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An international multicenter phase II randomised trial evaluating and comparing two intensification treatment strategies for metastatic neuroblastoma patients with a poor response to induction chemotherapy
A SIOPEN Study

VERITAS

V2.0 dated 08th August 2018

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

AGPHO	Austrian Group of Paediatric HaematoOncology
AIEOP	Associazione Italiana Ematologia Oncologia Pediatrica
ANZCHOG	Australia and New Zealand Children's Haematology/Oncology Group
BSPHO	Belgian Society of Peadiatric HaematoOncology
CRCTU	Cancer Research UK Clinical Trials Unit
GPOH	German Group of Paediatric HaematoOncology
NCRI CCL CSG	Neuroblastoma Group UK
HSPHO	Hellenic Society of Paeditric Haematology-Oncology
ISPHO	Israeli Society of Paediatric Haematology Oncology
NOPHO	Nordic Society for Paediatric Haematology and Oncology (Norway, Sweden, Denmark, Finland)
SFCE	Société Française des Cancers et Leucémies de l'Enfant et de l'Adolescent
SEOP	Spanish Society of Paediatric Oncology
SFOP	Société Française d'Oncologie Pédiatrique
SIAK	Switzerland

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

IMPORTANT INFORMATION FOR CENTRES PARTICIPATING IN THIS CLINICAL TRIAL

Centres wishing to participate in this study must agree to refer patients to approved specialist centres for those treatments which cannot be given locally.

Centres must have agreements in place with (1) an approved mIBG treatment centre and (2) a suitable paediatric stem cell transplant centre as per standard practice.

Centres will not be approved to open this study until these measures are in place. Patients must start treatment according to their randomisation to continue in the study.

SIGNATURE PAGE

VERITAS "An international multicenter phase II randomised trial evaluating and comparing two intensification treatment strategies for metastatic neuroblastoma patients with a poor response to induction chemotherapy"

EudraCT N°: 2015-003130-27

SPONSOR N°: 2015/2294

V2.0 dated 03rd May 2018

I have read and approve this protocol named **VERITAS**. 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 directive, 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.

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 VERITAS trial and provides information about procedures for patients taking part in the VERITAS trial. **The protocol should not be used as a guide for treatment of patients not taking part in the VERITAS trial.**

Study site :

Investigator Name and Title :

Investigator Signature :

Date of Signature :

1 Study Contacts

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2 TRIAL SYNOPSIS

Study's Title	VERITAS: An international multicenter phase II randomised trial evaluating and comparing two intensification treatment strategies for metastatic neuroblastoma patients with a poor response to induction chemotherapy
Coordinating Sponsor	Gustave Roussy 114 rue Edouard Vaillant 94805 Villejuif Cedex, France
EudraCT number	2015-003130-27
Coordinating Investigator	Dominique VALTEAU COUANET, MD, PhD Gustave Roussy (Villejuif, France)
Patients	Very High Risk Neuroblastoma Patients
Main Objective	The main objective is to evaluate the efficacy of two intensified consolidation strategies in very-high risk neuroblastoma (VHR-NBL) patients in terms of event-free survival from randomisation date. This evaluation will follow a hierarchical testing procedure: each experimental treatment will be first evaluated as a single-arm phase 2 study, and in case of positive conclusion, the relative efficacy of both arms will then be evaluated comparatively.
Secondary Objectives	a - To estimate and compare the overall survival (OS) of patients treated in the two treatment strategies b - To evaluate and compare the safety of the two treatment strategies in terms of toxic death and non-fatal toxicities rates. c - To estimate and compare the disease response after BuMel and at the end of treatment of the two treatment strategies d - To evaluate the between-treatment differences in Quality adjusted Time WIthout Symptoms and Toxicity (Q-TWiST approach) e - To evaluate the feasibility and document the logistical issues raised by 131I-mIBG and topotecan therapy in a multicenter setting f - To estimate and compare the Event-Free Survival of the two treatment strategies from the start of the intensified consolidation chemotherapy g - To estimate and compare the Event-Free Survival of the two treatment strategies from the date of the neuroblastoma diagnosis
Study Design	Prospective, open-label, randomised, multi-centre phase 2 trial
Inclusion Criteria	1 - Metastatic neuroblastoma (NBL) 2 - Patient previously treated within the ongoing High Risk Neuroblastoma SIOPEN study or treated with the current

	standard treatment for very high risk neuroblastoma off-trial 3 - mIBG scintigraphy positive at diagnosis and after induction chemotherapy (pre BuMel evaluation). 4 - Metastatic response after induction chemotherapy lower than the ongoing High Risk Neuroblastoma SIOPEN trial criteria to be eligible for High Dose Chemotherapy (metastatic response worse than partial response (< PR) or SIOPEN score > 3) 5 - Females of childbearing potential must have a negative serum 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 are listed in Appendix. Female patients who are lactating must agree to stop breast-feeding. 6 - Written informed consent from parents/legal representative, patient, and age-appropriate assent before any study-specific screening procedures are conducted according to local, regional or national guidelines. 7 - Patient affiliated to a social security regimen or beneficiary of the same according to local requirements.
Main Non Inclusion Criteria	1 - Parenchymal brain metastasis (even one) 2 - Progressive disease at study entry 3 - Previous high-dose therapy and Autologous Stem Cell Reinfusion 4 - Performance status (Karnofsky, Lansky) <70% 5 - Patient having received other therapy for cancer treatment than those allowed as per the ongoing High Risk Neuroblastoma SIOPEN trial or as defined in the future frontlines protocol (for HRNBL1 trial : after induction + 2 TVD) 6 - Impaired organ function (liver, kidney, heart, lungs) • Shortening fraction <28%, or ejection fraction <55%, or clinical evidence of congestive heart failure or uncontrolled cardiac rhythm disturbance • Dyspnoea at rest and/or pulse oximetry <95% in air • ALT, Bilirubin > 2 ULN • Creatinine clearance and/or GFR < 60 ml/min/1.73m^2 and serum creatinine ≥ 1.5 mg/dl 7 - Any uncontrolled inter-current illness or infection that in the investigator's opinion would impair study participation 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 - Known hypersensitivity to dacarbazine 14 - Concomitant use with St John's Wort

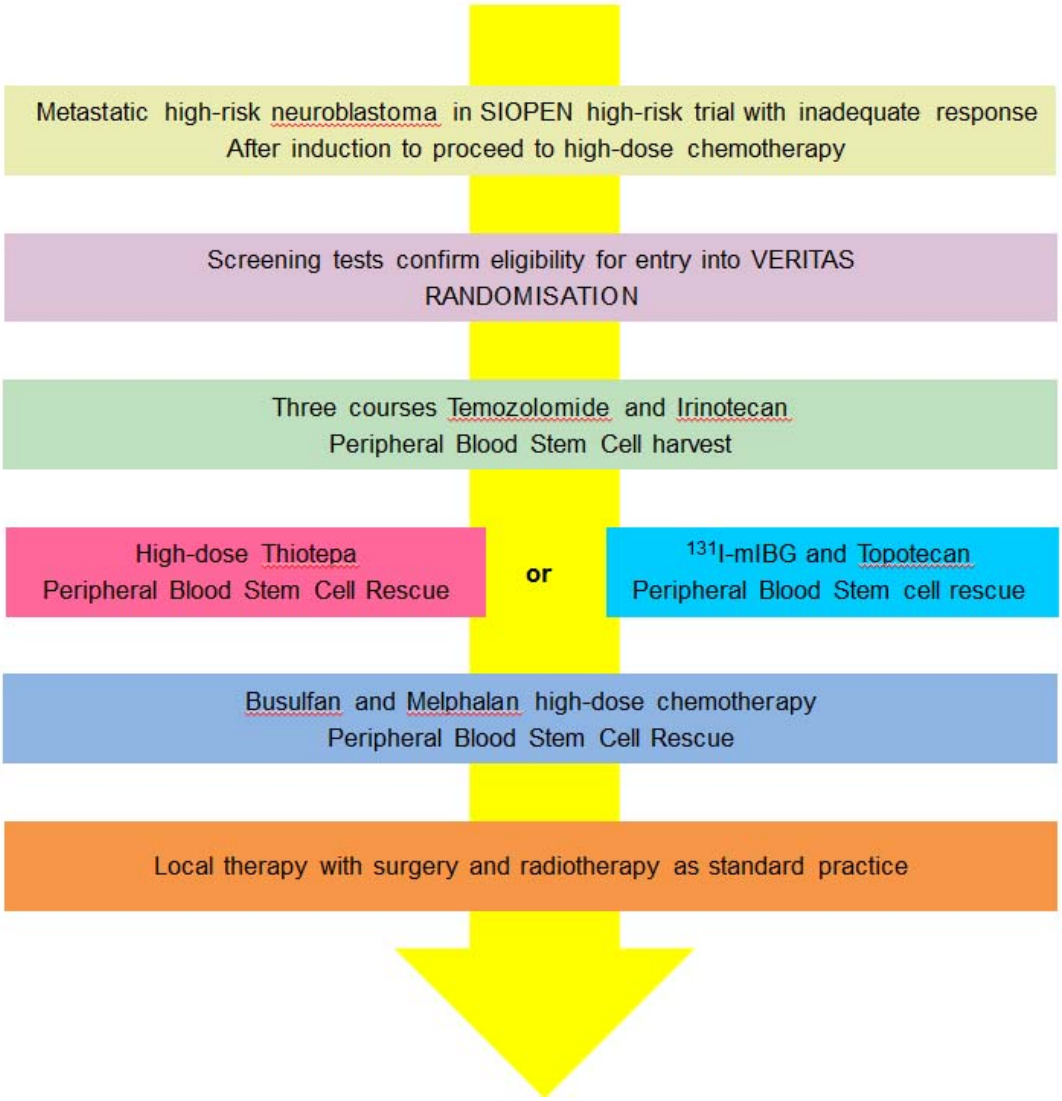
Evaluated Treatments	The trial will evaluate two randomised arms. Each arm includes - three cycles of Temozolomide-Irinotecan, similar in both arms, - a specific consolidation course detailed hereinafter, - a BuMel sequence, followed by an ASCT, similar in both arms, - external radiotherapy as appropriate, and/or local surgery of the tumour residues as appropriate. The specific consolidation courses differ between the randomised arms as follows: **Arm A: High administered activity ^{131}I- mIBG and Topotecan** Day 1 ^{131}I-mIBG course 1: 444MBq/kg with *in vivo* whole-body dosimetry Day 1-5 Topotecan 0.7 mg/m2 daily Day 15 ^{131}I-mIBG course 2: the target is to deliver a combined whole-body radiation dose of 4 Gy Day 15-19 Topotecan 0.7 mg/m2 daily Day 25-29 ASCT as soon as the radiation levels allow it **Arm B: High dose Thiotepa** Day 1-3 Thiotepa 300 mg/m²/day Day 4 ASCT The Investigational Medicinal Products (IMPs) are ^{131}I-mIBG, Topotecan and Thiotepa.
Primary endpoint	3-years Event Free Survival (EFS) from the date of randomisation into the VERITAS trial
Secondary Endpoints	a - Overall survival b - Adverse events, evaluated using NCI-CTCAE v5.0 toxicity grading system, reported by treatment phase and overall over the whole treatment duration (maximum grade). The stopping rule for toxicity will be based on the occurrence of adverse events leading to ventilation in an ICU and treatment-related deaths. These events will be specifically monitored over the first 6 months after randomisation. c - Disease response after BuMel and at the end of treatment d - For the Q-TWiST analysis: time spent with severe toxicity after randomisation and before progression/relapse (duration of hospitalisation will be used as a surrogate of time with toxicity); time spent without progression/relapse and without toxicity; and time from progression until death. e - Logistical issues raised by each strategy of intensified consolidation chemotherapy f - Event-Free Survival from the date of start of the consolidation

	phase g - Event-Free Survival from the date of the neuroblastoma diagnosis
Randomisation	Patients randomised in a 1:1 ratio to: Arm A: ^{131}I-mIBG and Topotecan + ASCT Arm B: High Dose Thiotepa + ASCT A minimisation procedure will be used to balance treatment arms 1:1 according to the stratification factors listed below: • country • MYCN-amplification • age at NBL diagnosis (below 5 years old, or 5 years and above) • metastatic sites (with or without bone/ bone marrow involvement)
Statistical Analysis **Sample Size**	**Main analysis** The main analysis will be based on the EFS analysis based on all patients included in the randomised trial (intention-to-treat). A hierarchical testing procedure will be followed. 1) Each arm will first be analysed as a single-arm phase-2 study comparing the 3-year EFS to the null hypothesis p0=15%, with a one-sided alpha=0.05 (one-step Fleming design). In each arm, if at least 17 of the 75 patients are alive free of event three years after randomisation, we will conclude a benefit, as the 3-year EFS is significantly higher than 15%. If some EFS times are censored in the first three years, the 3-year EFS rate will be estimated using Kaplan-Meier method and its one-sided 95% confidence interval will be tested against the null hypothesis. 2) If both treatment arms appear associated with a benefit, the EFS curves of both experimental groups will then be compared. EFS curves will be estimated using Kaplan-Meier method and compared using a log-rank test. The relative treatment effect (hazard ratio for event, Arm A versus B) will be estimated in a multivariable Cox model, assuming proportional hazards, and controlling for the stratification variables. For a pragmatic reason of feasibility (very rare setting) a two-sided alpha=0.20 will a priori be used. With this approach, the power of the comparison remains acceptable. **Secondary analyses** The whole EFS curve of each treatment group will also be compared to historical curves drawn from HR-NBL1 study, after selection of similar patients (high risk neuroblastoma with poor response to induction chemotherapy), In addition to the frequentist hypothesis-driven approach, a Bayesian approach will be considered to describe treatment effect distribution, with several priors including a non-informative prior. Heterogeneity of relative treatment effect (Arm A versus B) according to the stratification variables will also be explored using forest plots and two-sided interaction tests. For exploratory purpose, we will also compare the EFS curves from the start of consolidation phase, excluding patients who have progressed or died before the start of the consolidation phase allocated by randomisation. EFS curves will also be estimated from the date of

	neuroblastoma diagnosis. Overall survival curves will be estimated using Kaplan-Meier method and compared using a log-rank test. The relative treatment effect (hazard ratio for event, Arm A versus B) will be estimated in a multivariable Cox model, assuming proportional hazards, and controlling for the stratification variables. Adverse events, evaluated using NCI-CTCAE v5.0 toxicity grading system, will be reported by treatment phase and overall over the whole treatment duration (maximum grade). Incidence of grade >2 extra-haematological toxicity will be estimated by treatment arm and compared between treatment arms, overall and by system organ class (SOC). A Q-TWIST analysis will be performed (Gelber and Goldhirsch). Duration of hospitalisation will be used as a surrogate of time with severe toxicity. Overall survival will be partitioned into three states: toxicity (time with severe toxicity after randomisation and before progression/relapse); time without symptoms of disease progression/relapse and without toxicity; and time from progression until death. Between-treatment differences in the mean duration of each state will be calculated. Q-TWiST will be obtained as a sum of time spent in the three health states, weighted by utility scores. The bootstrap method will be used for testing statistical significance. Threshold analysis and gain functions will allow a group comparison for different utility values. **Interim analyses** No formal interim analysis of efficacy endpoint is planned. A close monitoring of treatment-related deaths and adverse events leading to ventilation in an ICU will be performed over the first 6 months after randomisation (stopping rule for toxicity). **Sample size** Pragmatic considerations have driven the sample size calculation (very rare disease setting). If the accrual could be increased compared to the anticipated rate, sample size would be re-estimated (adaptive design), blinded to observed treatment effect, to allow for a smaller alpha (higher strength of evidence). Overall, a total of **150 patients** is achievable over 5 years of accrual. For each treatment group considered as a single-arm study, 75 patients yield a 94%-power to conclude a significant benefit compared to p0=15% if the true 3-year EFS is 30%, with a one-sided alpha=0.05, assuming no censored data in the first three years. The number of 150 patients (119 events) allows for a randomised Phase II trial with the following hypotheses/ parameters: - comparison of EFS-curves of both experimental treatments, - exponential EFS distribution, - two-sided alpha=0.20, - 80%-power to detect a 14.4%-increase of 3-year EFS (30% versus 44.4%, HR=0.675).

Planned Calendar	Planned recruitment period: 5 years Treatment period per patient: ≈ 1 year Follow-up : 3 years after randomisation Planned study duration: 8 years A long-term follow-up is also planned.

Figure 1 – Overall Succession of Main Treatments and Procedures



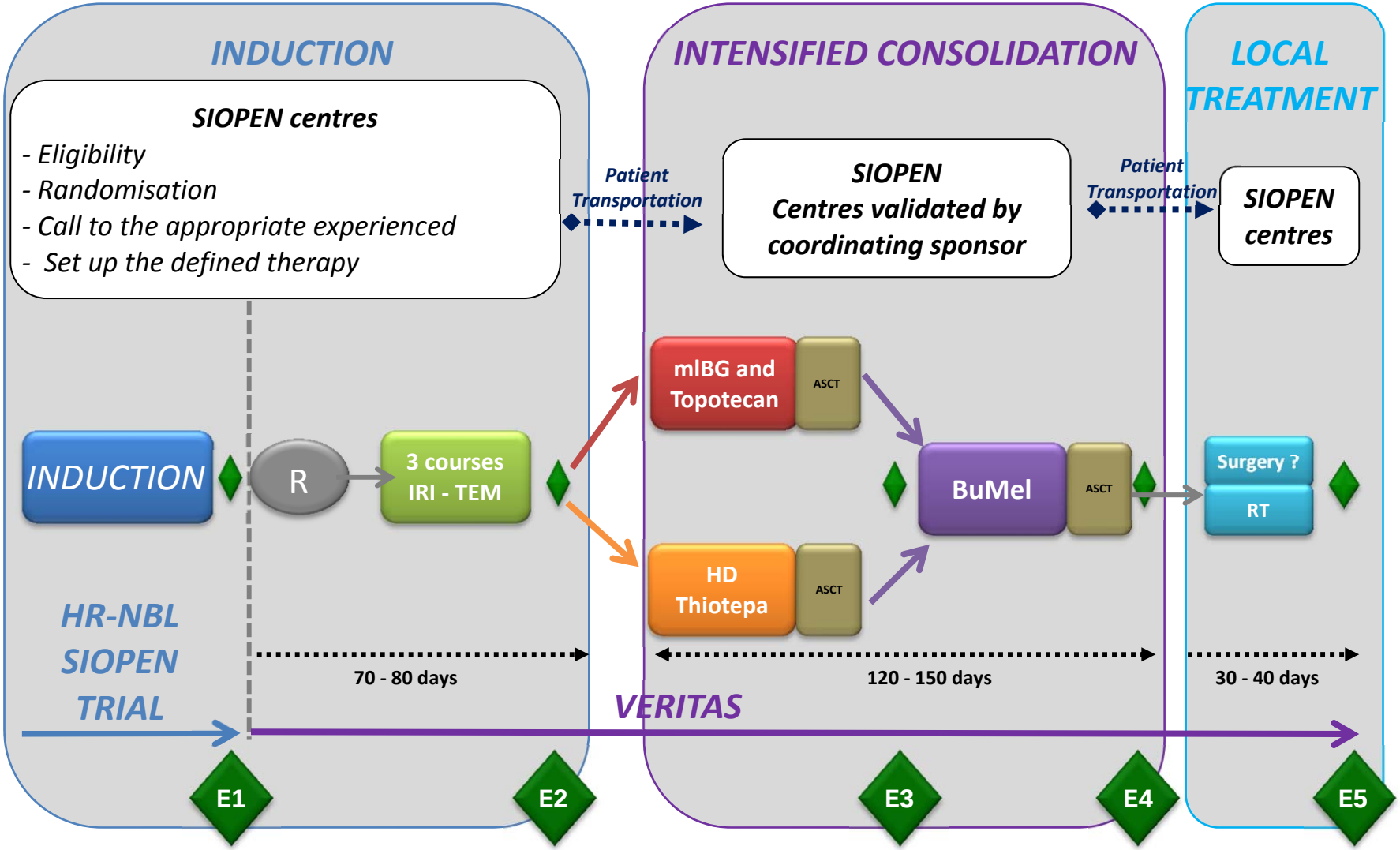


Figure 2- Detailed overall study flowchart (including treatments and disease evaluations (depicted as green diamond shapes)

Extensive efficacy assessments E1, E2 and E5 (see **Erreur ! Résultat incorrect pour une table.**) include the primary tumour imaging + a bone marrow evaluation +^{123}I-mIBG scintigraphy + urinary catecholamine metabolites.

E4 comprises ^{123}I-mIBG scintigraphy + primary Tumour imaging + urinary catecholamines

E3 comprises only the primary tumour imaging by echography and and the urinary levels of catecholamine metabolites

Figure 3 bis- Detailed study flowchart

Evaluations	E1 (Study entry)		IRI-TEM			E2	Arm A: 131m-IBG / Topotecan + ASCT		Arm B: HD Thiotepa + ASCT	E3	Bu-Mel + ASCT	E4	Local RT +/- Surgery	E5	Follow-up visits
	within 28 days	within 7days	D1	D21	D42	Prior to intensified chemotherapy	D1	D15	D1	Prior to BuMel 6 to 9 wks after the 2nd inj. of 131I-mIBG	D1	Post-BuMel, prior to local treatment		End of local & study treatment	Q3M during the first 3 y. after rando.
Eligibility criteria	x														
Medical history		x													
Full clinical examination		x	x	x	x	x	xxxxx	xxxxx	xxxxx	x	xxxxxxx	x	x	x	x
Rercord AEs	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
TREATMENTS															
Temozolomide			xxxxx	xxxxx	xxxxx										
Irinotecan			xxxxx	xxxxx	xxxxx										
^{123}I-mIBG							x0000	x0000							
Topotecan							xxxxx	xxxxx							
HD Thiotepa									xxx00						
Busulfan											xxxxx000				
Melphalan											000000x0				
ASCT							D25 to D29		D4		D8				
EVALUATIONS															
^{123}I-mIBG scintigraphy (a)	x					x						x		x	x (a)
Primary tumour imaging (MRI or CT) (b)	x					x						x		x	x (j)
Primary tumour imaging (echo) (b)										x					x (j)
Cerebral imaging (MRI or CT) (b)	x														x (k)
Blood MRD testing (c)	x					x						x		x	
BM MRD testing (c)	x					x								x	
BM (trephine biopsy) (d)	x					x								x	
BM (aspirates) (e)	x					x								x	x (k)
Urinary catecholamine metabolites (f)	x					x				x		x		x	x
biochemistry (g)	within 72hours		x	x	x		x	x	x		x		x	x	x (l)
Haematology (h)	within 72hours		Weekly			x	2/weeks		2/weeks	x	3/weeks	x	Weekly	x	x
T3, T4, TSH (i)							x	x		x				x	at 6 months
Cortisol, ACTH (i)							x	x		x				x	at 6 months
Serum pregnancy test		x				x									
Pulmonary function	x					x				x				x	x (k)
Echocardiogram	x					x				x				x	x (k)
Urine analysis	x														
HBV and HIV testing	x					x									
Auditory function														x	x (k)

(a) At one year follow-up if positive at E5 and then yearly until negative or progression

(b) Imaging of the primary tumour and cerebral imaging: by MRI or CT scan as judged appropriate by the site. Ultrasound/echography imaging of the primary tumour is mandatory before BuMel

(c) The minimal residual disease (MRD) testing is performed using two types of methods: a quantitative reverse-transcriptase polymerase chain reaction (QRT-PCR), and by immunocytology (IC). For the disease evaluation, blood and bone marrow are tested via both methods to detect any signs of minimal residual disease.

(d) BM trephine: the pathologist performs a histological examination and provides the clinician with the information. Ten unstained sections are needed for central review by SIOPEN members (see Appendix 5).

(e) BM aspirates: to be shared between slides for staining, BM cytospin, and QRT-PCR (see Appendix 5)

(f) For patients with positive test for urinary catecholamine metabolites at diagnosis, the test will be repeated every 3 months the 3 first years, and then every 4 months the 4th year.

(g) Electrolytes (Na+, K+, Cl-), albumin, CRP, Renal function: urea, creatinine (and also the urinary level of creatinine, in order to calculate the creatinine clearance), liver function: AST or ALT, bilirubin.

(h) Haemoglobin level (Hb), platelets count, and white blood cells (WBC) with differential count (neutrophils, lymphocytes, eosinophils, basophils

(i) In patients of the arm A, treated with 131I-mIBG and topotecan

(j) Until full tumour shrinking.

(k) As clinically indicated

(l) GFR assessment should be determined at the end of treatment. In children who can give a reliable 24 hour urine collection, endogenous creatinine clearance is acceptable. Where this is not possible, then GFR estimation by DTPA or CrEDTA is preferred. Children who had an end of treatment GFR of less than 80ml/min/1.73m² should have a repeat GFR and serum magnesium at one year and 5 years off treatment. It is known that children receiving platinum based compounds, the GFR does not decrease with time as it does after ifosfamide. However, tubular toxicity may persist or appear years after treatment

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

Evaluations **Study steps** **Tests**	**E1** **Study entry**	**E2** **Prior to intensified chemotherapy**	**E3** **Prior to BuMel**	**E4** **Post-BuMel, prior to local treatment**	**E5** **End of local treatment = end of study treatment**
^{123}I-mIBG scintigraphy	❒	❒		❒	❒
Primary tumour imaging (MRI or CT)[a]	❒	❒		❒	❒
Primary tumour imaging (echography) [a]			❒		
Cerebral imaging (MRI or CT) [a]	❒				
Blood MRD testing [b]	❒	❒		❒	❒
BM MRD testing [b]	❒	❒			❒
BM (trephine biopsy) [c]	❒	❒			❒
BM (aspirates) [d]	❒	❒			❒
Urinary catecholamine metabolites	❒	❒	❒	❒	❒

a: imaging of the primary tumour and cerebral imaging: by MRI or CT scan as judged appropriate by the investigator. Ultrasound/echography imaging of the primary tumour is mandatory before BuMel

b: the minimal residual disease (MRD) testing is performed using two types of methods: a quantitative reverse-transcriptase polymerase chain reaction (QRT-PCR), and by immunocytology (IC). For the disease evaluation, blood and bone marrow are tested via both methods to detect any signs of minimal residual disease.

c: BM trephine: the pathologist performs a histological examination and provides the clinician with the information. Ten unstained sections are needed for central review by SIOPEN members (see Appendix 5).

d: BM aspirates: to be shared between slides for staining, BM cytospin, and QRT-PCR (see Appendix 5)

3 ABBREVIATIONS

AE	adverse event
AIPF	automatic immuno-fluorescence plus FISH
ANC	absolute neutrophil count
ARDS	acute respiratory distress syndrome
ASCR	Autologous Stem Cell Reinfusion
ASCT	Autologous stem cell transplant
BM	bone marrow
BuMel	Busulfan and Melphalan high dose chemotherapy regimen (myeloablative chemotherapy)
CCRI	Children's Cancer Research Institute (Vienna, Austria)
CCSG	Children's Cancer Study Group
CEM	carboplatin, etoposide and melphalan myeloablative chemotherapy regimen
CI	confidence interval
CR	Complete response
CRF	Case Report Form
CRP	C-reactive protein
CT	computer 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
EFS	Event-free survival
FISH	Fluorescence in situ hybridisation
G-CSF	granulocyte stimulating growth factor
GFR	glomerular filtration rate
GI	gastro-intestinal tract
GTV	gross tumour volume
Gy	Gray
HaChA	Human anti-chimeric Antibody
HDC	High-Dose Chemotherapy
HR-NBL-1.7	High-risk neuroblastoma 1.7 trial – a SIOPEN study
HVA	Homovanillic acid
^{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
LDH	lactate dehydrogenase
LI	local irradiation
LTI	long-term continuous infusion
MAT	myeloablative therapy

mIBG	meta-iodobenzylguanidine
MLC	multi-leaf collimator
MLPA	multiplex ligation-dependent probe amplification
MNA	MYCN amplified
MNC	mononuclear cell
MR	mixed response
MRD	Minimal Residual Disease
MRI	magnetic resonance imaging
MRI/CT	magnetic resonance imaging or computed tomography
MTD	maximum tolerated dose
NBL	neuroblastoma
NCA	nurse controlled analgesia
OS	Overall survival
PBSC	Peripheral blood stem cell
PBSCH	peripheral blood stem cell harvest
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
prn	pro re nata; means “as needed” in Latin language
PTV	planning target volume
QRT-PCR	quantitative reverse transcriptase polymerase chain reaction
RA	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
SUSAR	Suspected Unexpected Serious Adverse Reaction
SMZ	sulfamethoxazol
SPECT	single-photon emission computed tomography
TBI	total body irradiation
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

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5 BACKGROUND AND RATIONALE

5.1 Background on very high risk neuroblastoma

High-risk neuroblastoma remains one of the major challenges in paediatric oncology. Despite the introduction of high-dose chemotherapy with haemopoietic support, the outcome of these patients remains poor. This is particularly so for patients who respond poorly to initial chemotherapy.

Induction chemotherapy in the current SIOPEN high-risk clinical trial (HR-NBL-1.7 / NCT01704716) is randomised between Rapid COJEC and modified N7. If metastatic CR is achieved, patients proceed directly to BuMel peripheral blood stem cells transplantation (PBCST). Patients who have not achieved metastatic CR are further treated with two cycles of TVD.

- If there is an adequate response (negative bone marrow evaluation, ≤ 3 mIBG spots) after the two cycles of TVD, the patients proceed to BuMel PBSCR.
- the remaining patients, approximately one third, are considered to have an inadequate response to induction therapy and come off the high-risk trial.

These latter patients have primary refractory or poorly responding disease and need some sort of effective salvage treatment if they are to be cured. At the present time there is no salvage therapy generally recognised as sufficiently effective for this patient group, and the prognosis remains very poor. For these patients with primary refractory or poorly responding disease, there are no current guidelines for treatment. Audits of care have shown a range of different treatment approaches. Separate national or institutional groups have piloted alternative treatment strategies, some of which appear to be promising, and a formal comparison of these is appropriate.

5.2 Trial Rationale

5.2.1 Rationale for study design

High-risk metastatic neuroblastoma is not cured by a single treatment. All patients who have become long-term survivors have received sequential treatments with various drugs.

For this reason, this trial does not compare two single treatments, but compares two sequential treatment strategies. In these two strategies, most of the components are evidence-based best practice, although the level of evidence supporting each component varies. There is one experimental component in each strategy. Indeed, none of these two treatment schedules can be considered as standard therapy, and none has been previously compared with any standard therapy in a randomised trial.

Although it might be considered that this trial should have a standard therapy arm as a comparator, analysis of patients treated in the SIOPEN HR NBL trial 1 who have failed to meet the R1 criteria has shown a wide heterogeneity of treatments. Therefore, there is no recognised or accepted standard treatment in this very high-risk patient group, and no guidelines exist for poor responders. Survival in this very high-risk group is currently very poor. Considering all these points, it is considered ethical to compare two experimental schedules without a standard comparator.

This trial compares two such strategies in a randomised way. Patients are eligible for entry into the trial if they fail to have an adequate response to induction and therefore cannot proceed directly within the high-risk study to BuMel PBSCR. Eligible patients will be randomised at that time point, even though further standard treatment will be administered before the randomised element, and there may be circumstances when an individual patient although randomised to a particular strategy, is unable to receive the randomised element of treatment. For example, if it proves impossible to perform an adequate PBSC harvest. All randomised patients will be analysed on an intention to treat basis.

Following randomisation, all patients will continue with standard dose chemotherapy with irinotecan and temozolomide for three courses to allow for PBSC harvest (it is not mandatory to have clear bone

marrows before attempting a harvest) and to facilitate scheduling of the randomised element of the study which may necessitate referral to another centre.

The patients will then receive one of two investigational intensification therapies according to random allocation:

- high administered activity ^{131}I-mIBG and topotecan and ASCR.
- high-dose thiotepa and ASCR

Then all patients will proceed to second high-dose chemotherapy: BuMel and ASCR.

The intensified consolidation chemotherapy will be followed by external radiotherapy as appropriate, by local surgery of the tumour residues as appropriate.

Common to both strategies are:

(a) systemic treatments, prior to and after the experimental intensification chemotherapy, and

(b) local treatments.

The systemic treatments are, successively:

(1) Additional 'conventional' chemotherapy with temozolomide and irinotecan schedule, as this combination showed evidence of activity.

(2) high-dose chemotherapy with busulfan and melphalan with ASCR, for which there is level 1 evidence of benefit.

The local treatments will be interpolated at appropriate time points. They include:

(1) surgery, in an attempt to remove the primary tumour, and

(2) external beam radiotherapy, targeting the primary tumour and any mIBG-positive residual metastatic sites.

The evidence for their benefit is less strong and more circumstantial, but they are regarded as part of current standard practice.

5.2.2 Rationale for the whole intensified consolidation strategy (including the investigational intensification strategies and BuMel)

5.2.2.1 Rationale for intensified consolidation with BuMel chemotherapy

High-dose, myeloablative, chemotherapy has been recognised for years to improve the outcome of high-risk neuroblastoma patients(2), and more recently the combination of Busulfan and Melphalan has been demonstrated to be superior to another commonly used schedule (3).

However patients with refractory disease, that is less than a partial remission after two lines of conventional chemotherapy, carry a very high risk of treatment failure even after myeloablative therapy. The mIBG score enabled to identify very high-risk neuroblastoma (VHR-NBL) patients who have a poor long-term survival (4-6). An intensified high dose chemotherapy strategy with two high dose procedures, each of them supported by a PBCST, may further improve their survival prognosis.

Therefore, in the VERITAS protocol, for the second sequence of both arms of the intensified consolidation chemotherapy, the combination of busulfan and melphalan will be administered, immediately followed by a ASCR. The BuMel therapy includes 16 i.v. infusions of busulfan, delivered every 6 hours from day 1 to day 5 (dose calculated according to patient's weight), and melphalan at 140 mg/m² at day 7. This sequence is preceded by a limited evaluation (primary tumour, urinary catecholamine metabolites, and ^{123}I-mIBG scintigraphy), and followed by an extensive disease evaluation, including a bone marrow evaluation, before proceeding to the local treatment.

Of note, the intensified consolidation therapy is administered in highly experienced centres (centres validated by the coordinating sponsor), while the induction therapy and the local treatment are administered at the enrolling centre.

5.2.2.2 Rationale for experimental strategy of arm A: ^{131}I-mIBG and Topotecan in neuroblastoma, followed by BuMel chemotherapy

Most neuroblastoma tumours express the noradrenalin transporter molecule and take up metaiodobenzylguanidine (mIBG). mIBG can be radiolabelled with either ^{123}I for imaging, or ^{131}I for therapy (7). mIBG labelled with iodine-131 has demonstrated activity for targeted therapy of neuroblastoma in both relapsed and newly diagnosed patients; while the reported response rates vary widely, the median response rate in these studies is about 30% (8-12). Previous studies have proven the safety of incorporating ^{131}ImIBG therapy into myeloablative chemotherapy regimens. Gaze *et al* demonstrated that the use of mIBG radiotherapy combined with high-dose chemotherapy and total body irradiation was feasible and well tolerated (13). In a pilot study, side-effects of ^{131}I-mIBG treatment associated with high dose chemotherapy and immunotherapy also proved to be tolerable in 11 children (14). French, Matthay *et al* showed that the addition of ^{131}I-mIBG to a myeloablative CEM regimen did not significantly contribute to the toxicity profile of the CEM (15). Toxicity was similar to that seen with the CEM regimen alone, including myelosuppression, mucositis, infection, and sinusoidal obstruction syndrome. More recently, Matthay *et al* demonstrated that the combination of high dose ^{131}I-mIBG followed 6–8 weeks later by myeloablative consolidation with busulfan and melphalan with ASCT is a feasible regimen for patients with refractory neuroblastoma (16). In addition, in this study, the improvement in disease response after the mIBG therapy suggests that this strategy may improve outcomes for patients with refractory disease, since overall response prior to myeloablative consolidation has a significant impact on outcome.

Recent efforts have focused on combining ^{131}I-mIBG with agents that may augment its activity. Camptothecins have been shown to sensitize tumour cells, including neuroblastoma, to the effects of radiation (17, 18). Topotecan is a topoisomerase I inhibitor which has activity against neuroblastoma, and it is a radiosensitiser. There is evidence from laboratory studies that the combination of ^{131}I-mIBG and topotecan is synergistic when topotecan is given simultaneously or secondarily to ^{131}I-mIBG (18). However, this synergy has not yet been proven clinically.

The combination of high-administered activity Iodine-131 meta-iodobenzylguanidine (^{131}I-mIBG) with topotecan, secondarily supported by peripheral blood stem cell transplantation, has been demonstrated to be a feasible treatment in both patients with relapsed, heavily pre-treated neuroblastoma, and also in patients with primary refractory high-risk disease (19).

The use of this treatment in patients with high-risk neuroblastoma over one year of age who failed to achieve an adequate response to induction chemotherapy on the SIOPEN high-risk neuroblastoma protocol has been shown to produce a worthwhile response rate (19, 20). This schedule delivers the targeted 4 Gy whole-body radiation absorbed dose within two administrations of ^{131}I-mIBG given two weeks apart. Using two delivery shots limits the unpredictability of whole body dose seen after a single administration, and thereby standardised the dose-related toxicity. Patients are then eligible for further potentially curative treatment including busulfan and melphalan high dose chemotherapy (3, 21).

In 61% of refractory patients (n= 46 patients),further potentially curative treatment including MAT was delivered (20). For patients who received BuMel after MATIN, the EFS and OS were 0.25 ($\pm$0.07) and 0.37 ($\pm$0.09), respectively. The outcome in this cohort is sufficiently encouraging to push the SIOPEN to complement the investigational intensification therapy by a BuMel treatment, in order to target a sufficiently intensified consolidation therapy for these very high risk patients.

Furthermore, data analysis of a retrospective cohort of seven patients with refractory metastatic NBL has revealed that the toxicity of BuMel administrated with a median interval of 11 weeks after MITOP therapy was acceptable (22).

Therefore, in the VERITAS protocol, the first treatment sequence of the arm A of the intensified consolidation chemotherapy will comprise two cycles of the combination ^{131}I-mIBG-Topotecan, overall administered within 19 days, and followed by a ASCR as soon as the whole body remaining dosimetry permits it, usually around day 25-29. The overall irradiation dose by ^{131}I-mIBG is 4 Gy, and the dose of topotecan is 0.7 mg/m²/day for 5 days per cycle. This sequence is preceded by an extensive disease evaluation (primary tumour, urinary catecholamine metabolites, ^{123}I-mIBG scintigraphy, and bone marrow evaluation), and followed by a more limited evaluation (without bone marrow evaluation), before proceeding to the BuMel sequence of the intensified consolidation therapy.

5.2.2.3 Rationale for the experimental strategy of arm B : high-dose Thiotepa, followed by BuMel chemotherapy

A double high dose strategy has been tested in a pilot study among VHR-NBL patients (23).

The treatment consists in one cycle of Thiotepa (900mg/m²) followed by a combination of oral Busulfan (600mg/m2) or IV Busilvex (doses adapted to the weight) and Melphalan. Each HDC was followed by ASCT performed 24 hours after last chemotherapy administration. Planned interval between the 2 ASCT was 2 months if no major toxicity or disease progression occurred.

From 2004 to 2011, 24 of the 26 VHR-NBL diagnosed patients were able to complete the treatment, two patients having progress before BuMel. The most important grade 3 non hematologic toxicity was mucositis and liver toxicity. Grade ≥ 3 veno-occlusive diseases (VOD) were reported in 6 cases (25%). However, most patients were managed with supportive care therapy without any particular complication. The median follow-up was of 3.1 years and the 3 year-EFS and OS rates after diagnosis were 36% and 68%, respectively (24). This pilot study showed that the toxicity of this HDC strategy was acceptable and that the survival of this cohort was encouraging.

Therefore, in the VERITAS protocol, the first treatment sequence of the arm B of the intensified consolidation chemotherapy will comprise one cycle of Thiotepa delivered at high dose, and immediately followed by a PBSCR. The dose of thiotepa is 300 mg/m²/day for 3 days (D1-D3), i.e.,,900mg/m² overall. Similarly to the arm A, this sequence is preceded by an extensive disease evaluation (primary tumour, urinary catecholamine metabolites, ^{123}I-mIBG scintigraphy, and bone marrow evaluation), and followed by a more limited evaluation (without bone marrow evaluation), before proceeding to the BuMel sequence of the intensified consolidation therapy.

5.2.3 Rationale for the treatment administered before and after the intensified consolidation

5.2.3.1 Rationale for the Temozolomide - Irinotecan treatment

The irinotecan-temozolomide combination is widely considered a “standard” treatment for relapsed or refractory neuroblastoma.

When temozolomide is combined with Irinotecan for patients heavily treated such as the high-risk neuroblastoma patients, the tolerated dose of temozolomide is 100mg/m²/day, per os, for 5 days every 3 weeks.

In a previous study in children with relapsed or refractory neuroblastoma (25), irinotecan was used at low and protracted doses : 10 mg/m²/day x 5 days (D1-D5), two weeks out of three. The first week, temozolomide was administered before the irinotecan infusion (i.e., at the dose of 100 mg/m²/day x 5 days every three weeks). However, a trial conducted in children with rhabdomyosarcoma showed that a regimen with irinotecan 50 mg/m²/day x 5 days (D1-D5) every 3 weeks was shorter and more convenient for the patients and their families, with a similar efficacy and tolerance (26).

Therefore, the first sequence of treatment of this trial will combine temozolomide per os 100mg/m²/day, one hour prior the infusion of irinotecan 50 mg/m²/day, for 5 days (D1-D5) per cycle. Three cycles will be repeated every 3 weeks. This induction chemotherapy within VERITAS will be followed by an extensive disease evaluation (primary tumour, urinary catecholamine metabolites, ^{123}I-mIBG scintigraphy, and bone marrow evaluation), before proceeding to the intensified consolidation therapy.

Of note, the induction therapy is administered at the enrolling centre, while the intensified consolidation therapy is administered in highly experienced centres (centres validated by the coordinating sponsor) where the patient must be therefore transferred. This induction chemotherapy will allow administering chemotherapy with some evidence of efficacy while setting up the upcoming intensified consolidation chemotherapy in the highly experienced centres (centres validated by the coordinating sponsor.

5.2.3.2 Rationale for the local treatments (radiotherapy and surgery)

The local treatments are managed at the enrolling centre, and comprise:

(1) surgery to remove the primary tumour, and

(2) external beam radiotherapy, targeting the primary tumour and the remaining mIBG-positive metastatic sites.

Surgery is part of the treatment for these high risk tumours.

With regards to surgery of the primary tumour site, there is some evidence that complete surgical excision does benefit the patient although it has been difficult to consider this variable separately because of the continuing development and assessment of chemotherapy variables (27-31). However, a recent analysis of a group of neuroblastoma patients by the Children's Cancer Group found an association between incomplete resection of the primary tumour and relapse in that site (32).

Therefore, surgery is scheduled in this trial after the induction and the intensified consolidation chemotherapy, which aim to achieve the maximum response at the primary site and to clear as much as possible the metastatic disease. The aim of the surgery is to achieve complete excision of the tumour with minimal morbidity to improve local control. In order to minimise the inherent variability of surgery there must be a commitment by those participating in the study to attempt complete excision. If a centre does not feel able to give this commitment then the patient should be transferred for surgical treatment to one which does.

According to data of the Children Cancer Group, consistent local irradiation may improve the local control of the tumour (33). A careful planning of the radiotherapy fields and dose is needed with consideration given to response, local status after surgery to the primary tumour and neighbouring organs. Metastatic sites should not be systematically irradiated. Some patients may be considered unsuitable for radiotherapy by reason of the site of primary tumour and the volume which would require irradiation. The enrolling centre may consider referring the patient to a centre with more extensive experience of such radiotherapy.

Doses will be specified according to ICRU recommendations. The dose should be treated to 21 Gy in 14 fractions of 1.5 Gy over not more than 21 days, with high energy photons from a linear accelerator.

The time interval between the post-BuMel ASCT and the radiotherapy must be greater than 60 days, due to the risk of busulfan-enhanced radiotoxicity. A negative interaction between radiotherapy and isotretinoin (13-cis-RA) has been described; therefore the start of the systemic maintenance therapy with isotretinoin should respect a free interval of at least 10 days.

An extensive evaluation should be performed after the local treatment.

5.2.4 Justification for the patient population. Rationale for age treatment cohort

This clinical trial will be conducted in children and adolescents with a metastatic high-risk neuroblastoma, who are enrolled in the ongoing HR-NBL SIOPEN trial but poorly responding to the induction chemotherapy. The age criterion of HR NBL1 is 1 to 21 years at diagnosis (90% of the patients are less than 5 years at diagnosis).

5.2.5 Rationale for the stratification applied at randomisation

Several factors can play a confounding role in the patient outcome, which could be extrinsic to the patient, such as the country of the enrolling centre, or intrinsic to the patient and the disease, such as the age at the neuroblastoma diagnosis, the extension of the neuroblastoma to the skeleton or to the bone marrow, or the presence of an amplified oncogene (NMYC amplified tumour).

5.2.5.1 Stratification based on the country of the enrolling site

According to the country, differences can occur in the patient management, e.g., in terms of supportive care and medical environment.

Therefore, the analysis of the VERITAS trial results will be stratified according to the country of diagnosis and primary care of the patient.

5.2.5.2 Stratification based on the age of the patient at the neuroblastoma diagnosis

Age is a major prognosis factor for high-risk neuroblastoma patients even treated with a protocol designed for high-risk patients. In the HR-NBL-1.7 trial, the first results showed that the 5 year EFS was 0.46 +/- 0.05 for the patients below 1 year, versus 0.37 +/- 0.05 for those between 1 and 1.5 year, 0.28+/- 0.02 for those between 1.5 and 5 years, and 0.14+/- 0.02 in patients above 5 years. Moreover, patients > 5 years who could not reach a metastatic complete response at the end of induction conventional chemotherapy could not be cured.

Therefore, the analysis of the VERITAS trial results will be stratified according to the age at neuroblastoma diagnosis, below 5 years old versus 5 years and above.

5.2.5.3 Stratification based on the MYCN amplification

Among patients treated within the HR-NBL-1 trial, the analysis of the survival curves demonstrated the MYCN status, amplified or not, had no influence of the 5-year OS. However, when tumoral cells exhibit and amplified oncogene MYCN, the relapses occur earlier and the time interval between the relapse and the death is shorter. Stratifying the analysis on this factor will enable to further evaluate its influence on the EFS and on the OS in VHR-NBL.

Therefore, the analysis of the VERITAS trial results will be stratified according to the MYCN status, amplified or not.

5.2.5.4 Stratification based on the involvement, or not, of bone or bone marrow

The involvement of the bone marrow and the detection of bone metastases at diagnosis are recognized risk factors of poor survival. The better prognosis associated to extraskeletal metastases alone was suspected during CCG studies (38, 39), and was confirmed in a French study conducted among grafted stage IV neuroblastoma (2). In this latter study, the involvement of the bone marrow was also confirmed as a poor prognosis factor.

Therefore, the analysis of the VERITAS trial results will be stratified according to the involvement of the bone marrow and to the presence of skeletal metastasis or not.

6 AIMS, OBJECTIVES AND OUTCOME MEASURES

6.1 Objectives

The trial aims to evaluate the efficacy of two intensified consolidation chemotherapy strategies randomly allocated among patients with very high risk neuroblastoma. These patients are recruited among the patients who were initially enrolled and treated in the ongoing HR-NBL SIOPEN trial or treated with the current standard treatment for very high risk neuroblastoma off -trial but who had an inadequate response to the induction chemotherapy.

The two strategies for the intensified consolidation chemotherapy are:

- high administered activity ^{131}I-mIBG and topotecan,

or

- high dose thiotepa.

The intensification chemotherapy, regardless of the allocated strategy, will be preceded by a standard chemotherapy with temozolomide and irinotecan for 3 cycles.

The intensification chemotherapy will be further supported by a peripheral blood stem cell rescue, and then followed by busulfan and melphalan therapy, also supported by a peripheral blood stem cell rescue. After the full sequence of intensified consolidation chemotherapy, patients will receive standard local treatments (surgery and radiotherapy).

Primary objective:

The primary objective is to evaluate the efficacy of two intensified consolidation strategies in VHR-NBL patients in terms of event-free survival.
This evaluation will follow a hierarchical testing procedure:

- each experimental treatment will be first evaluated as a single-arm phase 2 study,
- in case of positive conclusion, the relative efficacy of both arms will then be evaluated comparatively.

Secondary objectives:

a - To estimate and compare the overall survival (OS) of patients treated in the two treatment strategies

b - To evaluate and compare the safety of the two treatment strategies in terms of toxic death and non-fatal toxicities rates.

c - To estimate and compare the disease response after BuMel and at the end of treatment of the two treatment strategies

d - To evaluate the between-treatment differences in Quality adjusted Time WIthout Symptoms and Toxicity (Q-TWIST approach)

e - To evaluate the feasibility and document the logistical issues raised by 131I-mIBG and topotecan therapy in a multicenter setting

f - To estimate and compare the Event-Free Survival of the two treatment strategies from the start of the intensified consolidation chemotherapy

g - To estimate and compare the Event-Free Survival of the two treatment strategies from the date of the neuroblastoma diagnosis

6.2 Outcome Measures

6.2.1 Primary endpoint:

The primary endpoint is the 3 years Event-Free Survival from the date of randomisation into the VERITAS trial, considering as events:

- disease progression or relapse
- death from any cause
- secondary malignancy.

Patients without event are censored at the date of their last follow up evaluation.

6.2.2 Secondary endpoints:

a - Overall survival, defined as the time from randomisation to death from any cause.

b - Adverse events, evaluated using NCI-CTCAE v5.0 toxicity grading system, reported by treatment phase and overall over the whole treatment duration (maximum grade). The stopping rule for toxicity will be based on the occurrence of adverse events leading to ventilation in an ICU and treatment-related deaths. These events will be specifically monitored over the first 6 months after randomisation.

c - Disease response after BuMel and at the end of treatment

d - For the Q-TWiST analysis: time spent with severe toxicity after randomisation and before progression/relapse (duration of hospitalisation will be used as a surrogate of time with toxicity); time spent without progression/relapse and without toxicity; and time from progression until death.

e - Logistical issues raised by ^{131}I-mIBG and topotecan or Thiotepa therapy in a multicenter setting

f - Event-Free Survival from the date of start of the consolidation phase

g - Event-Free Survival from the date of the neuroblastoma diagnosis

7 ELIGIBILITY

During the HR-NBL/SIOPEN trial, if the reassessment after the induction chemotherapy does not permit to proceed to BuMel high dose chemotherapy, the entry into the VERITAS trial will be proposed to these patients, if at all possible.

The patient will be randomised to one of the intensification consolidation treatment, and will proceed to a third line chemotherapy with temozolomide and irinotecan within six weeks of the randomisation.

To be eligible for randomisation, the patients must fulfil the list of eligibility criteria below (inclusion and non-inclusion criteria). These criteria are interpreted literally and cannot be waived. All clinical and laboratory data used for determining the patient eligibility must be available in the patient's medical/research record to serve as source document verification in case of an audit.

7.1 Inclusion criteria

1. Metastatic neuroblastoma (NBL)
2. Previously treated within the ongoing High Risk Neuroblastoma SIOPEN study or treated with the current standard treatment for very high risk neuroblastoma off-trial
3. ^{131}I-mIBG scintigraphy positive at diagnosis and after induction chemotherapy (pre BuMel evaluation).
4. Metastatic response after induction chemotherapy lower to the ongoing High Risk Neuroblastoma SIOPEN trial criteria to be eligible for High Dose Chemotherapy (metastatic response worse than partial response (< PR) or SIOPEN score > 3)
5. Females of childbearing potential must have a negative serum 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 are listed in Appendix 12. Female patients who are lactating must agree to stop breast-feeding.
6. Written informed consent from parents/legal representative, patient, and age-appropriate assent before any study-specific screening procedures are conducted according to local, regional or national guidelines.
7. Patient affiliated to a social security regimen or beneficiary of the same according to local requirements.

7.2 Non-inclusion criteria

1. Parenchymal brain metastasis(es) (even one)
2. Progressive disease at study entry
3. Previous high-dose therapy and ASCR
4. Performance status (Karnofsky or Lansky) <70%
5. Patient having received other therapy for cancer treatment than those allowed as per the ongoing High Risk Neuroblastoma SIOPEN trial or as defined in the future frontlines protocol (for HRNBL1 trial : after induction + 2 TVD)
6. Impaired organ function (liver, kidney, heart, lungs) according to the following definitions
    - o Shortening fraction <28%, or ejection fraction <55%, or clinical evidence of congestive heart failure or uncontrolled cardiac rhythm disturbance
    - o Dyspnea at rest and/or pulse oximetry <95% in air.
    - o ALT, Bilirubin > 2 ULN
    - o Creatinine clearance and/or GFR < 60 ml/min/1.73m^2 and serum creatinine ≥ 1.5 mg/dl
7. Any uncontrolled inter-current illness or infection that in the investigator's opinion would impair study participation
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. Known hypersensitivity to dacarbazine
14. Concomitant use with St John's Wort

8 SCREENING, CONSENT AND RANDOMISATION PROCEDURE

8.1 . Screening – pre-treatment procedures

No trial specific procedure should be carried out prior to signing the consent form for this study.

Once the appropriate informed consent forms are signed, the patient must undergo the following assessments:

- Disease evaluation **E1** (must be completed within 4 weeks prior to starting trial treatment):
  - Primary tumour imaging (appropriate imaging according to RECIST): MRI/CT scan,
  - ^{123}I-mIBG scan[1],
  - bone marrow evaluation (aspirates from four sites, and trephine biopsies from two sites)
  - urinary catecholamine metabolites (dopamine, VMA and HVA).
  - MRD testing by AIPF/immunocytology and QRT-PCR on blood and bone marrow (aspirate samples)
  - Brain MRI or CT scan (in order to detect possible brain metastasis)
- Complete medical history within 1 week prior to starting trial treatment.
- Full clinical examination (including vital signs, weight and height) within 1 week prior to starting trial treatment.
- Doppler Echocardiogram (ECHO) within 4 weeks prior to starting trial treatment.
- Full blood count (includes Haemoglobin (Hb), platelets and white blood cells (WBC) with differential count (neutrophils, lymphocytes, eosinophils, basophils) within 72 hours prior to starting trial treatment.
- Renal function: creatinine clearance (calculated based on blood and urinary level of creatinine)
- Blood biochemistry must be evaluated within 72 hours prior to starting trial treatment, and should include :
  - sodium, potassium, chloride,
  - ALT (SGPT), AST (SGOT), GGT, bilirubin,
  - CRP.
- Performance status
- A serum pregnancy test will be done on females of child bearing potential within the week prior to starting trial treatment. Results of the tests are needed to determine patient eligibility and the result from the pregnancy test must be obtained before starting trial treatment
- Hepatitis B Virus testing
- HIV testing

These procedures are required to be eligible in the VERITAS trial. If some of these procedure have already been carried out in conformance with the prespecified timepoints (as described above), there is no need to repeat the procedure.

8.2 Informed Consent

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 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.

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 Investigator should also stress that

[1] **A ^{123}I mIBG is standard**. A diagnostic ^{131}I mIBG will be accepted only if ^{123}I mIBG cannot be performed. ^{131}I mIBG therapeutic scans will **not** be accepted, because these types of scan cannot be baseline scans for response.

the patient parents or legal guardian are completely free to refuse to take part or withdraw from the trial at any time. The parent/approved guardian and /or patient should be given ample time to read the Information Sheet and to discuss their participation with others outside of the site research team. They must be given an opportunity to ask questions which should be answered to their satisfaction. The right of the parent/approved guardian and/or patient to refuse to participate in the trial without giving a reason must be respected.

If the parent/approved guardian and/or patient expresses an interest in the patient participating in the trial they should be asked to sign and date the latest approved version of the Informed Consent Form. The Investigator, or designate, must then sign and date the form on the same day as the patient/parent/legal guardian. A copy of the Informed Consent Form should be given to the parent/approved guardian and/or patient, a copy should be filed in the Investigator Site File (ISF) and the original placed in the hospital records.

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. 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 in accordance with national guidance/legislation.

If the patient/parent/legal guardian wishes to donate the residual biological samples collected as part of this study for future research, they need to complete the tick box on the consent form to indicate this.

8.3 Randomisation procedure

Patients already registered in the on-going High Risk Neuroblastoma SIOPEN study will be included in the randomised trial once the informed consent is signed and all eligibility criteria are checked.

A centralised randomisation procedure will be used to define treatment allocation A minimisation program will be used to balance treatment arms 1:1 according to the stratification factors listed below:

- country
- MYCN-amplification
- age at NBL diagnosis (below 5 years old, or 5 years and above)
- metastatic sites (with or without bone/ bone marrow involvement)

9 TREATMENT DETAILS

The successive sequences of treatment of the protocol are described on the overall flowchart of the study below.

With regards to the treatments, each sequence of treatment is detailed in the subsections of the section 9.1, and the dose modifications are detailed in section 9.3. All supportive care treatments are detailed in section 9.4.

The disease assessments and safety assessments to perform are detailed in section 9.2.

9.1 Trial Treatments

9.1.1 Temozolomide – Irinotecan

The first sequence of therapy within the VERITAS trial is induction chemotherapy specific to the study, and comprising three cycles. This sequence enables to set up the intensified consolidation chemotherapy and solve all logistical issues, while maintaining an antiproliferative pressure against the disease.

The VERITAS study-specific induction therapy combines temozolomide and irinotecan.

Temozolomide is administered orally, at the dose of 100 mg/m², at least one hour before the irinotecan infusion. Of note, preclinical data suggested schedule-dependant effects, so the compliance to this administration scheme is important.

Irinotecan is administered through infusion, 50 mg/m², from day 1 to day 5, with an overall dose per cycle is 250 mg/m² over 5 days.

The induction chemotherapy is described in Figure 3 and Table 2 below.

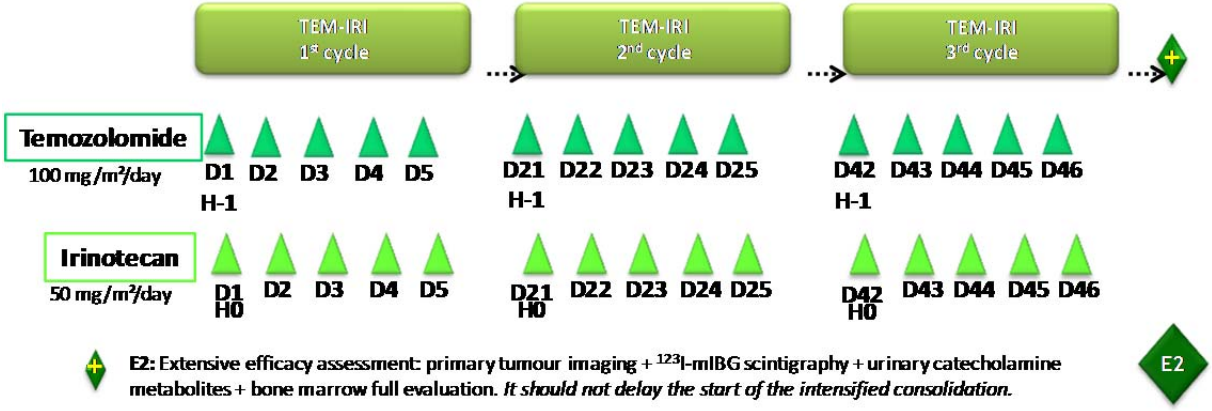


Figure 3 – Overall schedule of the Temozolomide – Irinotecan study-specific induction therapy

Table 2 - Detailed schedule of the VERITAS TEM-IRI induction therapy (3 cycles)

		1st cycle						2nd and 3rd cycles					
		1	2	3	4	5	//	21 42	22 43	23 44	24 45	25 46	//
Drug	**Dose**						//						//
Temozolomide	100 mg/m² daily - per os	▲	▲	▲	▲	▲	//	▲	▲	▲	▲	▲	//
Irinotecan	50 mg/m² daily - slow infusion	▲	▲	▲	▲	▲	//	▲	▲	▲	▲	▲	//
Nausea Prophylaxis and treatment	According to local policies												
Neutropenia prophylaxis	According to local policies												

Common side effects and recommended supportive care:

The combination temozolomide-irinotecan is well tolerated. However, the following grade 3-4 toxicities can be expected (25, 26): diarrhea (particularly late-onset diarrhea), nausea, and myelosuppression, particularly neutropenia (potentially complicated by fever and infection). Therefore, it is recommended to provide the appropriate supportive care. More specifically:

- Diarrhea should be managed by appropriately administering loperamide and by providing beverages with a high level of electrolytes. See further details in section 9.4.2.1). Loperamide should NOT be administered prophylactically.
- Nausea should be managed according to recommendations provided in section 9.4.1.1. A prophylactic treatment is recommended.

  In case of vomiting associated with severe diarrhea, the patient must be promptly hospitalised for appropriate medical management.
- Myelosuppression, including neutropenia, should be prevented and managed according to recommendations provided in section 9.4.1.2. In case of febrile neutropenia, see section 9.4.1.3, and Appendices.

9.1.1.1 Temozolomide

Preparation and administration:

Temozolomide is an oral drug, provided as hard capsules. They are available at the following dosage: 5 mg, 20 mg, 100 mg, 140 mg, 180 mg and 250 mg.

Capsules must be swallowed whole with a glass of water, without being chewed. For children who have difficulties in swallowing, temozolomide capsules should be placed in fruit juice or fruit compote and administered after the capsule has been allowed to soften.

If vomiting occurs after the dose is administered, a second dose should NOT be administered that day.

Dosage regimen:

Temozolomide is administered orally, at the dose of 100 mg/m² rounded off to the nearest ~~tablet~~ capsule size. It is administered at least one hour before the irinotecan infusion

Drug delivery:

Temozolomide is commercially available throughout the European Union. The oral formulation is provided by several manufacturers.

Further information is displayed in the protocol appendices, section 24.1.

9.1.1.2 Irinotecan

Preparation and administration (as per local practice) :

e.g : Irinotecan is formulated as a 20 mg/ml solution to be diluted for infusion. The appropriate amount of the concentrated solution must be diluted into 250 ml of normal saline solution or of glucose 5 % solution.

Drug-drug interactions or drug-herbal substance interactions:

Irinotecan is metabolised through the CYP450 3A4 pathway. Therefore, concomitant treatment with drug known to interact with the CYP450 3A4 must be avoided.

St John's wort herbal tea is prohibited during the irinotecan administration (drug-herbal substance interactions).

Dosage regimen:

Irinotecan infusion, 50 mg/m², through a daily one-hour infusion, administered from day 1 to day 5.

The overall dose per cycle is 250 mg/m² over 5 days.

Of note: in case of severe haematological toxicity, a dose reduction might be applied for the following cycles.

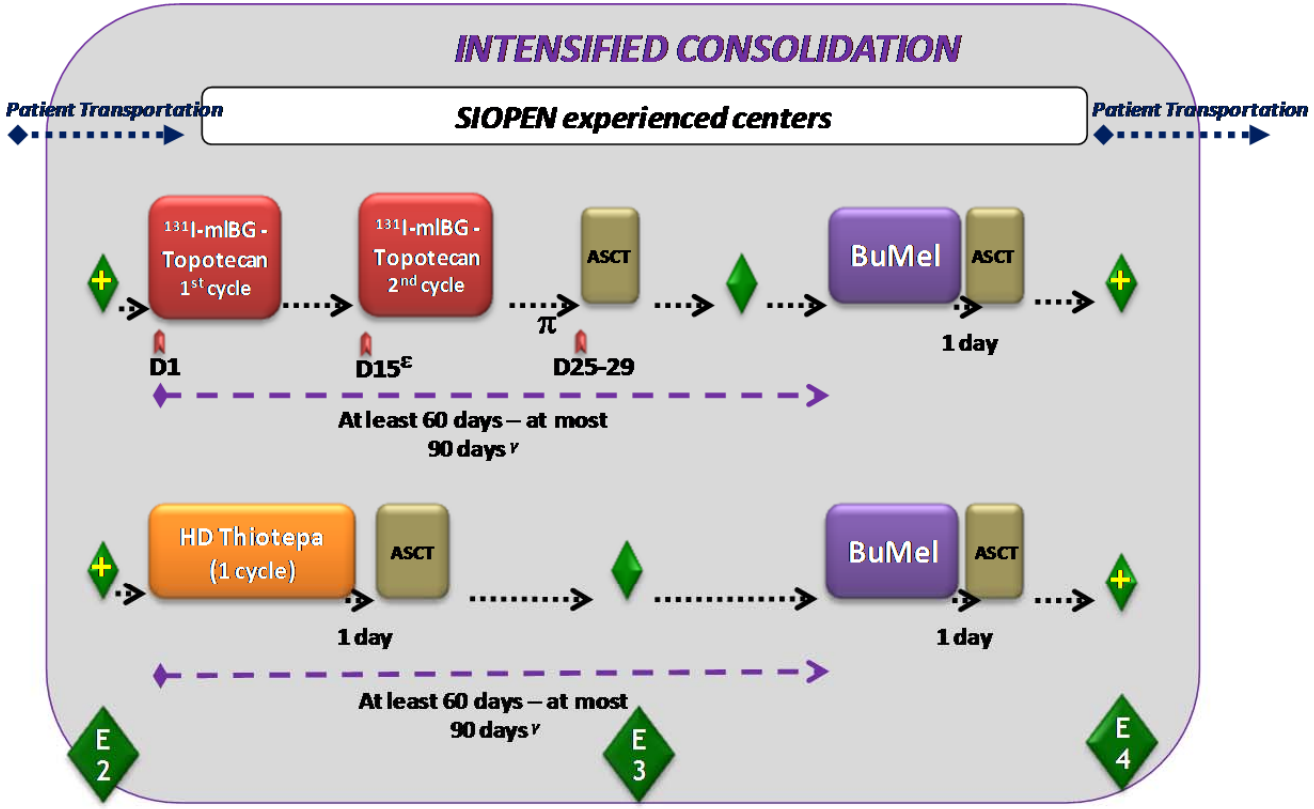
Drug delivery:

Irinotecan is commercially available. The 20 mg/ml intravenous formulation is provided by several manufacturers.

Further information is displayed in the protocol appendices, section 24.1.

9.1.2 Intensified Consolidation Therapy

Figure 4 - Overall scheme of the trial sequence of the intensified consolidation therapy



ε : the 2nd cycle of mIBG-topotecan must take place within 15 to 21 days after day 1

π : autologous stem cells infused when the whole-body activity of ^{131}I-mIBG is below 30MBq, probably by day 25-29

Efficacy assessment E3: primary Tumour imaging by echography + urinary catecholamines

More extensive efficacy assessments:

E2 includes the primary tumour imaging by MRI or CT scan +^{123}I-mIBG scintigraphy + urinary catecholamine metabolites + a full bone marrow evaluation. *It should not delay the start of the intensified consolidation.*

E4 includes primary tumour imaging (MRI or CT)+^{123}I-mIBG scintigraphy + urinary catecholamine metabolites

γ : BuMel should be started not earlier than 60 days after the first high dose therapy (MIBG or Thiotepa), and must be started by day 90 regardless of haematological recovery, unless a major organ toxicity or the disease progression leads to withdraw the patient from the trial.

A patient is eligible to start the intensified consolidation treatment if the following disease response criteria and the safety criteria are fulfilled:

- **No evidence of disease progression** at the **E2** disease evaluation
  - primary tumour imaging (MRI/CT scan)
  - ^{123}I-mIBG scintigraphy

Assessment of urinary levels of catecholamine metabolites and full bone marrow evaluation, including MRD test by QRT-PCR and IC, in bone marrow and in peripheral blood must also be performed (see Table 7 - Detailed schedule of the Disease Evaluations throughout the trial).

- **The main safety criteria** are :
  - Performance status greater or equal 70% (Karnofsky or Lansky)
  - Life expectancy of at least 12 weeks
  - Neurologic function: no significant neurologic deficit or objective peripheral neuropathy (grade ≥ 2). If seizure disorders, patients may be enrolled if well controlled with anticonvulsants
  - Cardiac function: shortening fraction ≥ 28% at echocardiogram, no signs of congestive heart failure or uncontrolled cardiac rhythm disturbance
  - 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).
  - Bone marrow: ANC > 1.10^9/L, platelet counts ≥ 50.10^9/L, and haemoglobin > 9 g/dL. Patients should not be platelets-transfusion dependent.
  - Liver function: ALT or AST < 5 x ULN and total bilirubin < 1.0 mg/dL
  - Renal function: serum creatinine < 1.5 mg/dL, or a creatinine clearance (or radioisotope GFR) > 60 ml/min.1.73m²
  - Parameters of the thyroid function : T3, T4, TSH
  - Parameters of the adrenal function : cortisol, adrenocorticotropin (ACTH)
  - HIV/Hbs Ag testing (blood)
  - At least $6x10^6$/kg CD34 cells from Peripheral Blood Stem Cells (PBSC) harvest, collected in a minimum of 4 bags
  - No active infection

9.1.2.1 High administered activity ^{131}I- mIBG and topotecan

^{131}I- mIBG and Topotecan are considered as Investigational Medicinal Products (IMPs).

There are a number of practical difficulties which may limit the ability to deliver the proposed schedule in practice. These include:

- The need to reserve a protected room for radionuclide therapy, ideally for a four-week duration, when there are limited facilities and competing clinical demands for resources.
- The need to provide paediatric nursing and medical cover for patient when confined to the radiation protected treatment room which may be located in hospitals without paediatric facilities.
- Limits imposed by the Environment Agency in the UK, or similar statutory authorities in other countries, on the amount of radioactive waste which may be discharged by hospitals into the public sewerage system.
- Sharing of care between a referring paediatric oncology centre where a patient will be assessed initially and cared for after mIBG/topotecan treatment, and the centre which administers the treatment.
- The ability to achieve an adequate yield of stem cells for the procedure to be undertaken safely.

However previous studies have shown that with careful planning, these difficulties can be overcome in some centres, and that it is feasible for patients to be referred from the original treating paediatric oncology centre to a remote centre for mIBG therapy.

The following Table 3 and Figure 5 describe the schedule of the 1st sequence of the arm A of the intensified consolidation therapy, the high administered activity ^{131}I-mIBG combined with topotecan.

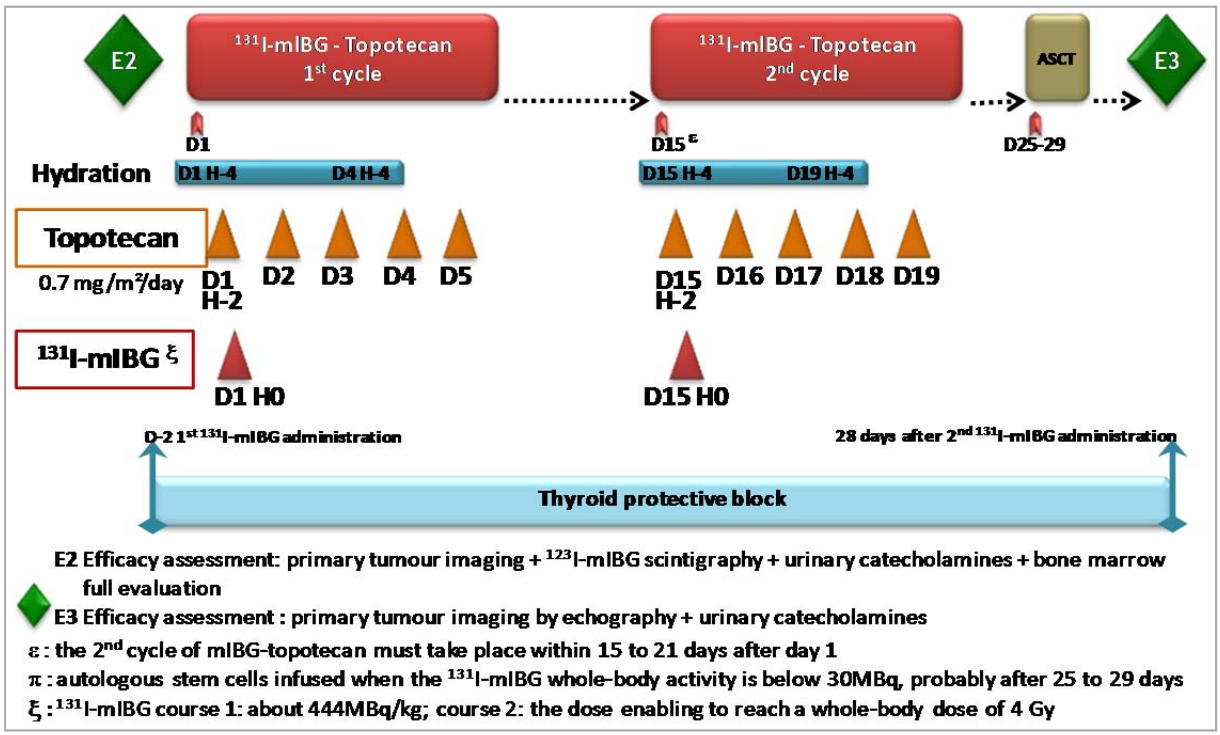


Figure 5 – Overall schedule of the 1st sequence of the arm A of the intensified consolidation therapy (^{131}I-mIBG - topotecan)

Table 3 - Detailed schedule of the 1st sequence of the arm A of the intensified consolidation therapy (^{131}I-mIBG - topotecan)

High-dose ^{131}I-mIBG-topotecan therapy day		**-2**	**1**	**2**	**3**	**4**	**5**	**//**	**15**	**16**	**17**	**18**	**19**	**//**	**25-29**	**43**
Drug	**Dose**							**//**						**//**		
^{131}I-mIBG	D1-D5: about 444MBq *μ 2nd course: up to a combined whole body irradiation dose of 4Gy		▲μ					//	▲					//		
Topotecan	0.7 mg/m² daily		▲	▲	▲	▲	▲	//	▲	▲	▲	▲	▲	//		
Peripheral Stem Cells	Minimum: 3x 10 6 /kg CD34+ i.v. §							//						//	◆	
Thyroid protective block	From day – 2 to 28 days after the 2nd mIBG infusion	⋇	⋇	⋇	⋇	⋇	⋇	⋇	⋇	⋇	⋇	⋇	⋇	⋇	⋇	⋇

*: 1st course of ^{131}I-mIBG: about 444 MBq/kg, with *in vivo* whole-body dosimetry.

μ: According to local regulations on the maximum dose, it may be capped by legal limits.

§: ASCT as soon as the radiations levels permit it, i.e., when the ^{131}I-mIBG whole-body activity is below 30MBq, probably around day 25 to 29.

9.1.2.1.1 Administration of ^{131}I-mIBG - topotecan:

The thyroid must be protected thanks to a thyroid block from two days before the first mIBG infusion up to 28 days after the 2nd mIBG infusion (see 23.2).

A general proposal is detailed below regarding the delivery of ^{131}I-mIBG. Local practice guidelines are allowed provided that they have been validated by the Coordinating Investigator before.

The start of treatment is day 1 (or day 15 for the 2nd cycle). Associated supportive care should be given in accordance with local practice guidelines.

- Prophylaxis of nausea may be started 12hours prior to the treatment start.
- After the appropriate analgesia, a urinary catheter and a peripheral line should be put in place.
- The child will be moved into the radiation-protected treatment room.
- At H-4, start a moderate prehydration (solution with 5% glucose, 3% NaCl, and 2% KCl, at the rate of 125ml/m²/hour) in order to ensure an adequate diuresis.
- Pretreatment (background) radiation dosimetry will be performed (section 21).
- After about 2 hours of prehydration, administer topotecan 0.7mg/m^2/day.

  Topotecan is reconstituted to a concentration between 25 to 50 micrograms per milliliter in normal saline solution and administered over about over 30 minutes.
- At H0, ^{131}I-mIBG 444 MBq/kg (12 mCi/kg) will be infused over 2 hours. More prolonged administrations should be avoided.

  Caution 1 : ^{131}I-mIBG should be administered using a peripheral venous line which will be removed as soon as the infusion is completed. The infusion of ^{131}I-mIBG through the central venous line is associated to a significant risk of having radiolabelled compound retained on the catheter.

  Caution 2: ^{131}I-mIBG must be prepared in a radio-protected area, according to the ongoing Good Practices of Preparation of Radiopharmaceutical compounds.

  Caution 3: Prior to^{131}I-mIBG administration, ensure emergency cardiac antihypertensive treatments are readily available. Ensure constant monitoring of the patient during administration.
- Before emptying the urine bag, the first dosimetric measurement must be performed. The urine bag should be placed close to the body for measurement.
- Then the urine catheter bag will be emptied, and another dosimetric measurement will be performed.

  In France, the urine catheter bag must be emptied every 3 hours (required as per regulation).
- Post-hydration should continue for 24 hours at the same rate (i.e., overall, the hydration is maintained for 72h).
- Dosimetry will be performed approximately every two hours over the first twenty four hours with the exact time accurately recorded. Subsequent dosimetric measurements should be performed every four to six hours. The urine catheter bag should be emptied, immediately prior to each measurement.

  Note: if the whole body irradiation dose reaches 4 Gy with the 1st cycle, the 2nd cycle is cancelled
- Topotecan is administered daily, at day 2 to 5, similarly to day 1
- Hydration can be discontinued after 24 hours (after an overall duration of 72h).

The dosimetric measurements will be used to calculate the whole body radiation absorbed dose from the first ^{131}I-mIBG administration (see section 22.1). The activity of ^{131}I-mIBG required for the second administration in order to give a total whole body absorbed dose of 4 Gy can then be calculated, and ordered in readiness.

If well, the child can be discharged home after the topotecan on day 5 subject to the residual level of radioactivity in accordance with local rules and under the supervision of the designated radiation protection adviser (see section 233). The full blood count should be checked every two days during the first week, prior to discharge and on day 7.

Tumour dosimetry should be performed wherever possible as described in Section 9.1.2.1.5.

Supportive care and concomitant treatments recommendations:

Appropriate supportive care, including packed red cells or platelets transfusions, and treatment of febrile neutropenia, should be carried out according to local policy.

Most concomitant treatments must be discontinued during the trial (see 9.5). Moreover, some drugs must be specifically interrupted during at least 4 half-lives prior to start the ^{131}I-mIBG – topotecan arm.

The thyroid block must be maintained from day-2 until 28 days after the second mIBG infusion (see 23.2).

2nd cycle:

The patient will be readmitted on day 14 if not before, and reassessed as on day 0. Prophylaxis of nausea may be started 12hours prior to the treatment start. On day 15 the second activity of ^{131}I-mIBG (as calculated above) will be administered as on day 1, with topotecan immediately prior and also on days 16 to 19. The full blood count will need to be monitored regularly during this period, and supportive care may be necessary.

Tumour dosimetry should be performed wherever possible as described in Section 9.1.2.1.5.

As the treatment is expected to be myeloablative, treatment should be given if the patient is well, *regardless* of the blood count. If the patient is ill, discretion about whether to proceed may be needed. If the responsible clinician has any doubts, the chief investigator should be consulted.

Again, the patient may be released from the radiation protection room either home or to a paediatric ward as soon as the radiation protection adviser allows, in accordance with local rules (see section 233).

9.1.2.1.2 Peripheral Blood Stem Cells Transplantation after ^{131}I-mIBG - topotecan:

The stem cells should be infused when the whole-body activity of ^{131}I-mIBG has fallen to less than 30MBq, probably between days 25-29. Standard supportive care continues according to local practice after autologous blood stem cell support until reconstitution has taken place (including discharge at home if clinically appropriate).

If there is anxiety about myelosuppression, stem cells may be returned if level is greater than 30 MBq at the clinicians' discretion. Consultation with the Chief Investigator or one of the co-investigators may also be appropriate.

9.1.2.1.3 Assessment after ^{131}I-mIBG - topotecan:

The disease assessment **E3,** comprising only primary tumour imaging by ultrasound (echography) and urinary catecholamine metabolites, should be undertaken to determine the response to treatment (see Table 7 - Detailed schedule of the Disease Evaluations throughout the trial).

Great care should be taken to ensure that any subsequent treatment takes into account any possible additive or synergistic toxicity between the proposed additional treatment and the mIBG/topotecan treatment. For example, if external beam radiotherapy is contemplated, the radiation dose already received by normal tissues must be considered; and any significant radiation dose to the lungs may increase the risk of radiation pneumonitis associated with busulphan and melphalan myeloablative therapy. Discussion with the study PI is advised in such cases.

9.1.2.1.4 Whole-body dosimetry guidelines

Whole-body dosimetry protocol

This is the protocol for data acquisition to perform whole-body dosimetry for ^{131}I-mIBG treatment of neuroblastoma. The calculations necessary are included as an appendix (see Appendix 6).

1) Acquire background reading prior to administration.
2) Ensure geometry is the same for all readings
3) Acquire first patient reading immediately following administration and before first void.
4) Acquire second reading immediately after first void.
5) Subsequent readings to be taken at least every 2 hours for the first 24 hours, every 4 - 6 hours thereafter. Patient must void before each reading.

Equipment

Whole body retention measurements should be taken using a compensated Geiger counter with a digital readout. The sensitivity range of the counter must be appropriate for the expected count rates.

The accuracy with which whole-body dosimetry can be determined is largely dependent on the reproducibility of the patient-counter configuration (see error analysis below). It is therefore advisable that the counter is fixed into the ceiling above the patient's bed. Care must be taken to ensure reproducibility of the patient position for each reading.

The counter must be allowed adequate time to warm up before each measurement. This will depend on the set-up in the individual institution.

A background reading should be taken prior to administration.

Note - it may be necessary to acquire more frequent background readings. These may be timed to coincide with when the patient leaves the room for a scan, for example. If the background is constantly changing, as may be the case when the waste is kept and frozen, background measurements should be acquired for each reading, although it will entail the patient being taken out of the room briefly.

Administration

It is important that the patient voids immediately prior to administration. This ensures that no activity is lost before the first whole body retention measurement.

If the patient has to void during administration the activity in this void must be measured and taken into account in subsequent calculations.

Data collection

In principle readings should be taken with the patient both supine and prone and the geometric mean of these measurements used in calculations. However in practice it is often difficult for the patient to lie prone. In this case two supine readings should be taken and the counts averaged.

The **1st patient reading** should be acquired immediately after administration and before the patient voids. If the patient cannot wait until the administration of ^{131}I-mIBG is complete before voiding, the urine should be collected and placed close to the patient for the first reading.

The **2nd reading** should be taken after the administration of ^{131}I-mIBG is complete, when the patient has emptied his bladder, and with any saved urine discarded. If urine has been collected in a catheter bag, this should be disposed of before the second reading.

The patient should void before all subsequent readings.

Further readings should be acquired regularly while the patient is in hospital. There should be a minimum of one set of readings roughly every two hours on day 1 (i.e. the day of administration), and one set of readings every 4 – 6 h thereafter.

It is unreasonable to wake the patient for readings overnight. Therefore readings should be taken last thing at night before the patient retires, as soon as they awake in the morning and at any time they

happen to wake up during the night. In practice this is easily achievable if parents, carers or nurses are trained to take readings whenever the patient voids.

For each reading the patient must be **positioned exactly as for previous readings** and must lie still for the duration of the measurement. It is important to impress upon the patient the need to maintain the bed in the same position and to neither raise nor lower it. It may help to mark the bed position on the floor before the administration to ensure that the bed doesn't move.

Readings of 1 minute duration should be adequate to provide good counting statistics but this should be extended if necessary.

All counts used in subsequent calculations should be background corrected.

9.1.2.1.5 Tumour dosimetry guidelines

The importance of tumour dosimetry

A key feature of both these studies is that individualised tumour dosimetry should be performed wherever possible. It is however acknowledged that not all centres delivering this treatment will be suitably equipped for this, and so inability to perform tumour dosimetry is not a bar to patient entry. All centres which have the expertise and facilities to collect data to allow dosimetry to be performed are strongly encouraged to collect individualised patient data. The investigators will provide all needed assistance in data interpretation.

Data acquisition for tumour dosimetry

Acquire a minimum of 3 SPECT scans of the region of interest following administration. Ideally a whole body scan will be performed, to allow dose calculation at various sites including the primary tumour (if present) and possibly also multiple metastatic sites.

The first scan should be acquired as soon as possible after administration, allowing for the camera's deadtime characteristics. This will typically be when the activity remaining in the patient has decayed to approximately 1000 – 1500 MBq.

- The minimum number of projections is 64.
- The matrix size should be 128x128.
- A high-energy collimator must be used. Energy windows should be set to enable triple energy scatter correction. This requires a peak 20% window situated at 364 keV and 6% windows set immediately below and above the peak window.
- The time for each projection will vary according the activity within the field of view and is dependent on camera sensitivity. The time will typically vary from 5 seconds per view when the patient retains 1000 MBq or more, to 20 seconds per view when 200 – 300 MBq are retained.

Acquire scans on at least two subsequent days. The timing of these scans will be dependent on local logistics and the timing of the therapy (for example it is usually not possible to scan at weekends). Ideally, scans will be acquired daily until the patient is discharged. Where possible, a corresponding CT scan should also be obtained. Ideally, images should be collected from a dedicated SPECT/CT unit.

To enable direct comparison between data acquired at different centres, it is **essential** that all parameters involved with scan acquisition and data processing are well documented. A form will be provided for this purpose. This form should be sent with the raw SPECT and CT image data to the coordinating centre.

9.1.2.2 Arm B: Intensified consolidation with High dose thiotepa

Thiotepa is considered as Investigational Medicinal Product (IMP).

For the intensified consolidation arm B, the dose of thiotepa delivered to the patient is 300 mg/m²/day for 3 consecutive days, i.e., 900 mg/m² overall.

The Figure 66 below depicts the intensified consolidation therapy with high-dose thiotepa.

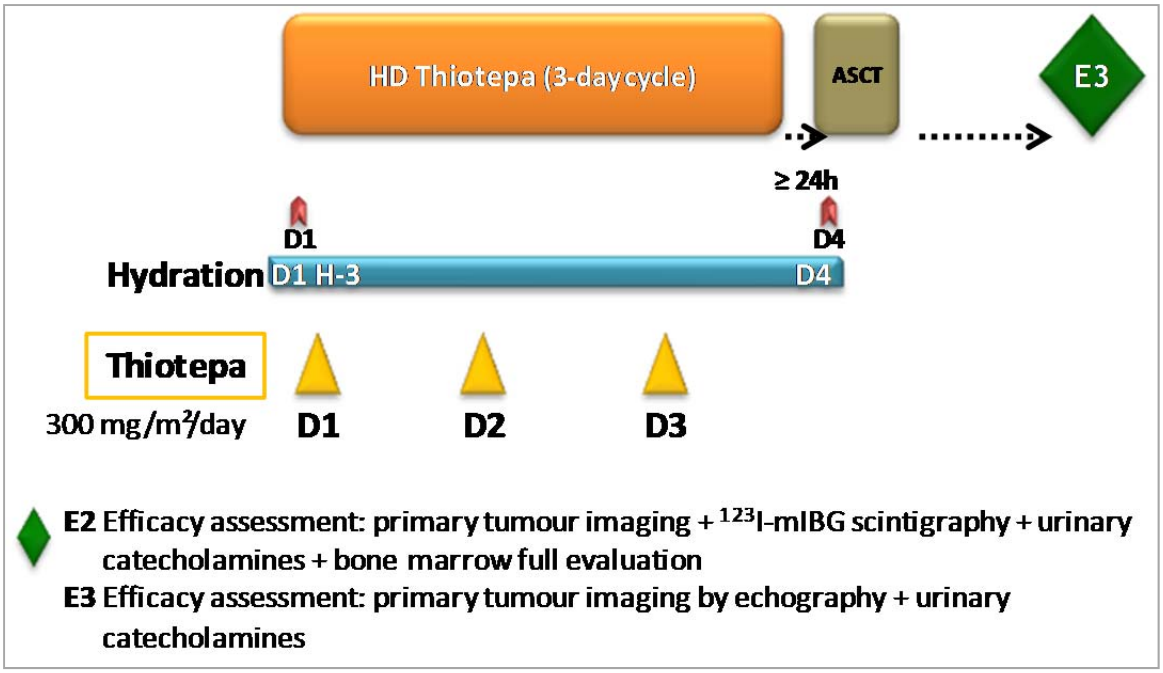


Figure 6 - Flowchart of the high-dose Thiotepa therapy (arm B - 1st part of the intensified consolidation)

Table 4 - Tabulated flowchart of the high-dose Thiotepa therapy (arm B - 1st part of the intensified consolidation)

High-dose thiotepa therapy day		**1**	**2**	**3**	**4**
Drug	**Dose**				
Thiotepa	300 mg/m^2/day	•	•	•	
Hydration	3l/m^2/day = 125 ml/m^2/hr	•	•	•	•
Peripheral Stem Cells	Minimum: 3x 10 ^{6} /kg/CD34+ i.v.				•

Drug delivery:

Thiotepa is commercially available throughout the European Union.

Preparation:

Thiotepa shoud be reconstituted as per local practice.

e.g : 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 shoud be administered as per local practice.

e.g : Dilution 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, haemorrhagic cystitis, and mucosal inflammation.

The full blood count should be performed every 2 days during the intensified consolidation phase.

Hydration:

Three hours prior to thiotepa administration, start the hydration with a polyionic solution[2] for infusion, at a rate of 125 ml/m^2/hr. Continue 24 hours after the end of the thiotepa day 3 infusion, i.e., until day 4.

Further information on this product can be found in the protocol appendices (see section 24.5).

Concomitant treatment:

Concomitant use with phenytoin and fosphenytoin is not recommended.

9.1.2.2.1 Peripheral Blood Stem Cells Transplantation after Thiotepa:

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

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 acetaminophen (10mg/kg p.o.) and diphenhydramine (1 mg/kg i.v.) (or chlorphenamine or generic antihistamine as per local practice)
- 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 where possible during stem cell infusion to avoid volume overload.
- Hydrate for 24 hours post stem cell infusion with 3000 ml/m²/day total IV fluids.

Dosage/Timing

A minimum of 3 x 10^6 CD34 stem cells/kg (optimum 5 x 10^6 /kg) must be available for this first stem cell transplantation of the intensified consolidation therapy.

Reinfusion of Stem Cells

Stem cells will be infused intravenously on Day 4, i.e., 24 hours after the end of thiotepa administration, and within 90 minutes of thawing.

9.1.2.3 Second sequence of the intensified consolidation therapy : BuMel High–Dose Chemotherapy

The BuMel therapy is the second phase of the intensified consolidation chemotherapy, and is the same regardless of the arm, A or B.

An interval of 60 days must be respected between the beginning of the first high dose therapy (mIBG for the arm A, or Thiotepa for the arm B) and the start of BuMel. Of note, BuMel must be started by day 90 regardless of haematological recovery unless there is major organ toxicity or progressive disease, in which case the patient will come off trial.

[2] An example of polyionic solution is the “B27” solution, containing sodium chloride (0.2%, i.e., 2 g/l), calcium gluconate (1%, i.e., 1 g/L), glucose (5.5%, i.e., 55 g/L), potassium chloride (0.15%, i.e.,1.5 g/L), and buffer (targeted pH 4 to 6.5).

A patient is eligible for the BuMel high-dose intensified consolidation if the following disease response criteria and safety criteria are fulfilled:

- **no evidence of disease progression** at the disease evaluation **E3,** compared to the evaluation prior to the 1st sequence of the intensified consolidation therapy (based on a disease evaluation comprising a primary tumour imaging by ultrasound (echography) and the measure of the urinary levels of catecholamine metabolites (without bone marrow evaluation) (see Table 7 - Detailed schedule of the Disease Evaluations throughout the trial).

- With regards to **safety criteria**, patients can start the BuMel/ ASCR sequence if their main organ functions fulfil the following criteria:
  - Liver: ALT and bilirubin blood levels ≤ 2 ULN
  - Renal function: creatinine clearance and/or GFR ≥ 60 ml/min/1.73m², and serum creatinine < 1.5 mg/dL. *Contact the study PI for dose modifications if GFR < 60 ml/min/1.73m², and serum creatinine ≥ 1.5 mg/dL*
  - Cardiac function: shortening fraction ≥ 28%, or ejection fraction ≥ 55%, and no congestive heart failure
  - Pulmonary function: normal chest X-ray and normal oxygen saturation.
  - Perform Doppler Echocardiogram

S

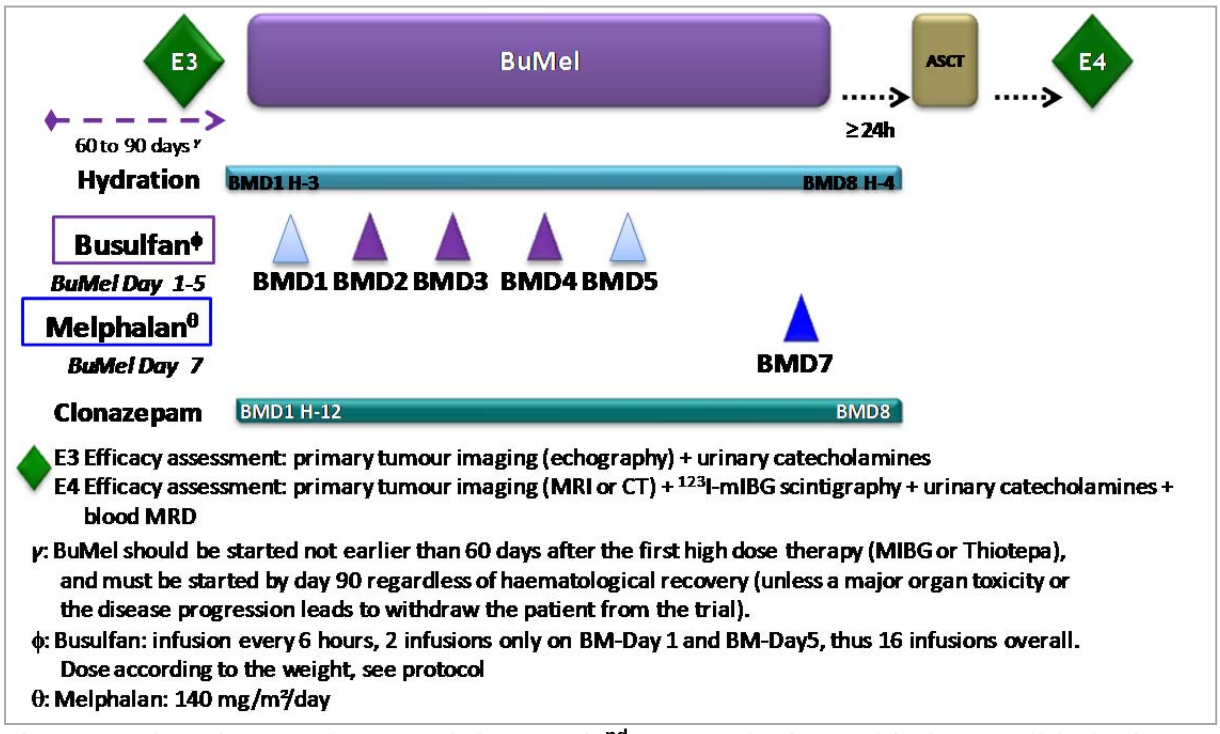


Figure 7 - flowchart of the BuMel therapy (2nd part of the intensified consolidation)

The BuMel therapy is similar in arm A and arm B of the study.

Common side effects and recommended supportive care:

- During busulfan treatment, no anti-emetic agents are indicated. 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, i.e., ondansetron 5mg/m² p.o. or i.v. every 12 hours as anti-emetic (max. single dose 8mg)
- 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/hr. To achieve this urine output, give i.v. hydration at 125 ml/m²/hr.

- **G-CSF 5µg/kg/day IV will be given daily beginning on Day +5** (40). G-CSF will continue until a stable increase of WBC > 5 x 10^9/l or ANC >0.5 x 10^9/l
- All blood products (packed red blood cells, platelets) must be irradiated with 15Gy and be leucocyte-depleted (ideally CMV negative). It is recommended that patients receive red packed blood cells to maintain haemoglobin > 8.0g/dl.
- Stop co-trimoxazole prophylaxis from the day of PBCST (BMD8) until at least 10 days post-ASCR, or until WBC ≥1.0 x 10^9/l.
- Prophylactic antifungal treatment with ketoconazole, itraconazole or fluconazole should be avoided, because of the increased risk of VOD with these drugs in particular in association with busulfan. For proven fungal infection, amphotericin would be used.
- 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.

Hepatic veno-occlusive disease may occur with BuMel therapy. No prophylaxis for HVOD is recommended. However, careful observation of patients during BuMel phase is required, and the management of HVOD is outlined in section 9.4.3.4.

Table 5 - Detailed schedule of the BuMel sequence

	BuMel Therapy DAY (BMD)	1	2	3	4	5	6	7	8
DRUG	DOSE								
Busulfan	< 9kg: 1.0 mg/kg 9 kg to < 16 kg : 1.2 mg/kg 16 kg to 23 kg : 1.1 mg/kg >23 kg to 34 kg: 0.95 mg/kg >34 kg: 0.8 mg/kg Infusions every 6 hours	•• (1)	••• • (2)	••• • (2)	••• • (2)	•• 1)			
Melphalan	140 mg/m^2 IV short infusion (15') **at least** 24 hr after Last busulfan dose							▲	
Hydration	3l/m^2/day = 125 ml/m^2/hr	Continuous until BMD-7 (24 hr after Melphalan)							
Clonazepam	0.025 – 0.1 mg/kg/day Total dose i.v as continuous infusion or Divided in 3 doses p.o/day	Continuous infusion BMD-1 until BMD8 (BMD8 = day of Stem Cells transplantation) If the child is excessively drowsy then reduce dose							
Peripheral Stem cells	Minimum 3X10^6/kg CD34+ i.v								◆

(1): on BMD- 1, BuMel is started at such a time that 2 infusions are administered that day; similarly, only two infusions, the 15th and the 16th infusions are administered on BMD- 5;

(2): from BMD-2 to BMD-4, busulfan infusions are administered every six hours.

9.1.2.3.1 I.V. BUSULFAN (BUSILVEX®)

The Busulfan formulation is a concentrate (6 mg/ml) for infusion; after dilution, 1 ml of solution contains 0.5 mg of busulfan.

Intravenous infusions should be delivered over 2 hours through the central catheter, every 6 hr, for a total of 16 doses.

The dosage follows the guidelines in the Table 6 below.

Table 6 - 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

Preparation and administration:

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 mid-day of day 1, up to mid-day of day 5.

Precautions

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

Renally impaired patients:

Studies in renally impaired patients have not been conducted. However, as busulfan is moderately excreted in the urine, dose modification is not recommended in these patients, but caution is recommended (see appendix 8 "drug information").

Hepatically impaired patients:

Busilvex as well as busulfan has not been studied in patients with hepatic impairment. Caution is recommended, particularly in those patients with severe hepatic impairment (see Appendix 8 "drug information"). Contact the study PI if any doubts.

Drug Delivery:

Busilvex® (busulfan) is commercially available throughout the European Union.

9.1.2.3.2 MELPHALAN

The total dose of melphalan is **140 mg/m²/day.** It should be administer **at least 24 hr after** Busulfan dose**.** There will be NO Melphalan dose adjustment based on GFR in BuMel HDC.

Preparation:

Melphalan for Intravenous administration, 50 mg vials.

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.

Hydration as per local practice:

Start hydration at a rate of 125ml/m^2/hr three hours prior to Melphalan administration. Continue until at least 12 hr post Melphalan. Start with sodium Chloride 0.9% (compatible with Melphalan). Change to Dextrose 2.5%, Sodium Chloride 0.45% post Melphalan administration. It is essential to establish a urine output of 4ml/kg/hr pre-Melphalan and for two hours post-Melphalan administration. Give increased fluids and /or furosemide to achieve this urine output.

9.1.2.3.3 Peripheral Blood Stem Cells Transplantation after BuMel:

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

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 acetaminophen (10mg/kg p.o.) and diphenhydramine (1 mg/kg i.v.).
- 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 where possible during stem cell infusion to avoid volume overload.
- Hydrate for 24 hours post stem cell infusion with 3000 ml/m²/day total IV fluids.

Dosage/Timing

A minimum of 3 x 10^6 CD34 stem cells/kg (optimum 5 x 10^6 /kg) must be available for each individual stem cell transplantation.

Reinfusion of Stem Cells

Stem cells will be infused intravenously on Day 8, i.e., 24 hours after the end of Melphalan administration, and within 90 minutes of thawing.

9.1.3 Local treatment

The local treatment should be performed as appropriate, targets the primary tumour, and may comprise surgery and radiotherapy procedures, and should be performed as per local practice.

A patient is eligible to pursue on local treatment if the following disease response criteria and the safety criteria are fulfilled:

- **No evidence of disease progression** at **E4** disease evaluation
  - primary tumour imaging by MRI or CT scan
  - ^{123}I-mIBG scintigraphy

Assessment of urinary levels of catecholamine metabolites and peripheral blood MRD testing must also be performed (see Table 7 - Detailed schedule of the Disease Evaluations throughout the trial).

9.1.3.1 Surgery Guidelines

Surgery objective

The aim of surgery in high risk neuroblastoma is to achieve complete excision of the tumour with minimal morbidity to improve local control. Chemotherapy is given to facilitate this.

There is no place for surgery other than biopsy before induction chemotherapy, since the risks of operation are higher and the outcome is not better.

This study will also collect data on the surgical procedure particularly on the completeness of excision (verification by early postoperative imaging –CT/MRI- within 24h to 48h postoperatively).

Timing

Surgery should take place after BuMel. *However, if nephrectomy is not going to be part of the procedure, then surgery may take place prior to BuMel.*

Important note: According to the tumour site and size, the potential complications of the surgery must be anticipated; if complications may occur, then the surgery must be postponed after the intensified consolidation full sequence. Undertaking a surgical procedure must not interfere with the intensified consolidation timelines.

If a patient needs additional surgery to the primary tumour site, this should be considered.

Further information on surgery, such as the definitions of the surgical procedures, the definitions of the major complications, additional information on surgery, and risk factors related to the primary tumour localisation can be found in appendices, section 24.88.

9.1.3.2 Radiotherapy Guidelines

Indication

In this protocol all patients will receive radiotherapy to the primary tumour site regardless of the extent and/or result of surgery. This includes patients presenting at diagnosis with a large abdominal or large pulmonary primary receiving BuMel HDC.

However, careful planning of the radiotherapy fields and dose is needed with consideration given to response, local status after surgery to the primary tumour and neighbouring organs. Discuss with the current Radiotherapy Panel.

Metastatic sites should NOT be systematically irradiated.

Some patients may be considered unsuitable for radiotherapy by reason of the site of primary tumour and the volume which would require irradiation. In these situations please discuss with your paediatric clinical oncologist (radiotherapist) and contact trial Co-ordinators for discussion.

Discussion about administration of radiotherapy should include consideration of referral to a centre with more extensive experience.

Timing of radiotherapy

Radiotherapy will be given after the intensified consolidation chemotherapy.

After BuMel HDC, the interval must be greater than 60 days after stem cell transplantation, due to the risk of busulfan-enhanced radiotoxicity.

Fields and dose of radiotherapy

CT Planning 3D conformal radiotherapy planning should be based on preoperative imaging. A planning CT scan at this time will allow the GTV to be identified accurately. Alternatively diagnostic CT or MRI scans performed at this time may be used. Postoperatively the surgical and pathological notes will also be taken into account.

Volume A virtual GTV should be defined on the planning CT-scan based on pre-operative imaging. This will include the post-chemotherapy primary tumour and any immediately adjacent persistently enlarged lymph nodes. This GTV 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 GTV should be expanded to form a CTV by adding a margin which will normally be 0.5 cm. It should be expanded further to take in the complete adjacent vertebrate. It may also be appropriate to include all areas of microscopic disease as indicated from the surgical report and the pathological examination.

The PTV takes into account uncertainties of positioning and possible organ movement. The margin from CTV to PTV should be based on departmental audit of movement. Usually it will be 0.5 to 1.0 cm.

The PTV should be encompassed by the 95 % isodose. The dose within the PTV should be between 95 and 107 %. . Beam shaping or MLC (or customised blocks) should be used to reduce unnecessary irradiation of normal tissues.

While 3D-conformal photon radiotherapy is the norm, there may be circumstances in which a more favourable dose distribution can be achieved by IMRT, electrons or proton bean therapy.

Dose Doses will be specified according to ICRU recommendations. The dose should be treated to 21 Gy in 14 fractions of 1.5 Gy over not more than 21 days. If a single-phase technique to treat the PTV to 21 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. Visible residual disease following the high dose chemotherapy regimen should not be boosted.

Fractionation: Conventional 1.5 Gy per fraction, 5 fractions per week. All fields will be treated daily.

Energy: High energy photons from a linear accelerator.

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 %. Care must be taken if liver function has been compromised by chemotherapy toxicity.

Spinal cord A dose of 21 Gy is acceptable for any length of spinal cord.

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 Gy if necessary to treat the PTV to the prescribed dose providing the opposite kidney's 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.

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 e.g. doxorubicin has been used.

Other sites Normal tissue tolerance is unlikely to be exceeded.

Quality control

A retrospective quality assurance audit has shown inappropriate deviations of protocol in a quarter of patients. These might have resulted in either a greater risk of local failure or avoidable late normal tissue toxicity. It is therefore proposed that radiotherapy plans should be reviewed prior to commencement of treatment. In this way it may be possible to correct deviations before treatment. To facilitate this it is recommended that proposed radiotherapy plans and the diagnostic imaging from which the target volume has been defined, should be uploaded onto the database or sent to the Sponsor for central upload ideally at least one week before the planned start of radiotherapy.

To ensure that the review recommendations are received prior to the start of radiotherapy please inform via Email the current radiotherapy panel about your upload (the review panel is open to increase the panel on expression of interest):

Mark Gaze, MD E-mail: mark.gaze@uclh.nhs.uk

Tom Boterberg, MD E-mail: Tom.Boterberg@UGent.be

Emails should include contact details of requesting centres and the short information that material has been uploaded for review ideally indicating the study number of the patient.

Following completion of treatment, data of the treatment actually given should be put on the system to allow review of radiotherapy by the Radiotherapy Panel.

The following information is required for radiotherapy quality control assessment:

- the CT/MRI scan used for the definition of the tumour volume
- field DRR's or simulator films
- isodose distribution in multiple planes and relevant levels
- dose/volume histograms of CRV, PRV and surrounding critical organs.

This information may be uploaded to the SIOPEN-R-NET via the Radiotherapy Sub-Study or alternatively sent to the Sponsor for central upload within a month of completion of radiotherapy treatment.

All information sent to the Sponsor should be clearly labelled with the patients study number and date of birth. In case any questions arise regarding the sent information a contact person, email address and phone number must also be supplied.

At the end of local treatment, the disease evaluation E5 must be conducted. **E5** should comprise a full bone marrow evaluation, including the detection of minimal residual disease based on QRT-PCR and immunocytology, and the urinary levels of catecholamine metabolites.

9.2 Disease response and safety Assessments

9.2.1 Disease assessments

The disease assessments throughout the study are depicted on the Table 7 below. The same table is displayed with the study flowcharts at the beginning of this protocol (see Table 2 bis).

Table 7 - Detailed schedule of the Disease Evaluations throughout the trial

Evaluations **Study steps** **Tests**	**E1** **Study entry**	**E2** **Prior to intensified chemotherapy**	**E3** **Prior to BuMel**	**E4** **Post-BuMel, prior to local treatment**	**E5** **End of local treatment = end of study treatment**
^{123}I-mIBG scintigraphy	❒	❒		❒	❒
Primary tumour imaging (MRI or CT)[a]	❒	❒		❒	❒
Primary tumour imaging (echography) [a]			❒		
Cerebral imaging (MRI or CT) [a]	❒				
Blood MRD testing [b]	❒	❒		❒	❒
BM MRD testing [b]	❒	❒			❒
BM (trephine biopsy) [c]	❒	❒			❒
BM (aspirates) [d]	❒	❒			❒
Urinary catecholamine metabolites	❒	❒	❒	❒	❒

a: imaging of the primary tumour and cerebral imaging: by MRI or CT scan as judged appropriate by the investigator. Ultrasound/echography imaging of the primary tumour is mandatory before BuMel

b: the minimal residual disease (MRD) testing is performed using two types of methods: a quantitative reverse-transcriptase polymerase chain reaction (QRT-PCR), and by immunocytology (IC). For the disease evaluation, blood and bone marrow are tested via both methods to detect any signs of minimal residual disease.

c: BM trephine: the pathologist performs a histological examination and provides the clinician with the information. Ten unstained sections are needed for central review by SIOPEN members (see Appendix 5).

d: BM aspirates: to be shared between slides for staining, BM cytospin, and QRT-PCR (see Appendix 5)

9.2.1.1 Imaging

A cross-sectional imaging of the primary tumour by MRI or CT-scan and a ^{123}I-mIBG scintigraphy will be performed at the following evaluations:

- at study entry (E1),
- before the 1st part of the intensified consolidation (^{131}I-mIBG – topotecan or thiotepa) (E2),
- after BuMel, after the intensified consolidation, and before the local maintenance treatment (E4),
- after the local treatment (E5). The latter evaluation is the end-of-treatment evaluation

An ultrasound imaging of the primary tumour by echography will be performed during the intensified chemotherapy, after the 1st part of the intensified consolidation (^{131}I-mIBG – topotecan or thiotepa), and before the BuMel (E3).

In addition, a cross sectional imaging of the brain will be performed at study entry, to ensure there is no brain metastasis.

9.2.1.2 Bone marrow sampling

A bone marrow aspirate from four body sites will be performed for bone marrow evaluation at each extensive disease evaluation:

- at study entry (E1),
- before the intensified consolidation full sequence (E2),
- at the end of local treatment (E5).

Any signs of MRD will be sought on the bone marrow (and concomitantly in the blood), using QRT-PCR test and IC test.

Pre randomisation

Aspirate and trephine biopsies will be sampled from four and two body sites, respectively, at study entry (with MRD tests).

If they were positive at the time, bone marrow trephine will be sampled again after BuMel.

Post BuMel

Bone marrow trephine (if positive at randomisation)

Please see the APPENDIX 5: BONE MARROW SAMPLING AND EXAMINATION GUIDELINES for detailed handling of the bone marrow samples.

9.2.1.3 Urinary catecholamine metabolites

The following urinary catecholamine metabolites will be measured: dopamine, homovanillic acid (HVA), and vanillyl mandelic acid (VMA)

At each evaluation: at randomisation (E1), before intensified chemotherapy (E2), before and after BuMel (E3 and E4) and after the local treatment (E5).

9.2.2 Safety assessments

9.2.2.1 Physical examination/symptom assessment

A physical examination (including PS (Karnofsky or Lansky)) with symptom assessment will be performed at study entry, prior to each treatment cycle, at the end of treatment, and at each follow-up visit.

A physical examination (including PS (Karnofsky or Lansky)) will be performed on a daily basis during the treatments days of the intensified consolidation therapy phase.

9.2.2.2 Blood and urinary chemistry

At study entry, and prior to each treatment cycle (TEM-IRI, 1st par to the intensified consolidation, BuMel part of the intensified consolidation, local treatment, and at the end of treatment visit, the blood levels of the following parameters is measured:

- Electrolytes (Na+, K+, Cl-), albumin, CRP
- Renal function: urea, creatinine (and also the urinary level of creatinine, in order to calculate the creatinine clearance)
- Liver function: AST or ALT, bilirubin

Some biomarkers are measured at specific time-point:

- Biomarkers of the thyroid function (T3, T4, TSH) and of the adrenal function (ACTH, adrenocorticotropin) for patients in the ^{131}I-mIBG – topotecan arm:
    - before the start of each cycle of ^{131}I-mIBG – topotecan (i.e., D1 and D15),
    - prior to BuMel (i.e., around 6 to 9 weeks after the 2nd injection of ^{131}I-mIBG),
    - 6-8 months after the completion of the ^{131}I-mIBG – topotecan – ASCT sequence, i.e., at the end-of-treatment evaluation
    - 12-15 months after the completion of the ^{131}I-mIBG – topotecan – ASCT sequence, i.e., at the 6-month follow-up evaluation.
- In female of childbearing potential, a pregnancy test must be performed at study entry and prior to the intensified consolidation phase.

9.2.2.3 Haematology

The full blood count (includes Haemoglobin level (Hb), platelets count, and white blood cells (WBC) with differential count (neutrophils, lymphocytes, eosinophils, basophils) will be performed at least at the following times and frequency, and adapted to the patient' clinical status:

- prior to each study treatment cycle,
- on a weekly basis during the VERITAS induction TEM-IRI
- every two days during the intensified consolidation phase
- weekly during the radiotherapy

MRD tests (QRT-PCR and IC) will be performed on blood at each extensive evaluation (concomitantly to BM MRD tests): at study entry, prior to and after the intensified consolidation full sequence, at the end of local treatment.

9.2.2.4 Imaging for safety purposes

Prior to BuMel, an ultrasound of abdomen is required, if the cross sectional imaging of the primary tumour has not included the liver.

9.2.3 Summary of the End-of-treatment assessment

At the end-of-treatment assessment, an extensive disease and safety evaluation will be performed:

Disease assessment (E5):

- primary tumour imaging
- ^{123}I-mIBG scintigraphy
- urinary levels of catecholamine metabolites
- bone marrow evaluation
- MRD test by QRT-PCR and IC in bone marrow and in peripheral blood

Safety assessment:

- General clinical examination, vital signs, height and weight
- Performance status
- Cardiac function: echocardiogram
- Liver function: ALT or AST, and total bilirubin blood levels
- Renal function: serum and 24-hour urinary creatinine for calculating the creatinine clearance
- Pulmonary function: perform a functional pulmonary test if pulmonary dysfunction was experienced and/or if clinically indicated
- Thyroid and adrenal function (T3, T4, TSH, ACTH, and cortisol).
- Auditory function: the appropriate test according to the children age must be performed

9.3 Dose Modifications

Maintaining the dose intensity as close as possible of what is scheduled in the protocol is an important objective.

9.3.1 Dose modification of TEM-IRI

Neutropenia should be managed thanks to G-CSF administration (see section 9.4.2).

Table 8 - Dose modifications of temozolomide and irinotecan during the VERITAS induction chemotherapy

Type of toxicity	Dose modification at 1st occurrence	Dose modification at 2nd occurrence
ANC < 0.75 x 10^9/L Or platelet count < 75 x 10^9/L Recovered on day 21 after the start of a cycle	**No dose modification**	**No dose modification**
ANC < 0.75 x 10^9/L Or platelet count < 75 x 10^9/L Recovered between day 22-28 after the start of a cycle	**No dose modification**	**Decrease temozolomide by 20%** (i.e., 80 mg/m²/day for 5 days)
ANC < O.75 x 10^9/L Or platelet count < 75 x 10^9/L Recovered between day 29-34 after the start of a cycle	**Decrease both temozolomide and irinotecan by 20%** (i.e., TEM : 80 mg/m²/day for 5 days, IRI: 40 mg/m²/day for 5 days)	**Decrease both temozolomide and irinotecan by 40%** (i.e., TEM : 60 mg/m²/day for 5 days, IRI: 30 mg/m²/day for 5 days)
ANC < O.75 x 10^9/L Or platelet count < 75 x 10^9/L Recovered on day 35 after the start of a cycle	**Discontinue study treatment**	Not applicable
Grade 3 and 4 diarrhoea > 3 days despite maximal loperamide therapy	**Decrease irinotecan dose by 20% (40 mg/m²/day)** • If the same level of toxicity persists > 2 weeks despite suitable symptomatic treatment, discontinue study treatment • If diarrhoea is ongoing on day 21, delay next cycle for up to 2 weeks until diarrhoea resolves to < grade 1 • If the diarrhoea does not resolve after a 2-week delay, the patient should discontinue study treatment	**Decrease irinotecan dose by 40%, i.e., 30 mg/m²/days** • If the same level of toxicity persists > 2 weeks despite suitable symptomatic treatment, discontinue study treatment • If diarrhoea is ongoing on day 21, delay next cycle for up to 2 weeks until diarrhoea resolves to < grade 1 • If the diarrhoea does not resolve after a 2-week delay, the patient should discontinue study treatment
Other grade ≥3 non haematological toxicity not recovered to grade ≤2 before day 21	**Decrease both irinotecan and temozolomide by 20%** (i.e., TEM : 80 mg/m²/day for 5 days, IRI: 40 mg/m²/day for 5 days)	**Discontinue study treatment**

Note: All platelets cut-off values require no platelet transfusions within 72 hours of starting the cycle. All neutrophil cut-off values require being off G-CSF for at least 72 hours. For those patients with known bone marrow involvement, the cut-off values required are ANC ≥0.5 x 10^9/L and platelets ≥50 x 10^9/L

9.3.2 Dose modification of ^{131}I-mIBG – topotecan

The 2nd ^{131}I-mIBG injection will be cancelled in case of liver failure (ALT or AST, AND bilirubin > 2.5 ULN) or of renal failure (blood level of creatinine > 1.5 x ULN and/or creatinine clearance < 50 ml/min/1.73m²

The 2nd ^{131}I-mIBG injection will also be cancelled if the whole-body dose of 4 Gy is reached at the 1st infusion.

No dose modification of topotecan is recommended, as the doses used are low, and the haematological toxicity will be addressed by the ASCR.

9.3.3 Dose modification of thiotepa

No dose modification of thiotepa should occur.

9.3.4 Dose modification of BuMel

In case of low weight and/or renal function impairments, PK evaluation should be discussed for BuMel Adaptation. In such case, contact the study PI for dose adaptation and/or busulfan pharmacokinetic evaluation.

9.4 Supportive Treatment

For clarity purposes, supportive care in this section are described according to the chemotherapy which will most likely require them, either as general supportive care if any kind of chemotherapy may require their use, or supportive care more likely associated to TEM-IRI (section 9.4.2), to the intensified consolidation chemotherapy (see section 9.4.3), and more specifically those associated to the intensified chemotherapy with ^{131}I-mIBG and topotecan (see section 9.4.4).

9.4.1 General supportive treatment recommendations

9.4.1.1 Anti-emetics

It is recommended to administer antiemetic as needed, and particularly during Thiotepa and Melphalan administration according to institutional guidelines, e.g. Ondansetron 5 mg/m² (maximum single dose 8 mg) p.o./i.v. every 12 hours.

Busulfan is usually well tolerated.

Anti-emetics treatment should be adapted to patient's symptoms.

Systematic prophylactic anti-emetic treatment is not recommended.

9.4.1.2 Blood component therapy

Due to the risk of graft versus host reactions in patients on chemotherapy (especially in the case of high-dose therapy) all blood products should be irradiated with 25 Gy prior to transfusion, according to national policies. The use of leukocyte filters for leukocyte depletion is advised.

Red blood cells transfusion recommendations:

The objective is to keep the haemoglobin level above 8g/dL.

Platelets substitution recommendations:

Platelet substitution is advised when the platelets are < 10 or 20 x 10^9/L according to national transfusion policies, and/or there is clinical evidence of bleeding.

All blood products must be deleucocyted blood product in order to prevent post-transfusional CMV infection.

9.4.1.3 Treatment guidelines for febrile neutropenia

a. Prophylactic antifungal and antiviral treatment is not recommended

b. If there is fever (>38°C) the centre's usual combination of broad-spectrum antibiotics should be started after blood cultures and urines samples in addition a chest X-ray (CXR) should be carried out.

c. If fever persists (>38°C) for 48 hours despite broad- spectrum antibiotics, a new infection evaluation should be performed and antifungal therapy should be started, regardless of the clinical condition of the patient.

The preferred antifungal therapy is liposomal amphotericin (Ambisome®) at a dose of 1mg/kg/day.

However, if this is not available then amphotericin B 0.5mg/kg for the first dose and then increased to 1.0mg/kg after 24 hours should be given.

In the case of impaired renal function liposomal amphotericin is recommended.

Other antifungal therapy e.g. fluconazole, Itraconazole and voriconazole are NOT permitted after BuMel because of the hepatic toxicity and increased risk of VOD.

Antibiotic therapy will be adapted to positive results without narrowing the activity spectrum.

9.4.1.4 Renal Function Monitoring

Serum creatinine should be monitored prior to each chemotherapy course. Glomerular function is to be assessed according to national / group guidelines, applying either isotope clearance, or calculated creatinine clearance (only acceptable during induction phase, not allowed to be used for creatinine clearance determination prior to intensified consolidation therapy).

According to Schwartz's formula , creatinine clearance (C crea) can be calculated from single serum samples:

$$\text{C crea} = \frac{\text{F x Height [cm]}}{\text{Crea serum [mg/dl]}} [\text{ml/min/1.73m}^2]$$

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

- Male, 1-16 years **F** = 0.55
- Female, 1-21 years **F** = 0.55
- Male, 16-21 years **F** = 0.70

Normal values (ml/min/1.73m²): ≥ 1 year: 120

9.4.1.5 Pain management

The standard pain prophylaxis of this study aims to manage efficiently patient's pain without the use of high doses of intravenous morphine.

Concomitant pain medication according to WHO recommendations:

Non-steroidal anti-inflammatory drugs (NSAIDs):

Paracetamol is approved for use from birth onwards. The analgesic potency of paracetamol is rated by many authors as lower than that of ibuprofen or other NSAIDs. The low analgesic potency and the narrow therapeutic range should be considered when using paracetamol.

Recommended dosages:

Paracetamol (i.e.: Mexalen®) 10 - 15 mg/kg PO q6h prn, or iv (i.e.: Perfalgan®) q6h prn 15mg/kg (100mg/100ml)

The **NSAID** most widely used in pediatrics is **ibuprofen**. It is approved from age 6 months onwards and has a longer duration of action (8 hours).

Recommended dosages:

Ibuprofen (i.e.: Ibuprofen-oral suspension at 2% or 4%) 10 -15 mg/kg PO before the treatment is administered and q4h prn

Metamizole

Because of its spasmolytic properties **metamizole** is particularly suitable for visceral pain or colicky pain. More useful than repeated short infusions is a long-term infusion with a dosage of 2.5 to 3.0 mg/kg/h, always with close monitoring of blood pressure values Metamizole is approved for use from the age of three months onwards (remark: not permitted in the UK). A risk assessment for agranulocytosis in children receiving metamizole therapy is not possible at present – only one such case has been reported to date (Ref: Mayer 1999).

Recommended dosages:

Metamizol (i.e.: NOVALGIN®) 10 mg/kg PO q6h prn or long-term infusion with a dosage of 2.5 to 3.0 mg/kg/h

Indomethacin (i.e.: INDOCIN®) is supplied in three dosage forms. Capsules INDOCIN for oral administration contain either 25 mg or 50 mg of indomethacin. Suspension INDOCIN for oral use contains 25 mg of indomethacin per 5 mL should not be used in children younger than 2 years

Recommended dosages:

Indomethacin 0.5 mg/kg PO q6h prn for high fever (UK recommendation).

9.4.1.6 Special warning for patients treated with immunosuppressive therapy

Cases of reactivation of Hepatitis B virus (HBV) have occurred in patients who are chronic carriers of HBV after they received immunosuppressive therapy. Some cases of HBV reactivation resulted in acute hepatic failure or fulminant hepatitis leading to liver transplantation or a fatal outcome. Recommendations:

- Patients must be tested for HBV infection before initiating treatment with immunosuppressive therapy
- Consult experts in liver disease before treatment in patients with positive HBV serology (including those with active disease) is initiated and for patients who test positive for HBV infection during treatment
- Closely monitor patients who are carriers of HBV requiring treatment with immunosuppressive therapy for signs and symptoms of active HBV infection throughout the treatment and for several months following the end of treatment.

9.4.2 Supportive treatment most specifically for TEM-IRI

9.4.2.1 Management of irinotecan-related diarrhea

Early-onset diarrhoea may occur during irinotecan infusion or within 8 hours following completion of the infusion.

Patients who have such an early onset of diarrhoea should receive a dose of atropine 0.02 mg/kg (max 0.25 mg) intravenously. Early diarrhoea may be accompanied by abdominal cramps and other cholinergic symptoms. If this happens, prophylactic atropine (0.02 mg/kg orally or IV) could be used before the next course of irinotecan.

Delayed-onset diarrhea is diarrhea occurring at least 8 hours after the irinotecan infusion completion, and up to 5 days after irinotecan administration.

For delayed onset diarrhoea occurring >8 hours after irinotecan administration, children should receive loperamide. Loperamide should continue until a normal pattern of bowel movements returns.

Loperamide should be administered at high dose, but no more than 48 hours:

- Loading dose of 4 mg

- Then 2 mg every two hours until 12 hours after the last liquid stool (but not longer than 48 hours)

In children, the loperamide maximal daily dose is 12 mg (6 capsules at 2 mg).

A treatment with loperamide lasting more than 48 hours is associated with a high risk of paralytic ileus.

Of note, loperamide must NOT be used prophylactically.

Oral hydration with large volumes of water and electrolytes should be prescribed during whole diarrhoea episode. Clinically significant diarrhea is associated with the need for parenteral support for dehydration

In the absence of any contraindications such as allergies, treatment with cefixime 8 mg/kg once a day (max daily dose 400 mg) could be considered and started 2 days before chemotherapy and continued daily until day 7, following local policies for the management of irinotecan-related diarrhoea.

If the delayed diarrhoea recurs, then cefixime should be given with the following courses.

In case of neutropenia < 500/mm" concomitant to diarrhea, anti-diarrheic agents might be associated to prophylaxis with broad spectrum antibacterial agents.

In addition to antibacterial agents, patients may be hospitalized in case of

- febrile diarrhea
- severe diarrhea requesting parenteral hydration
- persisting diarrhea, despite a 48-hour treatment with loperamide at the appropriate dosage.

9.4.2.2 Management of Pneumocystis Pneumonitis Prophylaxis

Patients should be considered for prophylactic sulfamethoxazole/trimethoprim (5 mg TMP/kg/day given orally 3 days a week). For sulfa-intolerant patients it is recommended that inhaled pentamidine only is used as prophylaxis. PJP prophylaxis using a pentamidine nebuliser at three-weekly intervals can be encouraged for children who are able to co-operate with jet inhalation (necessary to be effective) which is usually only the case for children of school age.

9.4.3 Supportive treatment most specifically for the intensified consolidation chemotherapy

It is anticipated that the double stem cell transplant strategy in this protocol will result in significant toxicity. It is therefore recommended that this treatment should only be administered in centres performing more than 10 paediatric stem cell transplants per year.

Prophylactic defibrotide with BuMel may be used according to institutional practice. Especially in HDT patients, multi-lumen central lines are essential for PSC sampling and supportive care.

9.4.3.1 Hydration

Sufficient hydration (2 to 3 l/m²) should be administered from 12 hours before beginning of high dose chemotherapy until stem cell reinfusion.

9.4.3.2 G-CSF after HDC (Thiotepa and BuMel)

The administration of G-CSF post ASCR is highly recommended in this study.

G-CSF : 5µg/Kg/day from day 5 post stem cell transplantation until a stable increase of WBC > 5 X 10^9/l or ANC > 0.5 X 10^9/l on two consecutive blood cell counts with a 48h interval (40). Protective isolation per local institutional guidelines

Please apply the local institution guidelines.

9.4.3.3 Diarrhoea management during intensified consolidation therapy

Diarrhea occurring during the intensified consolidation therapy must be managed promptly according to local guidelines.

A treatment with loperamide lasting more than 48 hours is associated with a high risk of paralytic ileus.

Of note, loperamide must NOT be used prophylactically.

Clinically significant diarrhea, defined by at least one of the following signs:

- an increase of seven stools per day,
- incontinence of stool,
- need for parenteral support for dehydration

In case of neutropenia < 500/mm" concomitant to diarrhea, anti-diarrheic agents might be associated to prophylaxis with broad spectrum antibacterial agents.

9.4.3.4 Prophylaxis, diagnosis and management of hepatic veno occlusive disease (HVOD)

9.4.3.4.1 Prophylaxis of HVOD Guidelines

The prophylaxis of HVOD is recommended after BuMel

Defibrotide

Dose: 25 mg/kg/d i.v.

Defibrotide is administered in 5% D-Glucose (Dextrose) water to a maximum concentration of 20 mg per 1 ml given IV in 4 divided doses (every six hours) each infused over 2 hours.

The administration should begin at day of conditioning until day +30, or until discharge from inpatient care (with a minimum treatment of 14 days) if VOD does not occur.

The duration of infusion can be reduced to a minimum of 30 minutes if venous access is limited. Although it should be noted that longer durations of infusion are preferable due to the short half-life of the drug.

The solution is stable at room temperature for 24 hours and exposure to light should be avoided.

Further information on defibrotide is provided section 24.100.

9.4.3.4.2 Diagnosis of HVOD

Clinical features

According to McDonald (43, 44) VOD is clinically defined by the combination of at least two of the three following criteria:

- liver enlargement and/or pain in the right hypochondrium
- jaundice
- ascites and/or unexplained weight gain exceeding 2.5% of baseline value

Pleural effusion may be observed, and can contribute to respiratory distress. Fever even if rarely described in the literature is frequent; nevertheless an extensive infection screen should be carried out, and repeated in order to eliminate an infectious cause of the above signs.

Hepatic function is often disturbed, with hyperbilirubinaemia in more than 90% of cases. Elevated transaminases occur in 60-70%. Coagulation abnormalities are less frequent, reduction in Factor VII & X levels are most frequent, and may occur before any clinical signs.

A decrease in urinary sodium output (< 10 mmol/l) is an early and constant finding; its normalisation is often the first sign of clinical recovery. Moderate abnormalities in renal function may be observed, often as a result of the restricted fluid regimen imposed.

There is a significant increase in platelet transfusion requirements (45).

Ultrasonography

The ultrasound findings are liver and spleen enlargement, ascites, gall bladder wall thickening, reduction of hepatic vein diameter, enlargement of the portal vein diameter and visualisation of the

para-umbilical vein. Doppler ultrasound can be useful to confirm the diagnosis. The results can be predictive and of prognostic relevance (46)

Histological features

Hepatic biopsy should NOT be performed.

9.4.3.4.3 Management of HVOD – symptomatic treatment

These children are usually very unwell and require supportive care until the hepatic lesions improve.

The following measures may assist in this:

- Fluid restriction (60 ml/kg/day), adjusted according to renal function.
- Strict sodium restriction,
  Caution: platelet transfusions contain considerable amounts of sodium.
- Defibrotide is strongly encouraged (if not already administered).
- Spironolactone (5 mg/kg/day), 5 days a week (with a 2 day break because of the long half life, reducing problems related to the accumulation of metabolites). However, this diuretic treatment is not always effective.
  Caution: Furosemide is ineffective, and, in addition, may exacerbate renal failure. However, it may sometimes be useful after blood product transfusions.
- Platelet transfusions should be given, to maintain the platelet count in excess of 20 x 109/l. This level may be difficult to maintain even with twice daily transfusions.
  Caution: Mind the amount of Na++ brought by the platelets transfusion
- Opiate analgesia may be required for abdominal pain.
- Abdominal paracentesis to drain ascites should only be carried out when the volume of ascites is causing serious respiratory distress, or in case of abdominal skin hypertension.
- Albumin administration is not recommended, but might be considered when the albumin level is profoundly low (< 15g/l).

9.4.3.5 Nutrition

Parenteral nutrition via the central venous line according to institutional standards is recommended after autologous stem cell transplantation.

9.4.3.6 Skin care

Delete every glue residues on the skin (plasters, electrodes, etc) at hospital acceptance in the Transplantation Unit.

Daily whole body cleaning.

Application of cold cream or sweet almond oil on the whole body once a day.

Application of Chlorhexidine twice a day on anal margin and cutaneous bends (armpits and groin) then dry with a hairdryer. In case of anal margin lesions, application of Dexbepanthenol cream.

9.4.3.7 Mucositis prevention and care

Mouth bath should be performed every 4 hours to prevent and treat mucositis according to institution guidelines.

Pain treatment should be established according to institution guidelines.

Opiate analgesia may be required. Pethidine (4 to 10 mg/Kg/day in continuous i.v. infusion) should be preferred to morphine and fentanyl.

9.4.3.8 Pneumocystis Pneumonitis (PCP) prophylaxis

Pneumocystis jirovecii pneumonitis prophylaxis with SMZ/TMP should not be stopped during treatment except in case of HVOD with hepatic dysfunction or neutropenia duration > 20 days. It should be maintained until 6 months post stem cell transplantation **and** lymphocytes count >500 mm^3

9.4.4 Supportive treatment most specifically associated with the intensified consolidation with ^{131}I-mIBG

Appropriate supportive care, including packed red cells or platelets transfusions, and treatment of febrile neutropenia, should be carried out according to local policy.

Most concomitant treatments must be discontinued during the trial (see 9.55). Moreover, some drugs must be specifically interrupted during at least 4 half-lives prior to start the ^{131}I-mIBG – topotecan arm.

A thyroid block must be maintained from day-2 until 28 days after the second mIBG infusion (see 23.2).

9.5 Concomitant Medication

Patients must be instructed not to take any additional medications, including over-the-counter (OTC) products and herbal remedies, during the study without prior consultation with their doctor (local PI).

Palliative and supportive care for disease-related symptoms should be offered to all patients when appropriate. Relevant concomitant medications and blood products, as well as interventions (e.g., analgesic use, paracentesis) received by patients from screening until the end of the SAE reporting period should be documented in the patient's medical notes.

No chemotherapy, hormonal anticancer therapy, or experimental anticancer medications other than those that are study-related will be permitted while the patient is receiving study treatment. In case of disease progression requiring withdrawal from study treatment, no further trial medications will be given and investigators should give whatever anti-tumour therapy is considered appropriate.

Topical applications, inhaled sprays, eye drops or local injection (e.g., intraocular) of corticosteroids are allowed.

Treatment-related adverse events related to concomitant medication should be treated according to local practice. Utilisation of CYP 450 3A4 inhibitors or inductors is not allowed (including St John's wort plant).

Palliative radiotherapy to non-target lesions will be permitted. Immunisation with live vaccine or antimalarial vaccine will not be permitted.

9.6 Patient Follow Up

The disease and safety assessments at follow-up are described below. Additional visits might be scheduled as needed, to monitor any sustained unresolved adverse event or new clinical event.

9.6.1 Disease assessments at follow-up

In the event of progression/relapse, the type of progression/relapse will be recorded (local/metastatic/combined) as well as the new anticancer treatment that is started.

In case of clinical symptoms, an extensive evaluation should be performed, that will include primary site imaging, BM evaluation, ^{123}I-mIBG scintigraphy, and the measure of the urinary catecholamine metabolites.

9.6.1.1 Primary site assessment

The disease assessment of the primary site should be performed with ultrasound imaging or CT scan imaging as appropriate.

Assessments will be performed every three months until full tumour shrinking.

9.6.1.2 Metastatic assessment

In case of residual ^{123}I-mIBG scintigraphy positive at the E5 evaluation (end-of-treatment), repeat ^{123}I-mIBG scintigraphy at one year follow-up, and then yearly until negative or progression. BM aspirates should be performed only in case of clinical signs. .

MRD will be sought on blood and BM samples only at the E5 end-of-treatment evaluation.

9.6.1.3 Urinary catecholamine metabolites

For patients with positive test for urinary catecholamine metabolites at diagnosis, the test will be repeated every 3 months the 3 first years. The test will be then repeated every 4 months the 4th year as per standard of care.

9.6.2 Safety assessments at follow-up

The toxicity assessment needs to be related to the randomised treatment received by the patient. All children require renal, auditory and fertility follow-up.

Those who have had extensive abdominal or pelvic radiotherapy may have prolonged thrombocytopenia.

9.6.2.1 General assessment

All patients should have a physical examination, height, weight, and full blood count (with differential and platelets) every 3 month for the first 3 years. These exams should be, then repeated every 4 months the 4th year, at 4 ½ years, at 5 years, and then yearly as per standard of care.

9.6.2.2 Renal follow-up

GFR assessment should be determined at the end of treatment.

In children who can give a reliable 24 hour urine collection, endogenous creatinine clearance is acceptable.

Where this is not possible, then GFR estimation by DTPA or CrEDTA is preferred.

Children who had an end of treatment GFR of less than 80ml/min/1.73m² should have a repeat GFR and serum magnesium at one year and 5 years off treatment. It is known that children receiving platinum based compounds, the GFR does not decrease with time as it does after ifosfamide. However, tubular toxicity may persist or appear years after treatment.

9.6.2.3 Auditory follow-up

Chemotherapy-related ototoxicity is usually permanent or irreversible.

Any child under the age of 3.5 years during treatment should have pure tone audiometry when they have reached 3.5 years of age. An adequate assessment at the end of treatment or before going to school is strongly advised.

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

9.6.2.4 Cardiac follow-up

Patients should have echocardiogram at end of treatment and then as clinically indicated.

9.6.2.5 Pulmonary follow-up

All patients receiving MAT and having experienced pulmonary dysfunction should have pulmonary function tests as clinically indicated.

9.6.2.6 BuMel-related reduced Fertility

A fertility assessment should be performed for all boys after puberty.

Due to busulfan ovarian effect, a hormonal substitution should be initiated at puberty, with the appropriate follow-up.

9.6.2.7 Thyroid and adrenal functions follow-up (for ^{131}I-mIBG patients).

In patients of the arm A, treated with ^{131}I-mIBG and topotecan, the thyroid and adrenal function must be controlled at study end of treatment (i.e., around 8 months after the 1st ^{131}I-mIBG infusion) and 6 months later on.

9.6.2.8 Secondary malignancy.

Any second malignancy must be reported to the Pharmacovigilance Unit of the Sponsor.

9.7 Patient Withdrawal

Patients may withdraw from study treatment at any time at their own request or at the request of their parents or they may be withdrawn from study treatment at any time at the discretion of the treating Investigator or CI for safety, behavioural, or administrative reasons. If a patient does not return for a scheduled visit, every effort should be made to contact the patient. In any circumstance, every effort should be made to document patient outcome where possible.

The Investigator should:

- Enquire about the reason for withdrawal
- Request the patient return for a final visit, if applicable
- Follow up with the patient regarding any unresolved adverse events
- Perform a physical exam on the patient (including height, weight and blood pressure/pulse)
- Arrange for a tumour assessment and all safety labs (full blood count, biochemistry and clotting bloods) to be collected

Patients who withdraw from trial treatment will continue to be followed up as per the protocol for 3 years after randomisation.

If the patient withdraws from study treatment, and also withdraws consent please refer to section below.

9.7.1 Withdrawal of Consent

Patients/parents/legal guardians may withdraw consent at any time during the trial. The details of the withdrawal should be clearly documented and communicated to the Sponsor.

There are three types of withdrawal as detailed below:

- Patient or their parent(s)/legal guardian would like to withdraw the patient from the trial, but is willing for the patient to be followed-up according to the trial schedule (follow-up data can be collected and used in the trial analysis)
- Patient or their parent(s)/legal guardian does not wish for the patient to attend trial follow-up visits but is willing for the patient to be followed-up at standard clinic visits (follow-up data can be collected at standard clinic visits and used in the trial analysis)
- Patient or their parent(s)/legal guardian is not willing for the patient to be followed up for trial purposes at any further visits (any data collected prior to the withdrawal of consent can be used in the trial analysis)

Patients/parents/legal guardians may, without any resulting detriment and without having to provide any justification, withdraw from the clinical trial at any time by revoking his or her informed consent.

Without prejudice to Directive 95/46/EC, the withdrawal of the informed consent shall not affect the activities already carried out and the use of data obtained based on informed consent before its withdrawal.

The following should be clearly documented in the medical notes:

- The date the patient or their parent(s)/legal guardian withdraw consent.
- The reason, if given (e.g. toxicity to drug).
- Type of withdrawal

10 ADVERSE EVENT REPORTING

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)

Any new untoward medical occurrence or worsening of a pre-existing medical condition in a patient or clinical investigation subject administered an investigational (medicinal) product. An AE can therefore be any unfavourable 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 Adverse Reaction (AR)

Any untoward and unintended responses to an investigational medicinal product related to any dose administered. The definition implies a reasonable possibility of a causal relationship between the event and the IMP. This means that there are facts (evidence) or arguments to suggest a causal relationship

The definition covers also medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product.

10.1.3 Serious Adverse Event (SAE)

A Serious Adverse Event (SAE) is any untoward medical occurrence 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**
    - Development of secondary malignant neoplasm
    - Overdose (with or without associated AE/SAE)

* A life-threatening event is one where the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if more severe.

**A medically significant event is 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 (eg,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 inpatient hospitalization, development of drug dependency drug misuse or drug abuse, transmission of an infectious agent (organism, virus or infectious particle) by the study drugs. .

The following are not considered to be serious adverse events (SAE):

- Events exclusively related to tumour relapse/progression or treatment of tumour relapse/ progressions
- 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
- Observation or short-stay units
- Hospitalization due to diagnostic procedures or standard supportive care (e.g. implant of central venous catheter)
- 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 (e.g., subject has no place to sleep; hospice facilities)
- Administrative admission (e.g., for yearly physical examinations)
- Protocol-specified admission during a clinical trial (e.g., for a procedure required by the study protocol or for clinical research)
- Optional admission not associated with a precipitating clinical AE (e.g., for elective cosmetic surgery)

10.1.4 Suspected Unexpected Serious Adverse Reaction (SUSAR)

A Serious adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. investigator's brochure for an unauthorised investigational product or summary of product characteristics for an authorised product)'.

10.2 Recording and assessing adverse events

Any AE which occurs or comes to the attention of the investigator from the time consent is given up to 30 days after the last study drug administration whether or not considered related to the IMPs must be recorded in the CRF.

The following AE must be recorded in the CRF :

- Any AE which occurs or comes to the attention of the investigator from the time of registration up to 30 days after the last study drug administration, whether or not considered related to the IMPs.
- Any AE considered to be reasonably related to the study treatment(s) or the research which occurs or comes to the attention of the investigator after this period of 30 days up to patient's discontinuation of the study.

All late Serious Adverse Events (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).

If in any one subject the same AE occurs on several occasions, then the AE in question must be documented and assessed anew each time.

For SAEs, a SAE report must also be submitted to the sponsor immediately, without undue delay.
The following aspects must be recorded for each event in the CRF.

- A description of the AE in medical terms, not as reported by the subject;
- The date of onset (start date)
- The date of recovery (stop date)
- The grade as assessed by the investigator according CTCAE (v5.0) toxicity criteria
- Action taken on study drugs (e.g. none, medication discontinued, dose reduction, medication delayed, reduction of infusion rate…).
- Other action (none, corrective treatment given, surgery..).
- The outcome according to the following definitions:
    - Recovered with sequelae (nature of the sequelae).
    - Recovered without sequelae.
    - Ongoing
    - Died
    - Unknown
- Seriousness: yes or no
- The causal relationship assessed by the investigator.

The site investigator is responsible for assessing the relationship between the AE and study drugs.

Sites investigators must determine whether there is a reasonable possibility that the study agent(s) caused or contributed to an AE/SAE. The relationship assessment, based on clinical judgment, often relies the following:

- A temporal relationship between the event and the administration of the study drug(s).
- A plausible biological mechanism for the agent to cause the AE/SAE
- Previous reports of similar AEs/SAEs associated with the study drug(s), or other drug in the same class
- Recurrence of the AE/SAE after re-challenge or resolution after de-challenge (drug withdrawal), if applicable
- Another possible aetiology for the AE/SAE (concomitant drug, concurrent disease/condition, underlying cancer disease…)

The terms used to assess the relationship of an event to study drug(s) are :

- Related: there is a reasonable possibility that the AE/SAE may be related to the study drug(s)

- Not related: : there is not a reasonable possibility that the AE/SAE is related to the study drug(s)

If there is insufficient or incomplete evidence to make a clinical judgement of the casual relationship, the site investigator is allowed to qualify the event as 'not assessable'.

When an AE/SAE is assessed as 'not related' to study drug(s), an alternative aetiology, diagnosis or explanation should always be provided.

If new information becomes available, the relationship assessment of any AE/SAE should be reviewed again and updated, as required.

10.3 Reporting of Serious Adverse Events (SAE)

Any 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, without undue delay via my eclinical report, a web portal that allow electronic transmission of SAEs/AESI https://www.evereport.eu/myeclinical/form/IGR/login.php or if not possible by fax using a SAE report form: +33 (0) 1 42 11 61 50

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 Serious Adverse Events (occurring after this period of 30 days) considered to be reasonably related to the study treatment(s) or the study procedures must be reported (no time limit).

Information collected 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 SAE:

- SAE (verbatim)
- Description of SAE
- Grade according to CTCAE v5.0
- Start date and stop date
- Date Investigator became aware of SAE
- Seriousness criterion
- Date of hospitalization (if applicable)
- Date of discharge (if applicable)
- Probable cause of death (if applicable)
- Date of death (if applicable)
- If autopsy was performed, result (if applicable)
- Causality assessment in relation to study drugs and to study procedure(s)

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

- A copy of the summary of hospitalization or prolongation of hospitalization
- A copy of the post-mortem report (if applicable)
- 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

Follow-up patients after adverse event information

The investigator is responsible for following all SAEs 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, without undue delay. The investigator also transmits the final report at the time of resolution or stabilization of the SAE.

10.4 Reporting of exposure to study drug during pregnancy/lactation

In principle, women of childbearing potential must have a negative urine or serum β-HCG pregnancy test within 7 days prior to the administration of the first study treatment.

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 without undue delay (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 fetus.

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.

Paternal Exposure

Male patients should refrain from fathering a child or donating sperm during the study and for one year.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should, if possible, be followed up and documented.

Where a report of pregnancy is received, prior to obtaining information about the pregnancy, the Investigator must obtain the consent of the patient's partner.

10.5 Responsibilities of the Sponsor

10.5.1 SAEs assessment

The Pharmacovigilance Unit at GR will assess the SAE in terms of seriousness, severity (NCI-CTCAE 5.0), relationship to the study drugs and expectedness. All SAEs will be coded using MedDRA.

10.5.2 Suspected Unexpected Serious Adverse Reactions (SUSARs)

To comply with regulatory requirements, the coordinating sponsor will identify all SAEs that are related to the investigational medicinal product and unexpected (ie, not previously described in the investigator brochure). 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 ethics committees and to the Eudravigilance database. All SUSARs reports and all reports involving expected serious adverse drug reactions that are fatal will additionally be forwarded to all study investigators and to the IDMC.

10.5.3 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. This DSUR will be submitted to the concerned competent authorities and ethics committees according to national legislation.

11 DATA HANDLING AND RECORD KEEPING

11.1 Data Collection

Data entry ideally will be done online by each treating physician via internet.

Data reported on each form should be consistent with the source data. If information is not known, this must be clearly indicated on the form. All missing and ambiguous data will be clarified. All sections are to be completed before being submitted.

In all cases it remains the responsibility of the Investigator to ensure that the CRF has been completed correctly and that the data are accurate.

11.2 Archiving

It is the responsibility of the Principal Investigator to ensure all essential trial documentation and source records (e.g., signed Informed Consent Forms, Investigator Site Files, Pharmacy Files, patients' hospital notes, copies of CRFs etc) at their site are securely retained for at least 15 years after the end of the trial.

Do not destroy any documents without prior approval from the Sponsor.

12 QUALITY MANAGEMENT

The trial is being conducted according to the current guidelines for Good Clinical Practice (GCP). All sites will be monitored by their respective National Coordinating Centres to confirm compliance with the protocol and the protection of patients' rights as detailed in the Declaration of Helsinki.

Site Set-up and Initiation

All sites will be required to sign appropriate contracts with their National Coordinating Centre prior to participation. In addition all participating Investigators will be asked to sign the necessary agreements and supply a current CV with evidence of recent GCP training to the National Coordinating Centre.

All members of the site research team will also be required to sign a Site Signature and Delegation Log which lists the range of duties that have been delegated to them for the trial. This should be countersigned by the Principal Investigator and returned to the National Coordinating Centre.

Prior to commencing recruitment all sites will undergo a process of initiation. Key members of the site research team will be required to attend either a meeting or a teleconference covering aspects of the trial design, protocol procedures, Adverse Event reporting, collection and reporting of data, and record keeping.

Sites will be provided with an Investigator Site File and a Pharmacy File containing essential documentation, instructions, and other documentation required for the conduct of the trial.

The National Coordinating Centre must be informed immediately of any change in the site research team.

12.1 On-site Monitoring

Monitoring will be carried out as required, following a risk assessment and as documented in the Monitoring Plan. It is the responsibility of participating National Coordinating Centres to ensure that the level and process of monitoring described in the Monitoring Plan is in accordance with their national regulations and to inform the Sponsor of any issues.

Additional on-site monitoring visits may be triggered for example by poor CRF completion rates, poor data quality, low or high SAE reporting rates, excessive number of patient withdrawals or deviations.

If a monitoring visit is required, the National Coordinating Centre will contact the site to arrange a date for the proposed visit and will provide the site with written confirmation. Investigators will allow the VERITAS trial staff access to source documents as requested.

12.2 Central Monitoring

Trial staff from the Sponsor will be in regular contact with the National Coordinating Centre/site research team to check on progress and address any queries that they may have. Trials staff will check incoming CRFs for compliance with the protocol, data consistency, missing data and timing. Data Clarification Forms requesting missing data or clarification of inconsistencies or discrepancies will be sent electronically to sites.

National Coordinating Centres/Sites may be suspended from further recruitment in the event of serious and persistent non-compliance with the protocol and/or GCP. Any major problems identified during monitoring may be reported to the Sponsor and the relevant regulatory bodies. This includes reporting serious breaches of GCP and/or the trial protocol to the appropriate regulatory bodies.

12.3 Audit and Inspection

The Investigator will permit trial-related monitoring, audits, ethical review, and regulatory inspection(s) at their site, providing direct access to source data/documents.

National Coordinating Centres and research sites are also requested to notify the Sponsor of any inspections by the Competent Authorities.

13 END OF TRIAL DEFINITION

For the purposes of the Competent Authorities, the end of trial is defined as the Last Patient Last Visit which will occur 3 years after the randomisation of the last patient in the study.

The National Coordinating Centre will notify the National Competent Authorities and Ethics Committees that the trial has ended at the appropriate time and will provide them with a summary of the clinical trial report within 6 months of the end of trial.

A separate long term Follow-Up study is planned in order to gather data regarding OS and EFS. 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 and will not be part of the Clinical Study Report.

14 STATISTICAL CONSIDERATIONS

14.1 Main Analyses

The main analysis will be based on the EFS analysis on all the patients included in the randomised trial (intention-to-treat).

A hierarchical testing procedure will be followed.

1) Each arm will first be analysed as a single-arm phase-2 study comparing the 3-year EFS to the null hypothesis p0=15%, with a one-sided alpha=0.05 (one-step Fleming design). This null hypothesis has been defined based on previous experience and considering the burden of evaluated strategies: such intense treatment cannot be justified if it is associated with 3-year EFS lower than 15%. Assuming that the 3-year EFS is evaluable for 75 patients (no censored data before 3 years, leading to a binomial distribution of the 3-year EFS), the treatment will not be considered efficient enough if there are less than 17 patients alive free of event at 3 years. In each arm, if at least 17 of the 75 patients are alive free of event three years after randomisation, we will conclude a benefit, as the 3-year EFS is significantly higher than 15%. If some EFS times are censored in the first three years, the 3-year EFS rate will be estimated using Kaplan-Meier method and its one-sided 95% confidence interval will be tested against the null hypothesis.
2) If both treatment arms appear associated with a benefit, the EFS curves of both experimental groups will then be compared. EFS curves will be estimated using Kaplan-Meier method and compared using a log-rank test. The relative treatment effect (hazard ratio for event, Arm A versus B) will be estimated in a multivariable Cox model, assuming proportional hazards, and controlling for the stratification variables. For a pragmatic reason of feasibility (very rare setting) a two-sided alpha=0.20 will a priori be used. With this approach, the power of the comparison remains acceptable (see §14.4).

14.2 Secondary Analyses

The whole EFS curve of each treatment group will also be compared to historical curves drawn from HR-NBL1 study, after selection of similar patients (high risk neuroblastoma with poor response to induction chemotherapy),

In addition to the frequentist hypothesis-driven approach, a Bayesian approach will be considered to describe treatment effect distribution, with several priors including a non-informative prior. One treatment will be considered superior to the other one if there is a 90%-probability that it is more efficient.

Heterogeneity of relative treatment effect (Arm A versus B) according to the stratification variables will also be explored using forest plots and two-sided interaction tests.

As an exploratory analysis in addition to the main EFS comparison performed on the ITT-population from the date of randomisation, we will also compare the EFS curves from the date of start of the consolidation phase, excluding patients who have progressed or died before the start of the consolidation phase allocated by randomisation. Hence, some patients may progress or die during the three courses administered before the start of the consolidation phase allocated by randomisation. This may dilute the treatment difference if the distribution of these events is randomly balanced between compared groups, or may bias the true treatment effect estimate.

EFS curves will also be estimated from the date of neuroblastoma diagnosis.

Overall survival curves will be estimated using Kaplan-Meier method and compared using a log-rank test. The relative treatment effect (hazard ratio for event, Arm A versus B) will be estimated in a multivariable Cox model, assuming proportional hazards, and controlling for the stratification variables.

Adverse events, evaluated using NCI-CTCAE v5.0 toxicity grading system, will be reported by treatment phase and overall over the whole treatment duration (maximum grade). Incidence of grade >2 extra-haematological toxicity will be estimated by treatment arm and compared between treatment arms, overall and by system organ class (SOC).

A Q-TWIST analysis will be performed (Gelber and Goldhirsch). Duration of hospitalisation will be used as a surrogate of time with severe toxicity. Overall survival will be partitioned into three states: toxicity (time with severe toxicity after randomisation and before progression/relapse), time without symptoms of disease progression/relapse and without toxicity; and time from progression until death.

Between-treatment differences in the mean duration of each state will be calculated. Q-TWiST will be obtained as a sum of time spent in the three health states, weighted by utility scores. The bootstrap method will be used for testing statistical significance. Threshold analysis and gain functions will allow a group comparison for different utility values. If both arms appear superior to the historical reference (p0=15%) but the EFS curves do not significantly differ between treatment groups, this Q-TWiST analysis will be considered to define the trade-off between both experimental treatments.

As detailed in a subsequent section, pragmatic considerations have driven the sample size calculation for this randomised evaluation of two experimental treatments in this very rare disease setting. Considering the current accrual in HR-NBL-1 protocol, the observed proportion of VHR-NBL and the anticipated participation rate, an accrual rate of 30 patients/year in the proposed randomised trial seems achievable, leading to a total of 150 patients over 5 years of accrual.

If the accrual could be increased compared to the anticipated rate, the sample size would be re-estimated (adaptive design), blinded to the observed treatment effect, to allow for a smaller alpha, equivalent to a higher strength of evidence.

14.3 Planned Interim Analysis

No formal interim analysis of efficacy endpoint is planned as a sufficient follow-up is required to get a reliable estimate of EFS curves. Moreover there is no obvious reason that should lead to the early termination of the trial: early stopping for major efficacy is very unlikely, and early stopping for futility does not make sense in this context.

We will perform a close monitoring of treatment-related deaths and adverse events leading to ventilation in an ICU over the first 6 months after randomisation (stopping rule for toxicity). Each arm will be monitored separately. Statistical stopping rules will be defined based on past experience with high-dose chemotherapy in the HR-NBL1 trial.

14.4 Sample size and power Calculations

Pragmatic considerations have driven the sample size calculation for this randomised evaluation of two experimental treatments in this very rare disease setting. Considering the current accrual in HR-NBL-1 protocol, the observed proportion of VHR-NBL and the anticipated participation rate, an accrual rate of 30 patients/year in the proposed randomised trial seems achievable, leading to a total of 150 patients over 5 years of accrual.

If the accrual could be increased compared to the anticipated rate, the sample size would be re-estimated (adaptive design), blinded to the observed treatment effect, to allow for a smaller alpha, equivalent to a higher strength of evidence.

For each treatment group considered as a single-arm study, 75 patients yield a 94%-power to conclude a significant benefit compared to p0=15% if the true 3-year EFS is 30%, with a one-sided alpha=0.05, assuming no censored data in the first three years.

The number of 150 patients (119 events) allows for a randomised Phase-II trial with the following hypotheses/ parameters (East 5.3):

- comparison of EFS-curves of both experimental treatments,
- exponential EFS distribution,
- two-sided alpha=0.20,
- 80%-power to detect a 14.4%-increase of 3-year EFS (30% versus 44.4%, HR=0.675).

15 TRIAL ORGANISATIONAL STRUCTURE AND CONDUCT

15.1 Coordinating Sponsor

The trial is being conducted under the auspices of GUSTAVE ROUSSY, France.

The Coordinating Investigator in each country, known as the National Coordinating Investigator, on behalf of the Sponsor, is responsible for the application for an Ethics Committee/Institutional Review Board approval according to national and institutional guidelines. Furthermore, the National Coordinating Centre/Coordinating Investigator will provide all authorised institutions of the participating countries with all documents required for an IEC/IRB approval according to local law and regulation.

The authorised institutions will provide all further documents required by national law and for application at the responsible Ethics Committee.

Strict adherence to all specifications laid down in this protocol is required for all aspects of the trial conduct; the investigator may not modify or alter the procedures described in this protocol.

15.2 Independent Data Monitoring Committee

Analyses will be supplied in confidence at least annually 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 may meet prior to the trial opening 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.

Though unlikely in this early phase study, it is possible that the trial might also stop early if the interim analyses showed differences in activity between treatments that were deemed to be convincing to the clinical community.

16 ETHICAL AND ADMINISTRATIVE CONSIDERATIONS

The accepted basis for the conduct of clinical trials in humans is founded on the protection of human rights and the dignity of human beings with regard to the application of biology and medicine and requires the compliance with the principles of Good Clinical Practice (GCP) and detailed guidelines in line with those principles (Directive 2001/20/EC (2) and Directive 2005/28/EC (47, 48).

GCP (Good Clinical Practice) is a set of internationally recognised ethical and scientific quality requirements which must be observed for designing, conducting, recording and reporting clinical trials that involve the participation of human subjects. Compliance with good clinical practice provides assurance that the rights, safety and well-being of trial patients are protected, and that the results of the clinical trials are credible (Article 1 of Directive 2001/20/EC (47)).

The Sponsor and Investigators shall consider all relevant guidance with respect to commencing and conducting a clinical trial (Article 4 of the Directive 2005/28/EC) and take into account the consensus on harmonisation for GCP by the International Conference on Harmonisation – ICH (Directive 2005/28/EC (48)).

Before patients can be registered on to the study, each site must obtain all necessary regulatory approvals in accordance with their national laws. It is the Principal Investigators responsibility to ensure any subsequent amendments gain the necessary approval. This does not affect the clinicians' responsibility to take immediate action if thought necessary to protect the health and interest of individual patients.

Sites will not be permitted to register patients until written confirmation of the institutional approval has been received by the Sponsor and the National Coordinating Centre.

16.1 Confidentiality and Data Protection

Personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the appropriate laws in each participating nation state.

Patients will be identified using only their unique trial number, initials and date of birth on the CRFs and on correspondence between the Sponsor, the National Coordinating Centres and participating sites.

The National Coordinating Centre may receive from other parties and the Sponsor's business information, trade secrets and/or confidential or proprietary information belonging to other parties. All such information which is designated as **confidential information** shall not be used for any purpose other than participation in the clinical trial and performance of obligations under this contract.

The National Coordinating Centre shall not copy or disclose any confidential information to a third party with the exception of employees, subcontracted partners, and healthcare professionals that require access to this information to perform their transferred obligations.

The restrictions shall not apply to information that was already in the possession of the Sponsor in receipt of the confidential information concerned before disclosure (except as a result of a breach of this agreement); information obtained independently from a third party source that was free to disclose the same; information that is in the public domain (except as a result of a breach of this or any other agreement); information that is required to be disclosed by law or any governmental or regulatory authority.

16.2 Insurance and Indemnity

The National Coordinating Centres are responsible for obtaining insurance to set up and run the VERITAS trial in their respective countries and for ensuring that sites in their country are adequately covered.

16.3 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 Study Coordinators will write an article reporting on the results as soon as possible after the final analysis and will be the first and final authors of the publication. Other authorships authorship will be determined by mutual agreement, taking account of the contribution made by each investigator/site, according to the SIOPEN rules.

17 APPENDIX 1: NATIONAL COORDINATORS CONTACT DETAILS

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18 APPENDIX 2: TOXICITY

18.1 Common Toxicity Criteria Grading (NCI – CTCAE version 5.0)

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

18.2 Sinusoidal Obstruction Syndrome Toxicity

Table 9 - Toxicity after High Dose Chemotherapy (CTCAE v5.0)

	Grade I	Grade II	Grade III	Grade IV	Grade V
Sinusoidal Obstruction Syndrome (SOS)		Blood bilirubin 2-5 mg/dL; minor interventions required (i.e., blood product, diuretic, oxygen)	Blood bilirubin >5 mg/dL; coagulation modifier indicated (e.g., defibrotide); reversal of flow on ultrasound	Life-threatening consequences (e.g., ventilatory support, dialysis, plasmapheresis, peritoneal drainage)	Death

Severe SOS is defined as: CTCAE v5.0 Grade 4 SOS or Grade 3 SOS **PLUS** a specific organ failure listed below:

- CTC Grade 4 hepatic failure, OR
- Pulmonary dysfunction: Continuous oxygen support for >48 hours, ventilatory support not clearly attributable to another cause, OR
- Renal dysfunction: serum creatinine >3 times the ULN for age and sex (CTC Grade 3 creatinine), or the need for dialysis (CTC Grade 4 acute kidney injury), not clearly attributable to another cause.

19 APPENDIX 3: Performance Scales

19.1 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.

19.2 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.

20 APPENDIX 4: PERIPHERAL STEM CELLS HARVEST AND STORAGE

20.1 Paediatric apheresis procedure

20.1.1 General Principles in 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 paediatric use because they have smaller extra corporal volumes (ECV).
- In older children with a body weight greater than 30 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 therapy.

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

A third factor is prevention of hypocalcaemia that results from chelation of calcium by citrate in the anticoagulant.

PBSC harvesting can begin at best when the peripheral CD34+ count is > 20 cells/μL. The paediatric apheresis procedure should be performed by an experienced paediatric team[3].

20.1.2 Apheresis Machine

Apheresis machines equipped with continuous flow centrifugation, such as the Optia with Spectra, Optia IDL7 device and CCMN10300 program or the Fenwal CS 3000+, are recommended because these devices are better tolerated than the discontinuous flow machines. Equipment should be operated in compliance with the manufacturer's operating guidelines.

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

20.1.2.1 Blood Priming

Priming of the machine prior to collection should be with Acid Citrate Dextrose anticoagulant and saline according to manufacturer's directions.

- In haemodynamically instable patients or very small children (BW < 15 kg) the priming should be performed by 5% albumin solution instead of saline solution.
- For patients less than 25 kg, a secondary prime with IRRADIATED[4], leukocyte-poor red blood cells should be done.

This is described in the standard operating procedures for each machine. The blood prime will be performed with cross-matched, irradiated, filtered red cells.

20.1.2.2 Procedural Support

Use of an apheresis machine in-line blood warmer on the return line.

There is evidence in the literature that apheresis procedure could be performed in children with platelet counts below 20 x 10^9 /L (53).

[3] A very experienced paediatric team would be a team performing at least 20 apheresis procedures per year for children younger than 12 years old, and at least 5 apheresis per year for children below 10kg

[4] Blood cells must be irradiated for **ALL** patients, in order to prevent GvH reaction when graft potentially containing T lymphocytes will be infused to patients

20.1.2.3 Anticoagulant

Anticoagulant to be used is Acid Citrate Dextrose Formula - A (ACD-A) in a ratio sufficient to prevent extracorporeal clotting. Heparin anticoagulation is not recommended for use in PBSC collections except for patients with an allergy to citrate.

Note : Hypocalcemia is a well-recognised side effect of citrate. To prevent hypocalcaemia a prophylactic calcium gluconate infusion 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.

20.1.2.4 Whole Blood Flow Rate

The following rates are designed to avoid citrate reactions and thus boluses and continuous infusions of calcium can be avoided:

- <2 years (<15 kg) 15-20 ml/min (initial; may be increased to 25-30 ml/min by ratio ramping)
- 2-5 years (15-20 kg) 25-40 ml/min
- >5 years 35-50 ml/min

20.1.2.5 Collection Goals

During each leukapheresis procedure, the volume of whole blood processed should be approximately 240 to 480 ml/kg (3 - 4 blood volumes). The total time necessary for the whole apheresis procedure should not exceed 5 h.

The optimal collection goal (total for all collections) is **more than 6 x 10^6 CD 34+ cells/kg**. The targeted number of cells can usually be obtained in 1-3 collection days.

20.1.2.6 Patient Monitoring

Patients should be observed continuously during the collection. Vital signs should be obtained every 15 minutes.

20.1.2.7 Laboratory Studies

For patients < 25 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 the following minimal lab values should be obtained: CBC with differential and platelet count, ionised calcium and magnesium.

20.1.2.8 Vascular Access

For continuous flow apheresis, two sites of venous access are required. In patients less than 25 kg use 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.

20.2 Cryopreservation of PBSC Products

Each collection should be processed and cryopreserved within 18 hours of collection using 10% dimethyl sulfoxide (DMSO) final concentration, controlled-rate freezer, and liquid nitrogen storage. Stem cells should be frozen at a final concentration of 0.5 to 1.2 x 10^8 nucleated cells/ml **in at least 3 bags (ideally 4 bags).**

20.3 Autologous Stem Cell Reinfusion

20.3.1 Fluid Management

Hydration with D5 0.45 NS +/- KCl or 0.9 NS should begin 2-4 hours prior to the infusion and be continued for at least 4 hours following infusion. Intravenous fluids on the day of PBSC infusion, excluding the volume of cells infused, should reach 3000 ml/m²/24 hours.

20.3.2 Premedication

If stem cells are not washed, the DMSO cryoprotectant may cause a histamine-like reaction when infused into the patient.

Therefore premedication with antihistamines (i.e. Benadryl) is recommended, to be performed according to local practices.

20.3.3 Thawing of PBSC

Thawing of PBSC should be performed according to institution's guidelines.

If performed at patient's bedside, the following is recommended:

- PBSC are thawed in a 37oC water bath which is monitored with a mercury thermometer to ensure temperature does not rise above 40oC.
- Only one bag of PBSC should be thawed at a time. In the event of bag breakage, every effort should be made to maintain sterility and salvage the PBSC component using a syringe with a large bore needle.
- When the infusion of one bag is completed, the next bag should be thawed.
- When the final bag of PBSC has been infused, the IV tubing should be flushed with normal saline.

21 APPENDIX 5: BONE MARROW SAMPLING AND EXAMINATION 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 **six** samples, **four aspirates and two trephines,** according to INSS guidelines.

The bone marrow evaluation takes place at study entry (E1), prior to intensified consolidation (E2), and at the end of local treatment (i.e., at the end of all VERITAS study treatment) (E5).

The following guidelines have been developed for the purpose of improving initial staging accuracy, treatment response evaluation, and, ultimately, patient care, by enabling a highly sensitive technique for detection and characterisation of rare neuroblastoma cells or tumour cell associated RNA. Bone marrow (BM) aspirates from four sites (left and right body sides) have to be performed. In addition, two trephine biopsies are mandatory.

With regards to immunocytology (IC) and QRT-PCR , there is a general international consensus on the necessity to establish an international standardisation of these two techniques, in order to make **results** from different centres **comparable** and to agree upon **cut-off values** for the clinical management of MRD (54). Therefore, although the results of IC and QRT-PCR are not directly comparable, a side by side comparison of both techniques with regard to their predictive prognostic power is relevant. Both the Bone Marrow Immunocytology and the Molecular Monitoring study committees therefore agreed upon a revised sampling procedure which includes **splitting** of the bone marrow sample in order to facilitate this request.

21.1 Bone Marrow Aspirations

21.1.1 Sampling and split of the samples

BM aspirations are necessary for bone marrow smears, immunocytology, QRT-PCR 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.

- **Priority aspiration and preparation of bone marrow smears**

Half a millilitre (0.5 ml) of BM is aspirated into the syringe and **immediately** dropped on a glass slide.

- **Priority aspiration** of 0.2 to 0.5 ml BM for 10 smears per side air dried for cytology (e.g. Pappenheim stained, keep at least 5 slides unstained).

- **Aspiration for immunocytology and QRT-PCR**

The appropriate amount of anticoagulant (e.g. 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 BM from each side into two single PAXgene™ tubes for QRT-PCR studies. Do not pool.
  - Send the filled PAXgene™ tubes to the national reference laboratory or to Prof. Sue Burchill, Leeds*.

- transfer 4.5ml (remainder) to National Immunocytology Reference Laboratory for processing of at least $3x10^6$ cells on cytospins by isolating mono nuclear cell suspension and using an adequate cytocentrifugation machine (e.g. Hettich)
  - ideally 2 times 3 x 10^6 cells on cytospins should be produced for quality controlled assessment of minimal disease.

- From the above bone marrow sample taken at diagnosis, place 1ml into a LAM tube (UK only).

Tubes are provided by the reference laboratory in Leeds (Prof. Sue Burchill or Dr Virginie Viprey.).

* In UK only; take 1ml of bone marrow aspirate each side into LAM tubes (provided by reference laboratory in Leeds). Send this sample to Leeds at room temperature, next day delivery (see below). Samples collected into LAM tubes (1ml each side at diagnosis, UK only) and heparin (remaining aspirate) should be mailed within 24h to country reference laboratory. If samples are taken on Friday, keep in fridge and mail on Monday.

21.1.2 Handling of the bone marrow cells in the laboratory

Separation of MNC, preparation and storage of cytospin preparations, immunocytological staining, evaluation criteria and reporting of results:

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 (54, 55).

Immunocytological staining can also be combined with FISH and evaluated using an automated scanning and relocation system (AIPF) (e.g. Metafer4/*RC*Detect, MetaSystems, Altlussheim, Germany) (56). Further detail on SOPs for QRT-PCR studies are described in Viprey *et al* (57).

21.2 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); 1 cm of cortical bone/cartilage and 2 mm of BM is inadequate for assessment. Trephine biopsies must be obtained by an experienced operator!

Overall, 10 unstained sections from each core biopsy and a copy of the local pathology report must be sent for central review to Dr. Klaus Beiske or to Dr. Angela Sementa.

21.3 Storage of Tumour Material, Slides and Bone Marrow Samples

It is mandatory to store material and slides from each tumour, biopsy and bone marrow and peripheral blood sample. This is important to conduct further/future biological and genetic analyses and to allow review and quality assessment studies.

In case of tumour resection and open and tru cut biopsies, the storage of **snap frozen material (at –70°C or below)** is most important. Besides this, it is advisable to store **touch preparations and cytospin preparations at –20°C** and **cell suspensions** (including DMSO), if available, **in liquid nitrogen**. Tumour cells obtained by fine needle biopsy can be stored either as cytospin preparations or cell suspensions.

The MNC fraction of BM and PB can be stored as cytospin preparation at –20°C and cell suspensions (including DMSO) in liquid nitrogen.

Furthermore, stained slides, IF/FISH images and QRT-PCR pictures have to be stored adequately for documentation and review purposes.

22 APPENDIX 6: WHOLE BODY AND TUMOUR DOSIMETRY CALCULATIONS

22.1 Whole body dosimetry calculations

Using the data collected as described in section 9.1.2.1.4, plot activity-time data and fit decay phases to the data. Integrate to determine the cumulated activity Ã. Determine the relevant S value from the patient weight. Calculate the whole-body dose using standard MIRD, i.e., D = ÃS.

22.1.1 Dose calculations

Whole-body absorbed dose calculations are made according to standard MIRD methodology. The activity-time data are plotted and integrated to determine the cumulated activity Ã. The number of phases to which the data fit must be chosen interactively. The potential error on this decreases with the number of data points.

The MIRD S value (wb -> wb) is determined according to the patient's weight. MIRD S values are available for newborn, 1 year old, 5 year old, and adults.

From these, an (empirical) equation may be generated to determine a patient-specific S value:

$$S = 1.96e-06 \times 70 \times Wt^{-0.918}\ \mathrm{MBq\,h}$$

where Wt is the patient's weight (kg). The absorbed dose is then given by D = ÃS

22.1.2 Error analysis

The accuracy of the absorbed dose estimate will depend largely on the accuracy of the measurements. The uncertainty on the absorbed dose can be determined according to methodology available in the literature.

Spreadsheets for this purpose are available on request from Glenn Flux, Dept. of Physics, Royal Marsden NHS Trust, London, UK (glenn@icr.ac.uk).

22.1.3 Calculation of the activity to administer for the second therapy

This is a straightforward calculation by ratio.

An example is given here:

If a first therapy administration of 7250 MBq resulted in a whole-body dose of 1.82 Gy, then the dose required from the second therapy is 2.18 Gy (to total 4 Gy).

Therefore :

$$\mathrm{Activity\,(2nd\,therapy)} = \frac{2.18}{1.82} \times 7250 = 8684\ \mathrm{Gy}$$

23 APPENDIX 7: 131I-MIBG GUIDELINES AND SAFETY

Caution : these are guidelines only. Please also consult your national guidelines and comply with your national regulations.

23.1 Radiation Protection Guidelines

23.1.1 Local rules

Every hospital will have a Radiation Protection Advisor who is responsible for policies and procedures related to radiation safety, called local rules, when using unsealed radioactive sources for cancer treatment, and to ensure that relevant legislation is fully complied with. The treating centre's Radiation Protection Advisor will therefore need to be kept fully informed.

23.1.1.1 Radiation protection of the patient

The patient must be in a radiation-protected room from the start of the infusion for around 10 days.

The amount of residual activity in the patient should be measured and charted each day.

The patient will be allowed to leave the protected room when the dose rate will be lower than 20 µSv/h at a distance of one metre.

23.1.1.2 Radiation protection of the parents

Parents or other responsible (non-pregnant) adults may consent to be designated as comforters and carers. These individuals may undertake routine child care such as feeding, washing toileting and dressing the child, in order to provide comfort, and to limit nursing exposure to essential nursing duties.

See also the Guidelines for parents during the hospital stay, section 23.5.1.

23.1.1.3 Radiation protection in case of emergency

In the event of an emergency, such as the need for an acutely unwell child to receive intensive care, the immediate needs of the child take priority over the limits set out below.

23.1.2 Radiation Restrictions

The radiation restrictions are modulated according to the remaining radiation activity emitted by the patient:

>800 MBq	Remain in hospital in Protected Room.
	Full precautions (apply the local rules).
400-800 MBq	Must be in Protected Room if needing medical/nursing care in hospital. If well may go out in private transport.
	Must avoid public places.
	Must avoid contact with other children or women who are or might be pregnant. Must sleep separately.
150-400 MBq	Must be in Radiation Protected Room if needing medical/nursing care. If well may go out.
	Must avoid prolonged contact with other people in public places.
	Must avoid contact with other children or pregnant women. Must still sleep separately

30-150 MBq	Must be in side room on general paediatric ward if needing medical/nursing care. If well may go out. Must avoid contact with other children or pregnant women.
<30 MBq	No restrictions. May be on open children's ward if unwell. May receive stem cells. If there is anxiety about myelosuppression, stem cells may be returned if level is greater than 30 MBq at clinician's discretion.

23.2 Thyroid blockade

Given the possible consequences of thyroid irradiation in children, a thyroid blockade is recommended (58).

The classic protection of the thyroid by potassium iodide, or by potassium perchlorate in case of allergy to iodine or if forgotten, turns out to have limited effectiveness. The visualisation rate of the thyroid on scintigraphy is 21%, and the rate of subsequent hypothyroidism can reach 82% within 2 years following treatment among surviving patients(59-61) .

The thyroid function must be controlled weekly during the 131I-mIBG therapy.

The thyroid blockade must be started two days prior to the 1st mIBG infusion, up to 28 days after the 2nd mIBG infusion, according to the protocol described below.

Table 10 - Thyroid blockade protocol

	Drug	***Dosing regimen***	***Treatment Start***	***Treatment end***
1	Thyroxin	100µg/m² once a day per os	D-2 [a]	28 days after the 2nd ^{131}I-mIBG administration
2	Neomercazole	10 mg/m² twice a day	D-2 [a]	28 days after the 2nd ^{131}I-mIBG administration
3a	Lugol solution [b]	1 drop/kg/day tid *Only if no allergy to iodine*	D-2 [a]	10 days after the 2nd ^{131}I-mIBG administration
Or				
3b	Potassium perchlorate (62)	< 5 kg : 100 mg/d 5 – 15 kg: 200 mg/d > 15 kg: 300 mg/d	1st administration before the mIBG infusion	15 days after the 2nd ^{131}I-mIBG administration

a : D-2 : i.e., 48h before the 1st ^{131}I-mIBG administration

b : iodine 1 g, potassium iodide 2 g, purified water qsp 100mL

23.3 Concomitant medications to be avoided

Table 11 - Drugs containing active substances that must be avoided during the ^{131}I-mIBG therapy

Antidepressant agents : Amitriptyline and derivatives Amoxapine Clomipramine Desuoraline Dosulepine Doxepine Imipramine and derivatives Maprotiline Loxapine Maprotiline Opiridamol Quinupramine Trazodone Trimipramine	Drugs used for cardiac disorders and that contains the following substances: Cordarone Digitoxine Israpidine Labétalol Nicardipine Calcium channels inhibitors: Nifedipine, Diltiazem, Verapamil Réserpine Bethanidine Debrisoquine Bretylium Guanethidine
Bronchodilatating agents : Fenoterol Salbutamol Terbutaline Xylometazoline	Agents lowering the nasal mucosa congestion : Phenylephrine Ephedrine Phenylpropanolamine

(extracted from Bombardier *et al,* 2010 (63))

23.4 Guidelines for Health Care Workers

23.4.1 Guidelines for Health Care Workers at the experienced centre

The following guidelines have been developed for the staff of the highly experienced centre (validated by the coordinating sponsor) which will administrate the ^{131}I-mIBG. Please use them as a template to be adapted according to your local regulations.

The usual precautions for radiation-limiting exposure of the staff in Curietherapy Unit also apply to this clinical trial. However, please take into account the additional features described in this document.

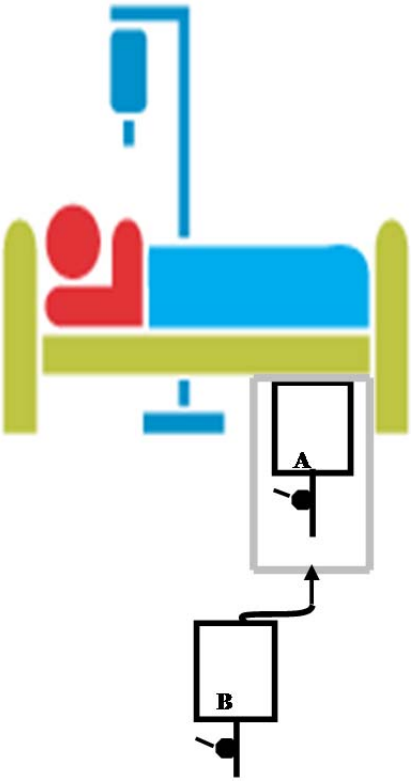
Patients included in the VERITAS trial received a first administration of ^{131}I-mIBG at a rate of 444 MBq / kg body weight (12 mCi / kg body weight) without exceeding 11 GBq (300 mCi).

A second administration takes place two weeks after the first one.

The slow infusion of the radiopharmaceutical, over 2 hours through a self-pulsed syringe disposed in a sealed truck, is made in the patient's room. The device is set up by the nuclear physician. When the injection is complete, there will be a signal; then please prevent the nuclear physician who will disconnect the injection system.

In order to limit the irradiation of caregivers, we will ask the family members to relay themselves at child' side, in order to provide routine care. These people will have a dosimeter to know at all times the radiation dose received. Your duty in the room will be limited to nursing and cleaning the room.

Intravenous hydration and a urinary catheter will be in place for the full duration of hospitalization in the curietherapy ward to prevent the bladder toxicity of this product.



For radiation protection and for asepsis purposes, a urinary system with two pockets in series has been approved for this trial. Please find below the instructions for draining urine:

1 When the proximal pocket (A), located in the sealed bin, is filled, connect a second pocket (B) at the valve of the bag (A)

Open the valve of the bag A, the urine flows into the distal bladder B. Keep open up to completely emptying the proximal pocket A.

3 Close the valve on the bag A which is located between the two pockets

4 Using a sterile dressing that will prevent contamination of soil with a few drops of urine, separate the two pockets at the connector level

Seal the upper opening 5 of the bag B and the valve of the bag A by using sealing film

6 Drain the pocket B in the appropriate toilet avoiding the risk of splashes. Then throw the empty bag and the pad used in the sealed bin.

Handling urine requires, as usual, wearing a mask and disposable gloves which are then thrown into the trash sealed.

23.4.2 Guidelines for Health Care Workers in case of hospitalization in another facility

The following guidelines should be provided to the parents for being provided to the hospital staff in case of hospitalization of the child in another ward, whatever the reason is.

They should be adapted according to local regulations.

All contact details of the investigating centre must be joined to these guidelines.

This child is included into a clinical trial. Within this trial, he/she received a chemotherapy with a radiopharmaceutical compound (^{131}I-mIBG).

He is now hospitalized in your facility. This radionuclide is naturally eliminated through the urine and / or feces. One patient who received such treatment may cause very low level radioactive waste at this stage of the elimination of the radiopharmaceutical.

Note that the risk of infection is the most significant risk associated with the waste of health-care, whether radioactive or not, therefore it is requested to handle them with gloves.

No special precautions are necessary for hospital staff or visitors.

Waste management:

The companies responsible for the collection of hospital waste are obliged to check for the presence of radioactivity at the waste, even at very low level. Please check they are appropriately informed.

This approach involves the following solid wastes:

- Linen soiled with urine.
- Empty Pockets urinary (throw the urine in the toilet and flush the toilet twice).
- Diapers, sanitary pads and any absorbent material.

Guidelines for the recovery and identification of radioactive waste:

Soiled linen should be stored for radioactive decay in plastic bags identified with the patient's name before any transfer to the washing service.

Disposable materials must be stored in a suitable local to decay in sealed plastic bags before being eliminated in the sector of household and similar waste (DADM), except where there is a risk of infection (HCW).

All bags must be clearly labelled with the patient's name, date of collection, and the nature of the radioactive element.

Instructions for the duration of collection and storage:

The duration of solid waste collection mentioned above shall be 15 days from the date of treatment. The waste will be stored in a suitable facility for a period of 80 days.

In case of transfer of your patient to another care facility, please send these instructions to the person responsible for his/her management.

23.5 Guidelines for parents

The following guidelines are provided as a template to be adapted according to your local regulations.

23.5.1 Guidelines for parents during the hospital stay

The following guidelines should be communicated to parents (please mind to explain them to ensure they are clearly understood):

The treatment your child includes a radioactive product, which is why he/she is hospitalized in a specific room to ensure his/her medical management while protecting people and environment from radiation.

In order to minimize your radiation exposure, please apply the following recommendations:

- Avoid prolonged physical contact with your child;
- Stand at least 1 m away from the bed of your child as often as possible;
- Do not sleep in the bed of your child;
- Avoid contact with the saliva of your child ... be careful when kisses! If necessary, wipe the saliva using a disposable tissue and throw it immediately in the trash of the chamber;
- Do not use the same toiletries as your child (toothbrush, glass, towel and washcloth ...)
- Wear disposable gloves when handling the bag of urine, blood or vomiting. Then discard the gloves in the trash of the chamber;
- Stay away of the bag collecting the urine;
- Wash your hands regularly;
- Always wear the dosimeter when you are in the curietherapy ward.

Why all these precautions ?

Part of the radiation associated to the therapy remains in the tumour to destroy it, but some will come out of your child's body in different modes:

- Radiation may be passing out through his/her body in all directions;
- Radioactive molecules will be eliminated in the urine, which therefore will emit radiation;
- Radioactive molecules will be present in the saliva, which will also be a source of radioactivity;
- A very small amount of radioactivity can also be found in sweat.

Human exposure to ionizing radiation can induce diseases whose frequency and severity are directly related to the amount of radiation received.

This is why we always try to reasonably limit irradiation. This cause - effect relationship has been demonstrated from a radiation dose of 100 mSv. Below 100 mSv, no direct effects have been observed in humans, but as a precaution, the labour laws in France imposes to all staff working in contact with radioactivity, an annual limit that must not exceed 20 mSv.

To limit the irradiation of caregivers, who are exposed to radiation throughout the year, your participation is essential in this context.

That's why we ask you to be present at your child' side, so that the radiation received by each is as minimal as possible. You will have a dosimeter, a small measuring device to know at all times the radiation dose received.

In similar conditions, the dose received by family carers never exceeded 1 mSv for the duration of hospitalization.

For comparison: the natural radiation exposure is about 2.5 mSv/year in most European countries. A scanner causes irradiation from 2 to 10mSv.

23.5.2 Guidelines for parents when discharged home

At hospital discharge, the following guidelines should be communicated to parents (please mind to explain them to ensure they are clearly understood):

Your child can be discharged from the curietherapy ward when the radiation he/she emits has significantly decreased; however, it is not yet at a zero level, so some precautions should be taken:

For 8 days, after the hospital discharge:

- Let your child drink plenty of water, to promote urinary excretion of radioactive molecules still present and protect the bladder irradiation,
- Make him take a shower and change his underwear every day
- Make sure that no one uses the toiletries of your child (toothbrush, glass, towel, washcloth, etc ...)
- Wash your hands when you take care of your child, especially when you are in contact with the urine,
- If you see traces of urine on the edge of the toilet bowl (or chamber pot), wipe thoroughly with a paper towel and throw it in the bowl,
- Flush the toilet twice after each use by your child;
- Use paper tissues
- Avoid contact with the saliva of your child (use caution when kisses ...)
- Do not stay more than three hours per day closer than 1 meter from your child.

For 15 days after the hospital discharge:

- Do not sleep in the same bed as your child
- Limit contact with children (<15 years): less than 3 hours per day, with an isolation distance greater than 1 meter.

For 30 days after the hospital discharge:

- Avoid your child's contact with pregnant women. An isolation distance of 1 meter will be respected as a minimum.

24 APPENDIX 8: DRUGS INFORMATION

24.1 Temozolomide

The European Summary of Product Characteristics of the approved formulations of temozolomide can be loaded from the EMA website, www.ema.europa.eu, => Find medicine => Human medicines => temozolomide, , or by following this link :
http://www.ema.europa.eu/ema/index.jsp?curl=pages%2Fmedicines%2Flanding%2Fepar_search.jsp&mid=WC0b01ac058001d124&searchTab=searchByKey&alreadyLoaded=true&isNewQuery=true&status=Authorised&status=Withdrawn&status=Suspended&status=Refused&keyword=temozolomide&searchType=inn&taxonomyPath=&treeNumber=&searchGenericType=generics.

24.2 Irinotecan

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

24.3 ^{131}I-mIBG

The Summary of Product Characteristics of the ^{131}I-mIBG (meta-iodobenzylguanidine - ^{131}I) can be loaded from the website of each National Health Authority.

24.4 Topotecan

The European Summary of Product Characteristics of the approved formulations of topotecan can be loaded from the EMA website, www.ema.europa.eu, => Find medicine => Human medicines => topotecan, or by following this link :

http://www.ema.europa.eu/ema/index.jsp?curl=pages%2Fmedicines%2Flanding%2Fepar_search.jsp&mid=WC0b01ac058001d124&searchTab=searchByKey&alreadyLoaded=true&isNewQuery=true&status=Authorised&status=Withdrawn&status=Suspended&status=Refused&keyword=topotecan&searchType=inn&taxonomyPath=&treeNumber=&searchGenericType=generics

24.5 Thiotepa

The European Summary of Product Characteristics of the approved formulation of Thiotepa can be loaded from the EMA website, www.ema.europa.eu, => Find medicine => Human medicines => Thiotepa (or)

or by following this link: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/001046/WC500090252.pdf

24.6 Busulfan

The European Summary of Product Characteristics of the approved formulation of Busulfan BUSILVEX® can be loaded from the EMA website, www.ema.europa.eu, => Find medicine => Human medicines => busulfan (or) BUSILVEX®, ,

or by following this link: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000472/WC500052066.pdf

24.7 Melphalan

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

24.8 G-CSF : Granulocyte Colony Stimulating Factor

G-CSF is commercially available from several suppliers. For further information, see the Package Insert. In addition the Summary of Product Characteristics of G-CSF can be loaded from the website of each National Health Authority.

24.9 Gabapentin

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

24.10 Defibrotide

Defibrotide is approved in the European Union, and its Summary of Product Characteristics can be loaded from the EMA website:

http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/002393/human_med_001646.jsp&mid=WC0b01ac058001d124

Please note this product is subject to additional monitoring. This will allow quick identification of new safety information. Healthcare professionals are asked to report any suspected adverse reactions.

25 APPENDIX 9: SURGERY

25.1 Definition of Surgical Procedures

Complete Excision

Complete excision is defined as the removal of all visible tumours, including the removal of abnormal lymph nodes and the sampling of normal lymph nodes.

It is important to assess the likelihood of microscopic residual tumour even if macroscopic complete resection has been achieved. This can be aided by pathological examination of biopsies taken from the tumour bed as well as examination of the tumour margins.

Excision With Minimal Residual Disease

(<5% OF ORIGINAL AND /OR <5ML VOLUME)

Macroscopic residual disease remains after operation.

The amount should be estimated by the surgeon in millilitres and as a percentage of the original tumour volume.

Incomplete Excision

More than 5% or 5ml of tumour remain after attempted excision.

The amount should be estimated by the surgeon in millilitres, and as a percentage and evaluated by post-operative imaging.

25.2 Definition of Major Surgical Complications

- Death within 30 days of operation, or obviously related to the operation at any time.
- Serious haemorrhage > 30% blood volume.
- Serious vascular injury leading to loss of tissue.
- Any spinal cord injury.
- Serious peripheral nerve injury leading to loss of function.
- Any organ failure.

Please report any of the above complications as SAE immediately to the Pharmacovigilance Unit of the Sponsor.

25.3 Aspects of Surgical Procedures

25.3.1 Surgery of the Primary Tumour

The aim of surgery is to remove the primary tumour completely. All suspicious tissue should be excised. Resection should be attempted after completion of induction chemotherapy, unless there is tumour progression or imaging suggests that complete excision is likely to be associated with a significant risk of death or serious mutilation, this latter situation is extremely rare. In those circumstances, the option of further chemotherapy or alternative therapy should be discussed with the national co-ordinator. Vascular encasement is not a contra-indication to surgery; indeed it is almost invariably present.

25.3.2 Lymph Node Evaluation

Depending on the site of the primary tumour, lymph nodes from the following regions should be examined and removed if they appear abnormal:

- lateral cervical region: jugular chain and supraclavicular area;
- chest: mediastinal lymph nodes above and below the tumour;

- Abdomen: coeliac nodes (infra diaphragmatic), mid-aortic (at renal level) and iliac region (bilaterally).

25.3.3 Intraspinal Extension

If feasible the extraspinal mass should be removed even though intraspinal disease remains. Macroscopic disease may be left in the intervertebral foramina, especially when there is a risk of leakage of spinal fluid and/or jeopardising the blood supply of the spinal cord.

25.3.4 Nephrectomy

Unilateral nephrectomy is acceptable if it is the only way to remove the primary tumour (but see below: 'Renal Preservation'. In this case the surgeon should first make sure that the contralateral kidney is normal and its vessels are free from tumour.

Renal preservation.

All three elements of the high risk protocol: chemotherapy, surgery and radiotherapy cause renal damage.

There are no data on the incidence of late renal loss following operation around the renal pedicle but surgeons have encountered this. Survival is not enhanced by nephrectomy as a planned procedure to facilitate more complete tumour excision, nevertheless this strategy has been accepted.

There are no data to indicate a maximum tumour volume which can be eradicated by radiotherapy but the consensus is that this should be as small as possible.

MAT presents a huge physiological stress to the patient and good renal function is important for recovery. Although radiation will impair renal function this effect is not manifest for three to five years after treatment.

Consensus 2004 (SIOPEN Neuroblastoma Annual Meeting Krakau):

After trying to balance the disparate risks of the different therapies the committees (Clinicians, Surgeons, Radiotherapy) agreed that preservation of renal function for the period of MAT was paramount!

The following conclusions were drawn for operations undertaken at the recommended time – after induction chemotherapy:

- The commitment to achieve complete surgical excision remains.
- This commitment should stop short of nephrectomy.
- A further operation may be considered after recovery from MAT if residual disease remains.
- Nephrectomy is acceptable at this stage if this is the only means to achieve complete excision.

25.3.5 Tumour Incision

After chemotherapy most tumours will be firm and compact, and spillage is therefore unlikely. Incision of the tumour is permissible if this aids excision.

25.3.6 Tumour Relation with Great Vessels

In order to gain further information on the accuracy of the pre-operative imaging, the intra-operative findings should be described in detail. Particular attention should be given to the technical difficulties encountered when the tumour is in contact with the vessels.

25.4 Risk Factors Related to Localisation

Data should be collected on the following for comparison with surgical complications.

Neck

- Tumour encasing vertebral and/or carotid artery
- Tumour encasing brachial plexus roots
- Tumour crossing the midline

Thorax

- Tumour encasing the trachea or principal bronchus
- Tumour encasing the origin and branches of the subclavian vessels
- Thoraco-abdominal tumour, peri-aortic fusiform tumour
- Lower left mediastinal tumour, infiltrating the costo-vertebral junction between T9 and T12

Abdomen

- Adrenal tumour infiltrating the porta hepatis
- Suprarenal tumour infiltrating the branches of the superior mesenteric artery at the mesenteric root
- Suprarenal tumour surrounding the origin of the coeliac axis, and of the superior mesenteric artery
- Tumour invading one or both renal pedicles
- Fusiform tumour surrounding the infrarenal aorta
- Tumour encasing the iliac vessels
- Pelvic tumour crossing the sciatic notch

Clips

Titanium or absorbable clips should be used if necessary to avoid interference with subsequent imaging.

25.5 Storage of Tumour Material, Slides and Bone Marrow Samples

It is mandatory to store material and slides from each tumour, biopsy and bone marrow and peripheral blood sample. This is important to conduct further/future biological and genetic analyses and to allow review and quality assessment studies.

In case of tumour resection and open and tru cut biopsies, the storage of snap frozen material (at –70°C or below) is most important. Besides this, it is advisable to store touch preparations and cytospin preparations at –20°C and cell suspensions (including DMSO), if available, in liquid nitrogen. Tumour cells obtained by fine needle biopsy can be stored either as cytospin preparations or cell suspensions.

The MNC fraction of BM and PB can be stored as cytospin preparation at –20°C and cell suspensions (including DMSO) in liquid nitrogen.

Furthermore, stained slides, IF/FISH images and QRT-PCR pictures have to be stored adequately for documentation and review purposes.

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27 APPENDIX 12: 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]
    - o oral
    - o intravaginal
    - o transdermal
- progestogen-only hormonal contraception associated with inhibition of ovulation [1] :
    - o oral
    - o injectable
    - o 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"