**	**6**	
Physical examination	●	●	●	●	●	●	●
Blood pressure	●	●	●	●	●	●	●
Full blood counts	●	●	●	●	●	●	●
Renal/liver function, electrolytes with Ca and Mg	●	●	●	●	●	●	●
Tubular function	●	●	●	●	●	●	●
GFR	●	●	●	●	●	●	●
Audiology	●		●		●		●
Echocardiogram		●		●		●	●

Note: for hearing function assessment, please see Appendix 2.

6.2.3 Overview of toxicity evaluation during consolidation phase

Toxicity Evaluation during Consolidation Phase			
Timing	**Before HD Thiotepa**	**Before HD Bu-Mel**	**End of consolidation**
Physical examination	●	●	●
Blood pressure	●	●	●
Full blood counts	●	●	●
Renal/liver function, electrolytes	●	●	●
Tubular function	●	●	●
GFR	●	●	●
Abdominal and Hepatic Ultrasound		●	●
Echocardiogram		●	●*
Chest Radiography	●	●	●

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

6.2.4 Overview of toxicity evaluation during maintenance phase

Toxicity Evaluation during Maintenance Phase						
Timing	**Before 1st cycle**	**Before 2nd cycle**	**Before 3rd cycle**	**Before 4th cycle**	**Before 5th cycle**	**End of treatment**
Physical examination	●	●	●	●	●	●
Blood pressure	●	●	●	●	●	●
Full blood counts	●	●	●	●	●	●
Renal/liver function, electrolytes	●	●	●	●	●	●
Chest radiography	●		●			●
ECG	●		●			●
Eyes Assessment	●		●			●

6.3 Follow-up assessment

After treatment discontinuation, progression-free patients will be followed 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 recommendend 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.

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 recommanded 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.
The collection of samples for research according to SOPs is strongly advised (see the section "Additional Research" below).

Primary Tumor 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.*

*If bone marrow aspirates are performed please collect additional material in EDTA and PAXgene^{TM} blood RNA tubes for follow up research (See Follow-up Research Study Manual).

RELAPSE
In case of new symptoms and findings, a full evaluation will be undertaken. Refer to Table 11 (Investigations and sample collections) and Table 12 (Liquid biopsy samples for multi-centre core-circulating biomarker studies in HR-NBL2/SIOPEN) for further details.
ADDITIONAL RESEARCH (see Follow-up Research Study manual + Laboratory manual)

Collection and storage of biological specimens according to SOPs for research is strongly advocated. Whenever blood sampling is performed it is strongly advisable that samples are also collected for research purposes.

6.4 Long term follow-up assessment

A long term Follow-Up study is planned in order to gather data regarding OS, EFS, long-term toxicities, late relapses and second malignancies. Information on patient outcomes, information on post-study anticancer therapies will be recorded in the CRF during the follow-up period as reasonably possible.
The investigator (or designee) will contact the patient in order to record data regarding progression. All efforts must be undertaken by the study sites to determine if there is progression but no additional protocol visit can be required. Results of this long-term follow-up will be reported separately, in the medical file of the patient, and will not be part of the Clinical Study Report.

Hearing (See Appendix 2)
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 trests. 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.

7 PATHOLOGY

7.1 General Remarks

The handling of the tumor 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 tumor areas for molecular-genetic/biological analysis. The pathologist should assess:
(a) the **representativity** of the chosen areas and report the percental distribution of viable tumor tissue, necrosis and preserved non-tumor tissue (i.e. fibrosis), and
(b) the **tumor cell content**, i.e. the percentage of tumor 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, tumor material from different tumor 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 tumor heterogeneities at the genetic level (i.e. for the *MYCN* 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 analysed using paraffin material.

7.2 Sectioning and securing tumor material in case of resected tumors

Ink the surgical margin and cut the tumor 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 tumor 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 tumor 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. tumor specimen A 1-4, specimen B 1-4). 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 tumor 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 tumor collection and freezing. Before using these for further analyses, making cryosections for the determination of the tumor cell content is mandatory.
- *Samples A4 and B4*: **put in sterile culture medium** (RPMI 1640) for preparation of tumor cell suspensions which may serve for evaluation of ploidy, drug sensitivity, etc. Tumor cell content should be checked by immunocytology on the cytospin preparations using appropriate antibodies.

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.

7.3 Sectioning and securing tumor 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 appropriate, put some of the fresh tissue in sterile culture medium (RPMI 1640) for preparation of tumor cell suspensions.
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 and/or put in sterile culture medium (RPMI 1640) for preparation of tumor cell suspensions depending on the amount of biopsy material received..

7.4 Securing tumor material in case of core needle (tru cut) biopsies

A minimum of four (preferentially five) core needle biopsies from different areas of the tumor 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 tumor cell content on frozen sections is required for each needle biopsy as they may originate from different tumor areas with different histological composition. Preparation of cell suspensions from core needle biopsy is not recommended.due to the paucity of the material.

7.5 Securing tumor material in case of fine needle aspirations (FNA)

Fine needle aspirations (FNA) yield cytological tumor cell samples and are generally not recommended because (a) the precise diagnosis and classification of the tumor according to the INPC is only possible on histological sections and (b) the material for biological investigations my

be scarce. However, tumor 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 tumor. 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 tumor cells in each vial, suspended tumor cells should be centrifuged on cytospins for cytomorphological, immunocytological and FISH analysis, spun down and snap frozen and/or used for analysis of ploidy by i.e. flow cytometry.

7.6 Histology report

At diagnosis

Resected tumor
Morphologic classification: The tumor 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 tumor residual is microscopic or macroscopic. The report must clearly indicate the estimated percentage of tumor 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 tumor material obtained is not necessarily representative of the whole tumor. 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 tumor, unclassifiable’. This term relates to a tumor which belongs unequivocally to the peripheral neuroblastic tumor 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 tumors giving rise to problems in classification, are: ‘neuroblastoma (Schwann cell stroma-poor), NOS’. This term is used for tumors 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 tumor with a stroma-rich/-dominant appearance containing areas of extensive calcification which may obscure a stroma-poor nodule.

After cytotoxic therapy

Tumor material
Sectioning of the tumor material in resected tumors or biopsies after cytotoxic therapy can be done following the same guidelines as for tumors 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 tumor 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 tumor. Therefore, assignment to the prognostic subgroups must not be made, although different areas of the tumor 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 tumor 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 tumor specimen, and morphologic description of the tumor 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 tumor 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 tumors. 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 tumors. 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 tumors 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 tumor cells in bone marrow and fine needle aspirates, anti-GD2 for bone marrow, and anti-CD56, anti-GD2 and anti-CD45 for tumor material are recommended. [Burchill S, Cancer 2017]

Exact determination of the tumor cell content

It is mandatory that the tumor cell content is evaluated in all samples used for molecular-genetic/biological investigations and DNA analyses. CD56, GD2, and common leukocyte antigen as well as the use of S-100 for unequivocal detection of Schwann cells are recommended. If the number of tumor cells in the touch preparations is low and obviously not corresponding to the tumor cell content in the paraffin material the imprints originate from, the touch preparations have to be checked by immunocytology for the presence of tumor/stromal cells.

8 BIOLOGICAL ANALYSIS

In HR-NBL, biological studies of the primary tumor, metastatic sites and liquid biopsies for analysis of prognostic and predictive biomarkers are of utmost importance. These studies require rigorous sample collections, according to well-defined standard operating procedures (SOPs).
Subject to patient consent and appropriate centre facilities, samples will be collected to evaluate the following core biological studies of the SIOPEN group:

- Genome and expression profile of DNA and RNA isolated from tumour at diagnosis (± relapse); constitutional DNA will be required for genomic studies.
- Prognostic and predictive value of DNA, mRNA and miRNAs in bone marrow and blood samples at diagnosis and through out the disease course.

Additional information on biological studies and sample collection is described in the accompanying Country Specific Laboratory Manual. The biological samples to be collected (clinical decision making and core research) for patients enrolled in the HR-NBL2 protocol are summarized in Tables 11 and 12.
Samples that are required for clinical decision making are mandatory and are distinguished from those that are for research; ancillary research might be mandatory, core-objectives of the SIOPEN group or optional studies. Furthermore, samples for ancillary studies that are planned in subsets of patients or at a national level are not included in the protocol, and rather are described in the Country Specific Laboratory manual which will accompany the HR-NBL2 protocol.

8.1 Primary tumor tissue

Analysis of tumor tissue of the primary tumor obtained at diagnosis is mandatory: following tissue sampling both frozen tumor tissue and formalin fixed paraffin embedded tumor tissue are to be collected at diagnosis according to SOPs.
In rare situations where no primary tissue can be obtained (due to the clinical situation of the patient, or sampling difficulties, or because no primary was identified) the following analyses may be done on a representative metastatic sample (invaded bone marrow, invaded lymph node), possibly following enrichment techniques.
The following investigations will be performed for all patients on primary tumor tissue obtained at diagnosis (mandatory analysis).
Pathological analyses:

- histological analysis (INPC)
- evaluation of tumor cell content in the sample

Genetic analyses:

- *MYCN* copy number status (clinical decision making)
- For patients 12-18 months old with stage M and MYCN non amplified tumors: Genomic copy number profile (high resolution aCGH and/or SNPa and/or lcWGS)

The following genetic analyses are highly recommended at diagnosis:

- Genomic copy number profile (high resolution aCGH and/or SNPa and/or lcWGS
- Telomere and ALT status
- ATRX status
- NGS panel sequencing (minimal consensus of 13 genes, including ALK) alternatively also whole exome sequencing (WES) or whole genome sequencing (WGS) approaches can be applied thus replacing copy number analysis

The same analyses as for the primary tumors should be done on relapse tumor samples with emphasis on WES or WGS to learn about acquired genomic aberrations with the option to apply targeted therapy approaches.

8.2 Liquid biopsy samples

8.2.1 Bone marrow samples

Bone marrow samples (aspirates and trephine biopsies) will be analysed for metastatic disease applying immunohistochemical, immunocytological and conventional cytomorphological investigations to assess disease status and response to treatment.[Burchill SA, Cancer 2017]

Highly recommended samples (Table 11; Table 12):

- bone marrow aspirates (one from right-side and one from left-side) in PAX gene™ RNA tubes
- bone marrow aspirates in EDTA tube for disseminating biomarker and tumour cell studies

Volumes: see Table 12

Bone marrow aspirates for SIOPEN ancillary research projects are *highly recommended* from all patients at the same time points as those required for standard clinical care: at diagnosis, mid induction, end of induction, after high dose chemotherapy, prior to maintenance therapy (if relevant), at end of treatment, and in case of relapse (7 time points).

8.2.2 Blood samples

Blood samples for analysis of circulating biomarkers and CTCs are highly recommended from all patients at the same time points as those required for standard clinical care: at diagnosis, mid induction, end of induction, after surgery, after HD Thiotepa (if relevant), after HD Bu-Mel, prior to maintenance therapy (if relevant), after the 2nd cycle of the maintenance treatment, at end of treatment, and in case of relapse (7 to 10 time points).
A blood sample for pharmacogenomics is higly recommended, once, at any time point.

For ancillary follow-up research questions, it will be valuable to collect blood samples during follow-up at regular intervals (i.e. 3 monthly intervals during 2 years); these samples are optional and according to national/local policies (See Follow-up Research Study Manual).

In addition, for immunological studies serum samples will be collected during maintenance with dinutuximab beta (twice, at each cycle: before administration and at the end of the administration) in order to evaluate the HACA response, and a blood sample will be collected for the analysis of FCGR/KIR polymorphism, once, at any timepoint.

Highly recommended samples (Table 12; Table 13):

- Blood samples in PAX gene™ blood RNA tubes (for CTC and circulating biomarker studies)
- Blood samples in EDTA tube (for circulating biomarker studies)
- Blood samples in EDTA tube (for pharmacogenomics and FCGR/KIR polymorphism)
- Serum samples

Volumes: see Table 12

For child participant with body weight between 1Kg to 6 Kg, the maximum volume of blood collection must be between 2.5 mL to 12 mL during one-off sampling of total blood volume (including routine blood specimens for clinical care) withing 3-months according to the Guideline for Paediatric Blood Volume for Research Purposes (version 1.1 _30/09/2015) from the HREC. To take in account the last recommendations of HREC, the volumes and the number of time point are adapted for child with body weight between 1Kg to 6 Kg (see table 14).

8.3 Where to send the samples

Quality control of samples for diagnostic and research procedures and the collaboration within the SIOPEN network are of high importance.

8.3.1 Tumor samples

Frozen and Formalin-fixed paraffin-embedded (FFPE) tumour to be sent to the Pathology or Biology reference laboratories depending on national networks/ setups
For sample preparation and shipping see Country Specific Laboratory manual.

8.3.2 Blood samples and bone marrow samples
A) Blood and bone marrow aspirates taken into PAX gene™ blood RNA tubes - to be sent to the Molecular Monitoring or Biology reference laboratories for detection of circulating CTCs, mRNAs, miRNAs and proteins to assess disease status and response to treatment(s). For sample preparation and shipping see Country Specific Laboratory manual.
B) Bone marrow trephine – to be sent to the local pathologists or to a relevant reference center. For sample preparation and shipping see Country Specific Laboratory manual.
C) EDTA blood (for preparation of plasma) and EDTA bone marrow samples – to be sent to Biology- or Molecular Monitoring- reference laboratories depending on national networks/ setups; for sample preparation and shipping see Country Specific Laboratory manual.
D) Serum blood samples – to be sent to Immunology, Molecular Monitoring or Biology reference laboratories depending on national network/setup. For sample preparation and shipping see Country Specific Laboratory manual.

Table 12: Investigations and sample collections

Evaluations	E1	E2	E3	E4	E5	E6	E7	E8	E9	(E10)
Study steps Tests	**Study entry**	**During induction**	**Post Induction**	**Post surgery**	**Post-Thio***	**Post Bu-Mel, prior to Rx**	**Before Maintance**	**After 2nd cycle**	**End of treatment**	**(Relapse)**
Blood for RTqPCR, ctDNA and biobanking – (see next page)	❐	❐	❐	❐	❐	❐	❐**	❐	❐	❐
Blood for FCGR/KIR polymorphism and pharmacogenomics	(❐)									
BM aspirate for cytomorphology, IC, RTqPCR[a)], and biobanking (see Table 12)	❐	❐	❐			❐	❐**	❐	❐**	❐
BM trephine	❐		❐				❐		❐	❐
Biology[b)] and biobanking of the tumor	❐									❐
Apheresis product[c)]		❐*	❐ *							
Serum samples [d)]							❐ →	❐ →		

a) for bone marrow infiltration estimation cytomorphology will be done, for quantification of the disseminate tumor cell load quantitative reverse-transcriptase polymerase chain reaction (RTqPCR) and quantitative immunocytology (IC) will be done
b) *MYCN, TERT and ALT*-FISH, SNParray/CGH/lcWGS, NGSpanel; Research projects on i.e. cell free DNA (cfDNA) will be carried out
c) for peripheral blood and apheresis product contamination RTqPCR, cfDNA studies and quantitative immunocytology (IC) will be done
d) for HACA levels evaluation
(❐) only once at diagnosis or can be later at any timepoint if missed at diagnosis
❐ → during maintenance at each cycle: before each antibody application and at the end of antibody infusion
* according to induction regimen
** *if bone marrow evaluation performed within clinical practice* (only if ≥ 8 weeks since last evaluation)

*Table 13: Liquid biopsy samples for multi-centre core-circulating biomarker studies in HR-NBL2 [For child with **body weight more than 6 Kg**]*

Sample Type	**Tube (volume of sample type)**	**INDUCTION**			**CONSOLIDATION**			**MAINTENANCE**			**Relapse**
		Study entry	During	End	Post surgery	Post Thio	Post Bu-Mel, pre RTx	Pre	Mid	End	
BM aspirate	PAX (2 x 0.5 ml from right and left; do not pool)	•	•	•			•	•*	•**	•	•
	EDTA[§] (4 ml from right and left; do not pool)	•	•	•			•	•*		•	•
Peripheral blood (PB)	PAX (2 ml)	•	•	•	•	•	•	•*	•	•	•
	EDTA (4-6 ml) #	•	•	•	•	•	•	•*	•	•	•
	EDTA (4 ml) ##	(•)									
	Serum (1 ml)							• →	• →		
PBSC	PAX (0.5 ml)	At time of harvest									
	EDTA (4 ml)	At time of harvest									

[§] Retain the cellular fraction for DTC studies
* To be repeated only if ≥ 8 weeks since last evaluation
** If bone marrow evaluation performed within clinical practice
[#] The collected plasma will be aliquoted into 0.5ml fractions and stored at -80°C for studies of neuroblastoma specific exosomes, miRNA, cfDNA
[##] The blood will be collected in two separated tubes: 2 ml for FCGR/KIR polymorphism and 2 ml for pharmacogenomics
(•) only once at diagnosis or can be later at any timepoint if missed at diagnosis; • → During maintenance, at each cycle: before each antibody application and at the end of antibody infusion

*Table 14: Liquid biopsy samples for multi-centre core-circulating biomarker studies in HR-NBL2 [For child with **body weight between 1-6 Kg**]*

Sample Type	**Tube (volume of sample type)**	**INDUCTION**			**CONSOLIDATION**			**MAINTENANCE**			**Relapse**
		Study entry	During	End	Post surgery	Post Thio	Post Bu-Mel, pre RTx	Pre	Mid	End	
BM aspirate	PAX (2 x 0.5 ml from right and left; do not pool)	•	•	•			•	•*	•**	•	•
	EDTA[§] (4 ml from right and left; do not pool)	•	•	•			•	•*		•	•
Peripheral blood (PB)	PAX (2 ml)	•	•	•			•	•*	•	•	•
	EDTA (4 ml) #	•	•	•			•	•*	•	•	•
	EDTA (2 ml) ##	(•)									
	Serum (1 ml)							• →	• →		
PBSC	PAX (0.5 ml)	At time of harvest									
	EDTA (4 ml)	At time of harvest									

[§] Retain the cellular fraction for DTC studies
* To be repeated only if ≥ 8 weeks since last evaluation
** If bone marrow evaluation performed within clinical practice
[#] The collected plasma will be aliquoted into 0.5ml fractions and stored at -80°C for studies of neuroblastoma specific exosomes, miRNA, cfDNA
[##] **Only if the body weight is ≥ 4Kg, the blood will be collected in two separated tubes: 2 ml for FCGR/KIR polymorphism and 2 ml for pharmacogenomics.**
(•) Only once at diagnosis or can be later at any timepoint if missed at diagnosis; • → **Only if the body weight is ≥ 4 Kg**: During maintenance, at each cycle: before each antibody application and at the end of antibody infusion
Please note the recommandations for this specific population: the priority blood sample are 1. Blood in Pax™gene Blood RNA and 2. Blood in EDTA tubes (4 mL) to generate plasma and cellular fraction.
Blood volume should not exceed 5% of the total blood volume during a one-off sampling of total blood volume (including routine blood specimens for clinical care), see appendix 13.

9 METHODOLOGY AND STATISTICAL ANALYSIS

9.1 Statistical design

The SIOPEN HR-NBL2 trial is a phase III trial including three sequential randomizations.
R-I is a two arm phase III trial designed to determine the most effective induction chemotherapy for HR-NBL. R-I will compare the EFS of two induction chemotherapy regimens, RAPID COJEC and GPOH. EFS is a well-established endpoint in pediatric oncology and it is accepted as a good measure of clinical efficacy. We will stratify the following randomizations on the received induction regimen, in order to equitably distribute the effects of post-induction regimens on EFS.
R-HDC is a phase III trial designed to determine the most effective consolidation chemotherapy for HR-NBL. Patients should have a sufficient metastatic response to induction chemotherapy in order to be eligible for R-HDC. Since it is not possible to exclude an interaction between induction and consolidation treatments, the R-HDC randomization will be stratified on the received induction treatment. Interactions between induction and consolidation chemotherapy will be analysed in exploratory analyses.
A question on local control will be assessed in a phase III setting (R-RTx) in patients with macroscopic residual disease after HDC and surgery. For these patients, the efficacy of 21.6-Gy radiotherapy to the preoperative tumor bed plus a sequential boost of an additional 14.4 Gy to the residual disease will be compared to 21.6-Gy radiotherapy to the preoperative tumor bed. Patients with no macroscopic residual disease will not be included in a randomized question and will receive 21.6-Gy radiotherapy to the preoperative tumor bed.

9.2 Randomization plan

Eligible patients, being informed and having signed the consent form, will be randomized centrally through on-line randomization software.
The R-I randomization will be made following a permuted block procedure with varying block size.
The randomization will be stratified on following factors: age, stage, *N-MYC* status, country.
The R-HDC randomization will be made following a permuted block design with varying block size.
The randomization will be stratified on following factors: age, stage, *N-MYC* status, induction regimen, response to the induction treatment, country.
The R-RTx randomization will be made following a permuted block design with varying block size.
The randomization will be stratified on following factors: age, stage, *N-MYC* status, induction regimen, HDC regimen, country.

9.3 Outcome definition

EFS is defined as the time duration from the date of randomization to occurrence of an event, including:

- Death from any cause
- Disease progression
- Relapse
- Second cancer

Patients lost to follow-up without event will be censored at their last evaluation date.

mCR is defined [at evaluation time, end of induction treatment] as :

- Absence of skeletal uptake on mIBG
- Negative bone marrow aspirates and biopsy
- Absence of other metastatic sites

OS is defined as the time duration from the date of randomization to the date of death (from any cause). Patients alive at last follow-up will be censored at their last evaluation.

Local relapse is defined [at evaluation time, 2 years after radiotherapy] as:
- Local-regional relapse as the unique site of relapse ('local only') or with distant metastasis ('combined')

9.4 Patient accrual and expected duration of the trial

Based on the SIOPEN and GPOH experience in conducting HR-NBL trials, the expected inclusion rate is of 250 patients per year for R-I, 150 patients per year for R-HDC, 55 patients per year for R-RTx once all centers are open.

R-I: induction regimens RAPID COJEC vs GPOH
Assuming a baseline 3-year EFS of 40%, with a sample size of 686 patients and a two-sided alpha=5%, this trial will have 90% power to demonstrate an improvement of 12% in 3-year EFS with a recruitment period of 3 years, and a minimum follow up of 1.5 years.

R-HDC: consolidation regimen Bu-Mel vs Thiotepa + Bu-Mel
About 60% of the expected annual recruitment of R-I patients will enter the second randomization. The 3-year EFS in the Bu-Mel arm (with immunotherapy) is estimated to be 55%. This study aims to show an improvement of 12% for the Thiotepa + Bu-Mel arm (3-year EFS of 67%). With a recruitment of 448 patients (224 in each arm) over a period of 3 years and a minimum follow-up of 2 years, the power to show a 12% difference is 80% (two-sided logrank test and α=5%).

R-RTx (macroscopic residual disease): 21.6 Gy radiotherapy vs 21.6 Gy radiotherapy + 14.4 Gy boost
About 22% of the expected annual recruitment of R-I patients will enter R-RTx. The 3-year EFS of patients with macroscopic residual disease after HDC + surgery treated with 21.6 Gy radiotherapy was 38%. With a sample size of 226 patients over a period of 4 years and a minimum follow-up of 4 years, the power to detect an improvement of 15% on 3-year EFS is 80% (two-sided log-rank test with α=5%).

9.5 Statistical analysis method

9.5.1 Description of study populations

Efficacy population
For each randomization, all included patients without major deviation of the eligibility criteria will be included in the population assessable for efficacy. According to the intention to treat principle, patients will be analysed according to the treatment arm that was assigned by randomization whether they actually received the allocated treatment or not. All cases of major deviation will be reviewed in order to decide if they will be included in statisitical analyses.
Safety population

The safety population will include patients from the time they receive a first administration of the study's treatment namely GPOH or COJEC induction chemotherapy for R-I, Bu-Mel single HDC or Thiotepa HDC for R-HDC, and radiotherapy for R-RTx. Each patient will be analysed in the arm according to the treatment that they actually received.

9.5.2 Analysis of primary endpoint

R-I: Efficacy assessment of induction treatments will rely on the EFS comparison using a Cox proportional hazard model adjusted on stratification factors. The 3-year EFS from R-I randomization will be described using the Kaplan Meier method. If no statistically significant survival benefit is observed in one of the two arms, recommendations for future practice will be based on toxicity outcomes.
R-HDC: Efficacy assessment of consolidation regimens will rely on EFS comparison between the single HDC (Bu-Mel) and the tandem HDC (Thiotepa followed Bu-Mel) arms in the efficacy population using a Cox's proportional hazard models adjusted on stratification factors. The 3-year EFS from R-HDC randomization will be described using the Kaplan Meier method.
R-RTx: Efficacy assessment of 21.6 Gy radiotherapy versus 21.6 Gy radiotherapy + 14.4 Gy boost will rely on the EFS comparison using a Cox proportional hazard model adjusted on stratification factors. The 3-year EFS from R-RTx randomization will be described using the Kaplan-Meier method.

9.5.3 Analysis of secondary and exploratory endpoints

For all included patients, the time-to-event endpoints (EFS and OS) will be described using the Kaplan Meier method. The median OS rates will be reported with 95% confidence interval and median follow-up will be calculated using the inverse Kaplan-Meier method.
In the efficacy population, OS will be compared between each arm using log rank test.
The EFS of patients with complete surgical resection will be described using the Kaplan Meier method. The median EFS rate will be reported with a 95% confidence interval. A proportional hazard model will be used to assess the effect of complete surgical resection on EFS adjusted on age, *MYCN* status, stage and received treatment.
The dose of radiotherapy will be correlated to the rate of local relapse using a proportional hazard model adjusted on age, *MYCN* status, stage and received treatments.
Laboratory data (LDH, ferritin), mIBG score, ALK status, CGH results and minimal residual disease data will be described and correlated to clinical features, response to treatment and survival. For quantitative variables, the mean and standard errors will be reported if normality assumption is satisfied else the median, the range and the quartiles will be reported. For qualitative variables, the frequency, the percentage and the 95% confidence intervals will be reported.

9.5.4 Safety analyses

The NCI-CTCAE v5.0 will be used to describe R-I, R-HDC and R-RTx treatments safety on the safety population. Specific objectives related to safety issues will be reported.
The acute toxicity of R-I induction chemotherapies will be compared with respect to febrile neutropenia and grade 3-4 infections, need of ICU care.
The acute and long term toxicities of the two consolidation regimens (single Bu-Mel HDC versus tandem Thiotepa + Bu-Mel HDC) will be compared with respect to febrile neutropenia and grade 3-4 infections, SOS/VOD incidence and severity and need of ICU care.

The long term toxicities of dinutuximab beta, including interaction with R-HDC treatments will be carried out on the safety population.

9.5.5 Interim analysis

Interim analyses will be performed at regular intervals depending on the accumulating data (see 11.2). A descriptive analysis will be performed and submitted for review by an Independent Data Monitoring Committee (IDMC). No interim efficacy analysis will be performed to preserve the overall type 1 error rate of the trial.

The IDMC will be asked if the accumulating data on compliance and safety justifies continuing recruitment of further patients or further follow-up.

Treatment-related deaths and adverse events leading to ventilation in an ICU over the first 6 months will be closely monitored and the IDMC should be asked about the first 6 months safety at each meeting.

Each randomization will be monitored separately.

No statistical defined stopping rule.

10 SERIOUS ADVERSE EVENTS

The Principal Investigator is responsible for ensuring that all staff involved in the study is familiar with the content of this section.

10.1 Definition

10.1.1 Adverse Event (AE)

An Adverse Event (AE) is any new untoward medical occurrence or worsening of a preexisting medical condition in a patient or clinical investigation subject administered an investigational (medicinal) product. An AE can therefore be any unfavorable and unintended sign (including abnormal laboratory findings for example), symptom, or disease temporally associated with the use of a medical product, whether or not a causal relationship (i.e.related/not related) with the treatment is suspected.

10.1.2 Serious Adverse Event (SAE)

A **Serious Adverse Event (SAE)** is any untoward medical occurence that at any dose:

- is fatal (results in death)
- is life-threatening
- requires or prolongs in-patient hospitalization
- results in persistent or significant disability / incapacity
- is a congenital anomaly / birth defect
- is medically significant (defined as any clinical event or laboratory result that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention (i.e. medical, surgical) to prevent one of the other serious outcomes listed in the definition above. Examples of such events include but are not limited to, allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasia or convulsions that do not result in patient hospitalization, development of drug dependency or drug abuse, drug misuse, suspected transmission via a medicinal product of an infectious agent (organism, virus or infectious particle).

Although overdose (with or without associated AE/SAE) and new cancer are not always serious by regulatory definitions, these events should be reported on a SAE report form and sent to the sponsor in an expedited manner.
A SAE judged as potentially related to a study drug qualifys as Serious Adverse Drug Reaction (SADR).

The following are not considered to be serious adverse events (SAE):
- Events exclusively related to tumor relapse / progression or treatment of tumor relapse / progressions are not considered as SAE.
- A visit to the emergency room or other hospital department that does not result in admission (unless considered an “important medical event” or a life-threatening event)
- Outpatient or same-day or ambulatory procedures
- Admission to observation or short-stay units
- Hospitalization due to diagnostic procedures or standard supportive care (i.e. implant of central venous catheter, transfusions)
- A pre-planned hospitalization for a condition which existed at the start of study drug and which did not worsen during the course of study drug treatment
- Social admission (i.e. subject has no place to sleep; hospice facilities)
- Administrative admission (i.e. for yearly physical examinations)
- Protocol-specified admission during a clinical trial (i.e. for a procedure required by the study protocol or for clinical research)
- Optional admission not associated with a precipitating clinical AE (i.e. for elective cosmetic surgery)

10.1.3 Expected Serious Adverse Event

An expected SAE is an event already mentioned in the most recent version of the investigator brochure or in the summary of product characteristics, for drugs with a market authorization.

10.1.4 Unexpected Serious Adverse Event

An unexpected SAE is an event not mentioned or different by its nature, intensity and/or, evolution with respect to the investigator brochure or to the product characteristic summary, for drugs with a market authorization.

10.1.5 Intensity criteria

Intensity criteria must not be confused with criteria for seriousness, which serve as guidelines for definition of reporting obligations.
Intensity of events will be estimated according to the NCI-CTCAE classification, version 5 (toxicity score grade 1 to 5). Intensity of adverse events not listed in this classification will be evaluated according to the following terms:

- Mild (grade 1): does not affect the patient's usual daily activity
- Moderate (grade 2): perturbs the patient's usual daily activity
- Severe (grade 3): prevents the patient carrying out his usual daily activities
- Very Severe (grade 4): necessitates intensive care or is life-threatening
- Death (grade 5)

10.2 Reporting of Serious Adverse Events (SAE)

Any grade 3-4 SAE which occurs or comes to the attention of the investigator at any time during the study since consent is given and within 30 days after the last administration of study drugs, independent of the circumstances or suspected cause, must be reported immediately, after becoming aware of it via my eclinical , a web portail that allow electronic transmission of SAEs *https://myeclinical.evedrug.eu/form/IGR/login.php* or if not possible by fax using a SAE report form to : +33 (0) 1 42 11 61 50.
Any grade 1-2 SAE will be reported in the CRF only.

Pharmacovigilance unit Fax : +33 (0) 1 42 11 61 50
Phone : +33 (0)1 42 11 61 00
(9 a.m. - 6 p.m. from Monday to Friday, except on bank holidays)
E-mail: phv@gustaveroussy.fr

All late SAE (occurring after this period of 30 days) considered to be reasonably related to the study treatment(s) or the research must be reported (no time limit).

The following Serious Adverse Events are excluded from the above mentioned time lines - unless being life threatening or fatal (in that case immediate SAE reporting is needed)

- **neutropenia**
- **thrombopenia**
- **anemia**
- **non complicated febrile neutropenia**
- **mucositis**
- **vomiting**
- **central line infection**

These are expected events with this chemotherapy regimen, thus they do not need urgent reporting. They will be reported in the CRF only.

Information collected in the SAE form is crucial to assess the case. For this reason diligence in collecting as much verifiable and reliable information is needed: both, quality and timeliness are key factors. If known, the diagnosis of the underlying illness or disorder should be recorded, rather than its individual symptoms.
The following variables will be collected for each AE:

- AE (verbatim)
- The date when the AE started and stopped
- Whether the AE is serious or not

In addition, the following variables will be collected for SAEs as applicable:

- Date AE met criteria for serious AE
- Date Investigator became aware of serious AE
- AE is serious due to : CTAE grade, other
- Date of hospitalization (if applicable)
- Date of discharge (if applicable)
- Probable cause of death (if applicable)
- Date of death (if applicable)

- Description of AE
- Causality assessment in relation to Study procedure(s)
- Causality assessment in relation to each study drug/treatment

The investigator must also attach the following to the serious adverse event report, wherever possible:

- A copy of the summary of hospitalization or prolongation of hospitalization
- A copy of all relevant laboratory examinations and the dates on which these examinations were carried out, including relevant negative results, as well as normal laboratory ranges.
- All other document that he judges useful and relevant.

All these documents will remain anonymous.
Further information can be requested (by fax, telephone or when visiting) by the monitor and/or the safety manager.

Follow-up information

The investigator is responsible for the appropriate medical follow-up of patients and for following proactively all AEs/SAEs until patient's death, until resolution, until the subject returns to baseline status, or until the condition has stabilized with the expectation that it will remain chronic, even if this extends beyond study participation.
Follow up information about a previously reported serious adverse event must be reported by the investigator to the Pharmacovigilance Unit immediately after becoming aware of it. The investigator also transmits the final report at the time of resolution or stabilization of the SAE.
She/he retains the documents concerning the supposed adverse event so that previously transmitted information can be completed if necessary.

Reporting of exposure to study drug during pregnancy/lactation

In principle, women of childbearing potential must have a negative serum or urine β-HCG pregnancy test within 7 days prior to the administration of the first study treatment and once a month during treatments and until the end of systemic exposure.
If a patient becomes pregnant during the course of the study, the treatments should be discontinued immediately. The Pharmacovigilance Unit of Gustave ROUSSY must be notified within 24h (via the pregnancy report form) and the subject followed by a multidisciplinary team during the entire course of the pregnancy and postpartum period. Parental and neonatal outcomes must be recorded even if they are completely normal and without AEs. Women who become pregnant should also be advised of the possibility of harm to the foetus.
Pregnancy itself is not regarded as an AE unless there is a suspicion that the treatment under study may have interfered with the effectiveness of a contraceptive medication. Congenital abnormalities or birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented even if the patient was discontinued from the study.

10.3 Responsibilities of the coordinating Sponsor

The Pharmacovigilance Unit at Gustave Roussy will assess the SAE in terms of seriousness, severity (NCI-CTCAE v5), relationship to the study drugs and expectedness). All SAEs will be coded using MedDRA.

10.3.1 Suspected Unexpected Serious Adverse Reactions (SUSARs)

To comply with regulatory requirements, the coordinating sponsor will report all SAEs that are related to the investigational medicinal product and unexpected (i.e. not previously described in the investigator brochure or in the Summary of Product Charasteristics). In the European Union, an event meeting these criteria is termed as suspected Unexpected Serious Adverse Reaction (SUSAR).

All SUSARs report will be reported to the concerned competent authorities and ethic committees and to the EudraVigilance database. All SUSARs reports and all reports involving expected Serious Adverse Drug Reaction that are fatal will additionally be forward to all study investigators.

10.3.2 Development Safety Update Report

The Pharmacovigilance Unit at Gustave Roussy will issue once a year throughout the clinical trial, or on request, the Development Safety Update Report (DSUR) of the study in accordance with the detailed guidance ICH E2F.
The DSUR will be submitted to the concerned competent authorities and ethics committees according to national legislation.

11 TRIAL MANAGEMENT

11.1 Steering committee

A Steering Committee will be set up for the study and will be composed of:

- the coordinating investigator: Dominique Valteau-Couanet
- the statistician of the study: Rachid Abbas
- induction phase: Lucas Moreno
- consolidation phase: Claudia Pasqualini
- maintenance phase: Cormac Owens
- local treatment phase: Sabine Sarnacki, Tom Boterberg
- biology: Gudrun Schleiermacher, Sue Burchill
- national coordinators: Angelika Eggert, Roberto Luksch
- representative of the sponsor (trial manager)

The Steering Committee will be responsible for:

- Monitoring the safety of the patients throughout the course of the study by reviewing the cumulating safety data, and determining actions to be taken (i.e. amendment, etc.)
- Monitor recruitment rates and encourage the study commitee to develop strategies to deal with any recruitment problems.
- Monitor completion of data sheets and comment on strategies from the study commitee to encourage satisfactory completion in the future.

- Monitor follow-up rates and review strategies from the study commitee to deal with problems including sites that deviate from the protocol.
- Approve any amendments to the protocol, where appropriate.
- Approve any proposals by the study commitee concerning any change to the design of the trial, including additional sub-studies.

The Steering committee will meet (physically or through a teleconference) every 6 months.
The Sponsor has the authority to make and implement all major decisions, such as, but not limited to, the termination of the study and amendments to the study protocol, possibly after discussion with the Independent Data Monitoring Committee.

11.2 Independent Data Monitoring Committee

Analyses will be supplied to an Independent Data Monitoring Committee (IDMC), which will be asked to give advice on whether the accumulated data from the trial (accrual, compliance and safety data), together with the results from other relevant research, justifies the continuing recruitment and treatment of patients.
The IDMC will operate in accordance with a trial specific charter based upon the template created by the Damocles Group. The IDMC meet prior to the trial opening after the randomization of 150 patients and annually thereafter during the recruitment and treatment phases of the trial.
Additional meetings may be called if recruitment is much faster than anticipated and the IDMC may, at their discretion, request to meet more frequently or continue to meet following completion of recruitment. An emergency meeting may also be convened if a major safety issue is identified.
The IDMC will report directly to the Sponsor.
The IDMC may consider recommending the discontinuation of the trial if the recruitment rate or data quality are unacceptable or if any issues are identified which may compromise patient safety.

12 STUDY, SITE AND INVESTIGATOR DISCONTINUATION

12.1 Overall study discontinuation

The study could be interrupted or terminated by the sponsor at any time in agreement with the coordinating investigator. Reasons may include, but are not limited to, the following:

- frequency and/or unexpected severity of the toxicity,
- if any information leads to doubt as to the benefit/risk ration of the clinical trial
- recruitment of patients too low,
- poor quality of the data collected,
- request of the Independent Data Monitoring Committee.

12.2 Site or investigator discontinuation

The Sponsor has the possibility to replace a site at any time. Reasons for replacing a site may include, but are not limited to, the following:

- Slow recruitment
- Poor protocol adherence / Serious breach to the protocol
- Inaccurate or incomplete data recording
- Non-compliance with the International Conference on Harmonisation (ICH) guideline for Good Clinical Practice.

The Sponsor can temporarily or permanently discontinue an investigator for participation in the clinical trial at any time. Reasons may include, but are not limited to, the following:

- Poor protocol adherence / Serious breach to the protocol
- Major deviation to the protocol
- Non-compliance with the International Conference on Harmonisation (ICH) guideline for Good Clinical Practice.

Serious breach is defined as any conditions, practices or processes that adversely affect the rights, safety or well being of the subjects and/or the quality and integrity of data.
Major deviation is defined as any conditions, practices or processes that might adversely affect the rights, safety or wellbeing of the subjects and/or the quality and integrity of data.
Minor deviation is defined as any conditions, practices or processes that would not be expected to adversely affect the rights, safety or well being of the subjects and/or the quality and integrity of data.

13 ETHICAL AND REGULATORY ASPECTS

13.1 Rules and regulations

The clinical trial is conducted in conformity with:

- Ethical principles stated in the Declaration of Helsinki 1964, as revised in Fortaleza, 2013
- Regulation (EU) 2016/679 of the europan parliament and of the council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation)
- Regulation (EU) 536/2014 of the european parliament and of the council of 16 april 2014 on clinical trials on medicinal products for human use, and repealing directive 2001/20/EC
- The European Directive (2001/20/EC and 2005/28/EC)
- Appendix 13 of the E. U. Guide to Good Manufacturing Practices (revised and adopted in February 2010 by the European Commission),
- The Good Clinical Practices guidelines (International Conference on Harmonization ICH E6) and Statistical Principles for Clinical Trials (ICH E9),
- The Clinical Safety Data Management guidance (ICH E2A),
- and any local Regulations including
    - French Public Healthcare Law (n° 2004-806) of August 9, 2004, a partial adaptation of the European Directive (2001/20/EC) on the conduct of clinical trials,
    - French Public Healthcare Law (n° 2016-41) of January 26, 2016, about modernisation of the health system,
    - Ordinance n°2016-800 of June 16, 2016 on medical research involving human subjects,
    - French Law n° 2002-303 of March 4, 2002 relative to patients' rights and to the quality of the healthcare system,
    - French Informatics and Liberties Law (n° 78-17) of January 6, 1978 modified by Law n° 2018-493 of June 20, 2018,
    - French decree N° 2018-687 of 1 August 2018 adopted for the application of Law No. 78-17 of 6 January 1978

13.2 Definitions of the start/end of a clinical trial and of the first visit of the first subject

The first act of recruitment (*i.e* start of the clinical trial) is defined as being of the first site initiation visit.
The first visit of the first subject is defined as being the date of signature of the consent form by the first patient, i.e. first inclusion in the trial.
The end of the trial is defined as being the last-protocol-specified visit of the last patient i.e. 5 years after the inclusion of the last patient.

13.3 Ethic Committee – Competent Authority

This protocol was submitted to the Ethic Committee which gave its approval on 25/09/2019. This protocol has also been approved by the Competent Authority on 26/07/2019.
Gustave Roussy has taken out a legal liability insurance policy (N°124895).
A clinical study report on the trial will be written at the latest 6 months after the end of the trial. Results will be sent to the competent authority and to the Ethic Committee.
Results of the long term follow-up might be available in medical publication format after availability of the Clinical Study Report.
Gustave Roussy will maintain records of essential trial documentation in the Sponsor file for a minimum duration of 25 years after the end of the trial.

13.4 Information and Consent of Participants

It is the responsibility of the investigator or co-investigator (to whom the responsibility has been delegated by the Principal Investigator as captured on the Site Signature and Delegation Log) to obtain a written informed consent from the patient and/or an approved guardian prior to performing any trial related procedure. A parent/guardian and age-specific patient Information Sheets are provided to facilitate this process.

Timepoints for consents:

- HR-NBL2 registration and **R-I**: at diagnosis (or up to 21 days after one cycle of chemotherapy for patients with localized neuroblastoma with *MYCN* amplification).
- **R-HDC**: after the disease evaluation at the end of induction and after surgery of the primary tumor for those patients who will receive surgery before HDC
- **R-RTx**: after HDC/ASCR and surgery

Investigators must ensure that they adequately explain the aim, trial treatment, anticipated benefits and potential hazards of taking part in the trial to the patient.
The patient/legal representative will be provided with an information and consent form in comprehensive, clear, relevant and simple language. The child should participate in the informed consent process together with the parents/legal representative in a way that is appropriate to his/her age and maturity. Ample time shall be given for the parent/approved guardian and/or patient () to reed the Information Sheet and to to discuss his/her decision to participate in the clinical trial with others outside of the site research team. They must be given an opportunity to ask questions which should be answered to their satisfaction Patient (or legal representative) should be informed about his/her right to refuse to participate and the right to withdraw from the trial at any time without any resulting detriment and without having to provide any justification.

Having read the information notice, the patient (or legal representative) must date and sign the latest approved version of the Informed Consent Form if he/she accepts to participate**.** This consent form must also be signed by the investigator on the same day as the patient/parent/legal guardian. The original consent form must be kept in the study file by the investigator and the parent/approved guardian and/or patient should receive a copy.
Details of the informed consent discussions should be recorded in the patient's medical notes, this should include who was present, date of information regarding, the initial discussion, the date consent was given, with the name of the investigator.
Throughout the trial the parent/approved guardian and/or patient should have the opportunity to ask questions about the trial and any new information that may be relevant to the patient's continued participation should be shared with them in a timely manner
During a patient's participation in the trial, any update to the consent form and any update to the written information should be provided to the patient. On occasion it may be necessary to ask again for the patient consent, for example if new information becomes available or an amendment is made to the protocol that might impact the patient's participation in the trial. In this case the process above should be followed and the patient's right to withdraw from the trial respected.

If during a clinical trial the minor reaches the age of legal competence to give informed consent, patients should be re-consented at the age of majority before that patient can continue to participate in the clinical trial, in accordance with national guidance/legislation,

13.5 Principal Investigator Responsibilities

The principal investigator of each establishment concerned promises to conduct the clinical trial in conformity with the protocol which has been approved by the Ethic Committee and the competent authority.
The principal investigator should not modify any aspect of the protocol without prior written permission from the Sponsor nor without the approval of the proposed modifications by the Ethic Committee/IRB and the competent authority.

The Principal Investigator is responsible for:

- providing the Sponsor with his/her CV as well as that of co-investigators,
- ensuring co-investigators and other healthcare professionals should be sufficiently qualified by education, training and experience to perform their tasks,
- identifying members of his/her team participating in the trial and defining their responsibilities,
- recruiting patients after receiving the Sponsor's approval.

Each investigator is responsible for:

- personally obtaining the informed consent form which has been dated and signed by the participant in the research prior to any specific trial selection procedure,
- regularly completing the case report form (CRF) for each patient included in the trial and ensuring that the Clinical Research Associate (CRAs) mandated by the Sponsor has direct access to source documents in order to validate information on the CRF,
- dating, correcting and signing the corrections on the CRF for each patient included in the trial,
- accepting regular visits from a CRA and possibly visits from auditors mandated by the Sponsor or inspectors from the regulatory authorities.

All documentation concerning the trial (protocol, consent form, case report form, investigator file, etc…), as well as the original documents (laboratory results, imaging studies, medical consultation reports, clinical examination reports, etc.) is considered confidential and should be kept in a safe place. The Principal Investigator should keep data as well as a list of patient-identifying data for at least 15 years after the end of the study, or more if specified by the local regulation.

14 DATA COLLECTION

Data management will be done by the Biostatistics and Epidemiology Unit of Gustave Roussy using TrialMaster®, a software compliant to 21 CFR Part 11. The same software will be used for patient randomisation. Personal identifiers (user ID / password) wil be provided to the person involved (investigators, CRAs …) who requested an access to the eCRF.

An electronic Case Report Form (e-CRF) with remote data entry will be used for recording all data required by the protocol for each patient. Data to be recorded should be limited to those needed to assess study objectives and to document the safety of the trial interventions. It is the responsibility of the Investigator to ensure that the e-CRF is properly and completely filled in. The e-CRF must be complete in due time as soon as the data are available in order to be informed without delay. The e-CRF must be completed for all patients who have given informed consent for any trial related procedure. Source documentation for patients should be the physician's patient records, and as such, will be maintained at the study site.

ECRF access will be strictly limited to the users who requested login credentials. Each user will have personal identifiers and data access will be strictly limited according toprofiles:

- Clinical Resarch Associate (CRA) profile allows data entry and queries resolution
- Investigator profile allows data entry, data review and electronic sign off of the complete eCRF
- Monitor profile allows source data verification.

Data collected in the e-CRF will be verified through use of programmed edit checks specified by the data centre. If necessary, discrepancies in the data will be brought to the attention of investigational site personnel and sponsor’s CRA. Resolutions of these issues will be reflected in the database. An audit trail within the system will track all changes made to the data.

15 QUALITY CONTROL AND QUALITY ASSURANCE

In order to guarantee the authenticity and the credibility of the data in conformity with good clinical practices, the Sponsor has installed a quality assurance system which includes:

- trial management in accordance with the procedures at Gustave Roussy,
- quality control of data at the investigating site by the Clinical Research Associate (CRAs) mandated by the Sponsor,
- possible auditing of investigating centres
- monitoring of sample collection for mandatory and core SIOPEN Molecular Monitoring and Biological research study objectives.

Quality control on the site will be ensured by the CRAs mandated by the Sponsor in accordance with the monitoring plan.
The CRA must check that the investigator’s file exists and that it is updated.
The CRA must verify the consent forms, that subjects fulfil eligibility criteria, the validity of evaluation criteria and treatment toxicity with the help of source documents.

16 DATA OWNERSHIP / PUBLICATION POLICY

The investigator promises, on his/her behalf as well as that of all the persons involved in the conduct of the trial, to guarantee the confidentiality of all the information provided by Gustave Roussy until the publication of the results of the trial.
All publications, abstracts or presentations including the results of the trial require prior approval of the Sponsor (Gustave Roussy).
All oral presentations, manuscripts must include a rubric mentioning the Sponsor, the investigators / institutions that participated in the trial, the cooperative groups, learned societies which contributed to the conduct of the trial and the bodies which funded the research.
The responsible of each treatment phase will be the first author of the related publication which will have to be submitted within 1 year after the final analysis. An investigator of Gustave Roussy will be last author of any clinical manuscript related to the protocol, exept for local treatment-related questions where will be second last author. An investigator of Gustave Roussy will be second last author of any ancillary question related to the patients included in the study.
Other authorships will be determined by mutual agreement, taking account of the contribution made by each investigator/site, according to the SIOPEN rules.

17 DATA PROTECTION

17.1 Confidentiality

Investigator agrees that the collection, processing and disclosure of personal data and medical information related to the Subject, and personal data related to Investigator and any investigational staff is subject to compliance with applicable personal data protection and security laws and regulations.

Investigator agrees to adhere to the principles of medical confidentiality in relation to Clinical Trial Subjects.

Investigator shall not disclose the identity of Clinical Trial Subjects to third parties without prior written consent of the Sponsor.

17.2 Investigator’s personal data

Investigator hereby expressly consents to the processing of Investigator’s personal data collected by Sponsor. Such consent shall authorize the transfer of personal data to countries other than the Institution's own country, for the following purposes:

a) the conduct and interpretation of the Clinical Trial;

b) review by governmental or regulatory agencies, Sponsor, and its agents, affiliates and collaborators;

c) satisfying legal or regulatory requirements;

d) publication on national and international public websites and other websites and databases that serve a comparable purpose;

e) upon request of individual patients and doctors provision to individual patients and doctors who may be interested in participating in a clinical trial at Institution;

f) storage in Sponsor’s databases for use in selecting sites in future clinical trials.

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APPENDIX 1: CRITERIA FOR ADVERSE EVENTS

NATIONAL CANCER INSTITUTE - COMMON TERMINOLOGY*

*Except for Hearing (see Appendix 2)

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

http://ctep.cancer.gov/

APPENDIX 2: 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 Resonses/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 [R]	4	Marked

[R] The results used are obtained by pure-tone audiometry, from the "better" ear < 40 dB at all lower frequencies.

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

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APPENDIX 3: 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 4 : 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 tumor response in the primary tumor, soft tissue and bone metastases, and bone marrow.
- Primary and metastatic soft tissue sites are assessed using Response Evaluation Criteria in Solid Tumors (RECIST) and ^{123}I–MIBG scans or [^{18}F]fluorodeoxyglucose–positron emission tomography scans if the tumor is MIBG nonavid.
- Bone marrow is assessed by histology or immunohistochemistry and cytology or immunocytology. BM with ≤ 5% tumor 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) Tumor 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

Tumor 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 5 : INRG & INSS CLASSIFICATION

INRG staging system (INRGSS)

L1	Localised tumour not involving vital structures as defined by the list of image-defined risk factors and confined to one body compartment.
L2	Locoregional tumour with presence of one or more image defined risk factors
M	Distant metastatic disease (except MS)
MS	Metastatic disease in a child under 18 months, with metastases confined to skin, liver and/or bone marrow

INSS staging system (including 1993 modifications)

Stage 1	Localised tumour with complete gross excision, with or without microscopic residual disease: representative ipsilateral lymph nodes neative for tumour microscopically: nodes attached and removed with tumour may be positive.
Stage 2a	Localised tumour with incomplete gross excision: representative ipsilateral nonadherent lymph nodes negative for tumour microscopically.
Stage 2b	Localised tumour with or without complete gross excision, with ipsilateral nonadherent lymph nodes positive: enlarged contralateral lymph nodes negative microscopically.
Stage 3	Unresectable unilateral tumour infiltrating across the midline (beyond the opposite side of the vertebral column) with or without regional lymph node involvement: or midline tumour with bilateral extension via infiltration (unresectable) or lymph node involvement.
Stage 4	Any primary tumour with dissemination to distant lymph nodes, bone, bone marrow, liver, skin and/or other organs (except as defined for stage 4s disease).
Stage 4s	Localised primary tumour (as defined for stage 1, 2a, or 2b disease) with dissemination limited to skin, liver, and/or bone marrow, limited to infants younger than 1 year (marrow involvement of less than 10% of total nucleated cells and MIBG scan findings negative in the tumour).

[Roly Squire; Journal of Cancer & Allied Specialties; August 2016]

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 tumor cell associated RNA.[Burchill SA, Cancer 2017]
With regards to RTqPCR, there is a general international consensus on the necessity to establish an international validation of this technique, in order to make results from different centres comparable and to agree upon its value for the clinical management of MRD.

Bone Marrow Aspirations
BM aspirations are necessary for bone marrow smears, immunocytology, RTqPCR 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.

1) Aspiration of half a millilitre (0.5 ml) of BM into the syringe and **immediately** dropped on a glass slide.
2) 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).

Aspiration for immunocytology and RTqPCR
The appropriate amount of anticoagulant (i.e. 0.5-1 ml heparin (5000IE/ml) in 3-5 or 10 ml BM, respectively) is aspirated into the syringe. Draw 5-10 ml bilateral aspirate into Heparin (5000IE/ml), and then shake immediately to allow the anticoagulant to mix with the bone marrow. This procedure is repeated for each puncture site.

- Transfer immediately, 0.5ml of BM from each side into two single PAXgene^{TM} tubes for RTqPCR studies. Do not pool.
- Send the filled PAXgene^{TM} tubes to the national Molecular Monitoring reference laboratory or to Prof. Sue Burchill, Leeds, United Kingdom.
- Transfer 4.5ml (remainder) to National Immunocytology Reference Laboratory or molecular Monitoring reference laboratory (depending on national organization) for processing of at least 3 x10^6 cells on cytospins by isolating mononuclear cell (MNC) suspension and using an adequate cytocentrifugation machine (i.e. Hettich). Ideally 2 times 3 x 10^6 cells on cytospins should be produced for quality controlled assessment of minimal disease.

Send samples at room temperature, next day delivery.

Handling of the bone marrow cells in the laboratory
The methods for preparation of mononuclear cells (MNC), processing, sending and storage of cytospins, 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/*RC*Detect, MetaSystems, Altlussheim, Germany).
Further detail on SOPs for RTqPCR studies are described in Viprey *et al*.[Viprey VF, Eur J Cancer 2007]

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).

Storage of Tumor Material, Slides and Bone Marrow Samples
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.
It is advisable to store touch preparations and cytospin preparations at –20°C and cell suspensions (including DMSO), if available, in liquid nitrogen.
Furthermore, stained slides, IF/FISH images and RTqPCR pictures have to be stored adequately for documentation and review purposes.

References:
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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 tumors, including neuroblastoma, phaeochromocytoma 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)
^{123}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 labetolol, 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 ^{131}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 throughly 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 ^{123}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 ^{123}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}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}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 tumor 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 ^{123}I-mIBG scan, especially in case of lesions adjacent to sites of high mIBG uptake (such as heart, liver, primary tumor).

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.
In HR-NBL2 trial, **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.

PET tracers

^{18}F-FLUORODEOXYGLUCOSE (FDG)

^{18}F-Fluorodeoxyglucose is a glucose analogue which is concentrated in sites of glycolysis, including most tumors and areas of infection/inflammation. ^{18}F-FDG is less specific for neuroblastoma than mIBG and is considered as a second line imaging agent. ^{18}F-FDG is most useful in neuroblastomas that fail to or weakly accumulate mIBG and is recommended as an option for evaluation of mIBG negative tumors.

Preparation, drug interactions, precautions
Patients should fast for at least 4 hours prior to ^{18}F-FDG injection. Any glucose containing IV fluids should be discontinued 4 hours prior to ^{18}F-FDG injection.

Administered activity and acquisition protocol
The injected activity can be calculated using the pediatric dosage card, which is available on its updated version on the EANM website.
The normal distribution of ^{18}F-FDG in children includes the brain, salivary glands, the Waldeyer's ring, the heart, the liver, the spleen, the bowel, the kidneys, and the bladder. Bone marrow activity is variable.

^{18}F-FDG uptake in neuroblastoma patients can be seen in both soft tissue and skeletal disease sites. Physiologic ^{18}F-FDG uptake in bone marrow is seen in the absence of tumor, especially in patients undergoing CSF stimulating factors. Cranial vault lesions can also be difficult to visualize due to adjacent brain activity although large skull lesions can be identified.
Appropriate paediatric CT settings should be utilized to minimize radiation dose.

^{18}F-DIHYDROXYPHENYLALANINE (DOPA)

^{18}F-Dihydroxyphenylalanine (DOPA) is a direct dopamine precursor. This radiopharmaceutical is actively transported into cells through the large amino acids transporter (LAT1) and then converted into dopamine by the amino-acid decarboxylase (AADC).
Some authors reported a greater sensitivity for ^{18}F-DOPA PET than ^{123}I-mIBG in the identification of disease relapse and for the assessment of response to induction therapy [Piccardo A. Eur J Nucl Med Mol Imaging 2012].
An exploratory study will be conducted in some centers to evaluate the diagnostic role of ^{18}F-DOPA PET/CT in comparison to ^{123}I-mIBG scan at the time of first disease presentation and at the end of the induction chemotherapy, and their impact on EFS and OS.

^{68}GA-DOTA-PEPTIDES

Autoradiography and immunohistochemistry studies showed that somatostatin receptors (SSR) can be expressed in 77-89% of neuroblastoma cells. Some authors suggested a greater sensitivity of ^{68}Ga-DOTA-peptide PET/CT in staging and restaging neuroblastoma compared to ^{123}I-mIBG scintigraphy,[Kroiss A, Eur J Nucl Med Mol Imaging 2011] although prospective data are lacking.

References:

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APPENDIX 8: HARVEST GUIDELINES

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

Timing

Peripheral blood stem cells (PBSC) mobilization is recommended according to the induction regimen schedule. Patients receiving GPOH induction will have the PBSC collection following Cycle 3 depending on bone marrow disease status. Documentation of clearance of tumor cells from the bone marrow is required for early collection according to the definition of MRD. If medical condition prohibits safe apheresis, it is appropriate to delay PBSC mobilization and harvest after subsequent induction course or end of induction therapy.

Patients receiving COJEC as induction 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 6 x 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 extra corporal 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 x 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 **>6 x 10^6 CD 34+cells/kg**, the cells to be subdivided **into ≥ 3 units to provide adequate stem cells for 2 transplants**. 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 10^8 nucleated cells/ml **in at least 3 bags.** The DMSO concentration in the infused bags should not exceed 1mg/Kg/day.

APPENDIX 9: NATIONAL COORDINATORS CONTACT DETAILS

The deployment of the HRNBL2 study in all countries will be gradual.

The following is a table of co-sponsors mentioning the countries that can start the study at first.

LADENSTEIN, RUTH, PROF. DR.	St. Anna Kinderkrebsforschung A - 1090 Vienna, Zimmermannplatz 10	Phone: +43 1 40470 4750 Fax: +43 1 40470 7430 Email: ruth.ladenstein@ccri.at	**AUSTRIA**
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APPENDIX 10: DECLARATION OF HELSINKI

WMA DECLARATION OF HELSINKI – ETHICAL PRINCIPLES FOR MEDICAL RESEARCH INVOLVING HUMAN SUBJECTS

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964
and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
59th WMA General Assembly, Seoul, Republic of Korea, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

1) Preamble

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

2) General Principles

3. The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient’s best interest when providing medical care.”

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician’s knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

3) Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

4) Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

5) Scientific Requirements and Research Protocols

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

6) Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

7) Privacy and Confidentiality

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

8) Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study

provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

9) Use of Placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention

and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

10) Post-Trial Provisions

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

11) Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared

in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

12) Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

APPENDIX 11: BIRTH CONTROL METHODS

1. **Birth control methods which may be considered as highly effective:**

For the purpose of this guidance, methods that can achieve a failure rate of less than 1% per year when used consistently and correctly are considered as highly effective birth control methods. Such methods include:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation [1]
    - oral
    - intravaginal
    - transdermal
- progestogen-only hormonal contraception associated with inhibition of ovulation [1] :
    - oral
    - injectable
    - implantable [2]
- intrauterine device (IUD) [2]
- intrauterine hormone-releasing system (IUS) [2]
- bilateral tubal occlusion [2]
- vasectomised partner [2,3]
- sexual abstinence [4]

2. **Acceptable birth control methods which may not be considered as highly effective** Acceptable birth control methods that result in a failure rate of more than 1% per year include:

- progestogen-only oral hormonal contraception, where inhibition of ovulation is not the primary mode of action
- male or female condom with or without spermicide [5]
- cap, diaphragm or sponge with spermicide [5]

3. **Birth control methods which are considered unacceptable in clinical trials**

Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhoea method (LAM) are not acceptable methods of contraception. Female condom and male condom should not be used together.

1 Hormonal contraception may be susceptible to interaction with the IMP, which may reduce the efficacy of the contraception method.

2 Contraception methods that in the context of this guidance are considered to have low user dependency.

3 Vasectomised partner is a highly effective birth control method provided that partner is the sole sexual partner of the WOCBP trial participant and that the vasectomised partner has received medical assessment of the surgical success.

4 In the context of this guidance sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject.

5 A combination of male condom with either cap, diaphragm or sponge with spermicide (double barrier methods) are also considered acceptable, but not highly effective, birth control methods.

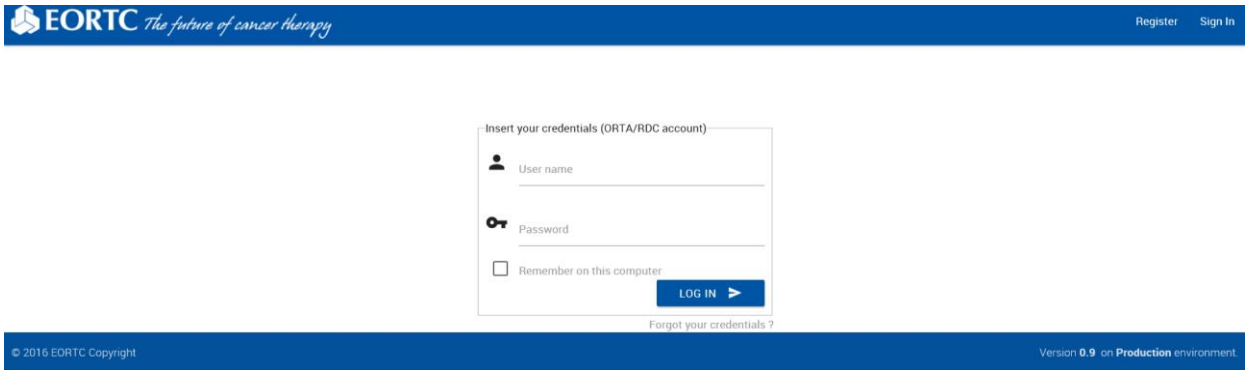
For more information, please refer to the CTFG Guidelines "recommendations related to contraception and pregnancy testing in clinical trials

APPENDIX 12: RADIOTHERAPY MANUAL

UPLOADING PROCEDURE

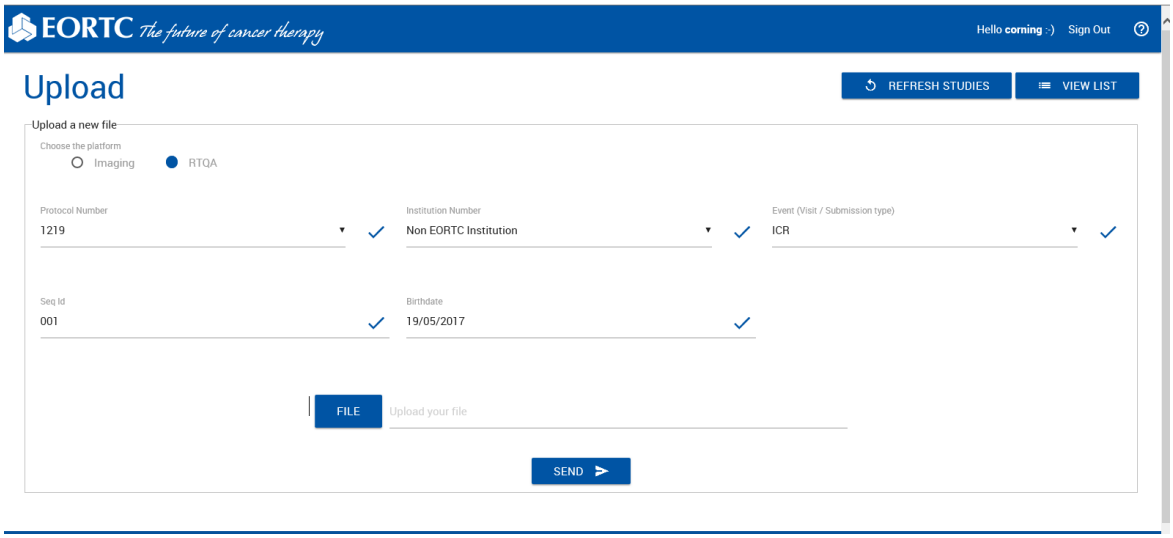
Dataset submission via EORTC RTQA Uploader at

https://www.eortc.org/tools



1. The file uploader requires a valid ORTA/RDC login. This is the same login used for CRF input. If you do not yet have an ORTA/RDC account, please request one by clicking on *Register* at the top of the page. If you already have a login but forgot your password you can click on *Forgot your credentials?*
2. Select *RTQA* under *Platform* and enter the following information :

Protocol Number	Select your study from the available drop down list.
Institution number	If you work in several institutions, take care to select the correct number. If your institution does not have an EORTC number, select Non EORTC Institution
Event(Visit/Submission Type)	Identify the data as Benchmark, Virtual Phantom Procedure or Individual Case Review
SeqId	The patient's SeqId or if not applicable 000
Birthdate	The patient's birthday in the format DD/MM/YYYY or todays date if not applicable

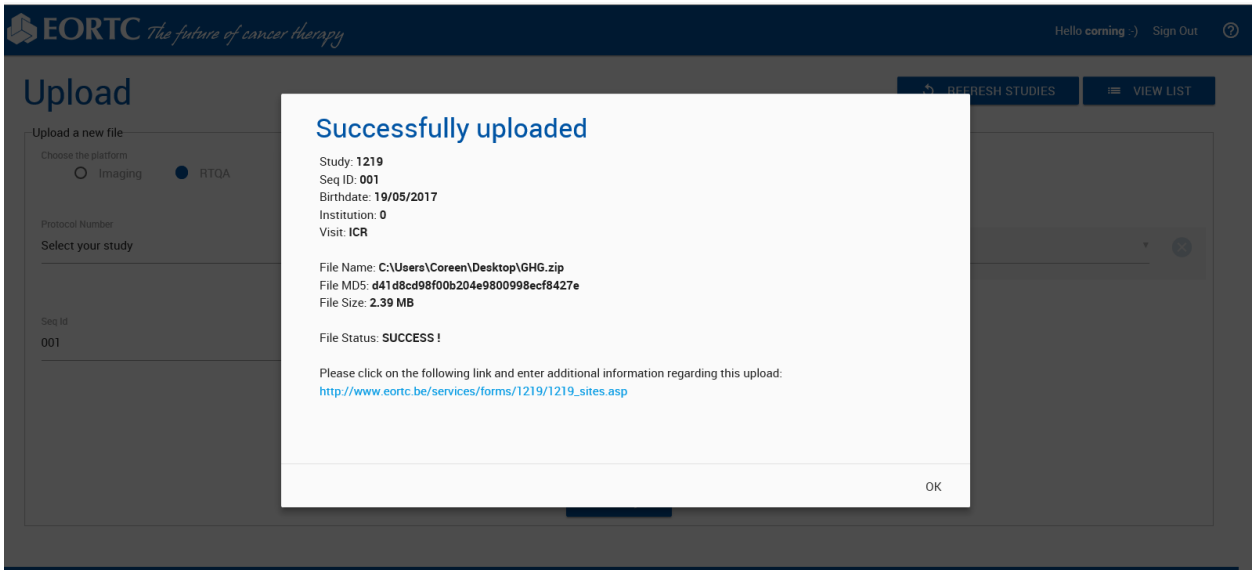


3. Click on “File” and select your compressed dataset (.zip) at your local computer.

Please avoid using spaces or special characters in the file name!

4. Click 

5. A *“Successfully uploaded”* screen will appear summarizing information about the upload and file and a confirmation email will be sent to your email address.



APPENDIX 13: GUIDELINE FOR PEADIATRIC BLOOD VOLUME FOR RESEARCH PURPOSES *(V1.1_30.10.2015)*

HEALTH RESEARCH ETHICS COMMITTEE (HREC)

UNIVERSITEIT•STELLENBOSCH•UNIVERSITY
jou kennisvennoot • your knowledge partner

Guideline for Paediatric Blood Volume for Research Purposes

Amended Document prepared by M Kruger

30 November 2015
V1.1

Public guideline

The following is the guideline for the SOP for researchers which should be available on the website:

- It is important to take the child's clinical condition into account when determining what volume can be used for research purposes.
- Blood volume should not exceed 5% of the total blood volume during a one-off sampling of total blood volume (including routine blood specimens for clinical care).
- Blood volume should not exceed 5% of the total blood volume within 3-months (including routine blood specimens for clinical care). *(US OHRP: 3 ml/kg or up to 50 ml total within 8 weeks).*
- If the blood volume necessary exceeds the above guideline, the research team need to submit additional motivation, which will be considered by the ethics review committee for final approval and may need expert opinion to guide the ethics review committee.

REC Member guideline

This guideline is to be used by REC members when there is a request for a larger blood volume to be taken from a child participant with adequate motivation by the principal investigator. This guideline also take into consideration the haemoglobin and is therefore a better guideline in the scenario dealing with impoverished communities and malnutrition.

CMRC IRB MAXIMUM ALLOWABLE TOTAL BLOOD DRAW VOLUMES (CLINICAL + RESEARCH)						
Body Wt (Kg)	**Body Wt (lbs)**	**Total blood volume (mL)**	**Maximum allowable volume (mL) in one blood draw (= 2.5% of total blood volume)**	**Total volume (clinical + research) maximum volume (mL) drawn in a 30-day period**	**Minimum Hgb required at time of blood draw**	**Minimum Hgb required at time of blood draw if subject has respiratory/CV compromise**
1	2.2	100	2.5	5	7.0	9.0 -10.0
2	4.4	200	5	10	7.0	9.0-10.0
3	6.3	240	6	12	7.0	9.0-10.0
4	8.8	320	8	16	7.0	9.0-10.0
5	11	400	10	20	7.0	9.0-10.0
6	13.2	480	12	24	7.0	9.0-10.0
7	15.4	560	14	28	7.0	9.0-10.0
8	17.6	640	16	32	7.0	9.0-10.0
9	19.8	720	18	36	7.0	9.0-10.0
10	22	800	20	40	7.0	9.0-10.0
11-15	24-33	880-1200	22-30	44-60	7.0	9.0-10.0
16-20	35-44	1280-1600	32-40	64-80	7.0	9.0-10.0
21-25	46-55	1680-2000	42-50	64-100	7.0	9.0-10.0
26-30	57-66	2080-2400	52-60	104-120	7.0	9.0-10.0
31-35	68-77	2480-2800	62-70	124-140	7.0	9.0-10.0
36-40	79-88	2880-3200	72-80	144-160	7.0	9.0-10.0
41-45	90-99	3280-3600	82-90	164-180	7.0	9.0-10.0
46-50	101-110	3680-4000	92-100	184-200	7.0	9.0-10.0
51-55	112-121	4080-4400	102-110	204-220	7.0	9.0-10.0
56-60	123-132	4480-4800	112-120	224-240	7.0	9.0-10.0
61-65	134-143	4880-5200	122-130	244-260	7.0	9.0-10.0
68-70	145-154	5280-5600	132-140	264-280	7.0	9.0-10.0
71-75	156-185	5680-6000	142-150	284-300	7.0	9.0-10.0
76-80	167-176	6080-6400	152-160	304-360	7.0	9.0-10.0
81-85	178-187	6480-6800	162-170	324-340	7.0	9.0-10.0
86-90	189-198	6880-7200	172-180	344-360	7.0	9.0-10.0
91-95	200-209	7280-7600	182-190	364-380	7.0	9.0-10.0
96-100	211-220	7680-8000	192-200	384-400	7.0	9.0-10.0

Based on blood volume of:		
kg	mL/kg	
1-2	100	Pre-term infant
> 2	80	Term infant - adult

This information is similar to that used by the Committee on Clinical Investigations, Children's Hospital in Los Angeles, CA; Baylor College of Medicine, Dallas, TX; and Cincinnati Children's Hospital Institutional Review Board, OH. These charts were adapted by: Rhona Jack, Ph.D. Children's Hospital and Regional Medical Center Laboratory, Seattle, WA in August 2001.

Reference: Rhona Jack; www.ucdmc.ucdavis.edu/.../Blood_Draws_Maximum_Allowable.doc - downloaded on 02 December 2010