HIGH RISK NEUROBLASTOMA
STUDY 1.5 OF SIOP-EUROPE (SIOPEN)

Lead Investigator: Study  Ruth Ladenstein / Austria
Lead Investigator: Infant issues  Adela Canete / Spain
Lead Investigator: Induction / TVD issues  Alberto Garaventa / Italy

2002 February:  STUDY activated with R0 and R1
Amendment 1: 2005 November: R0 closed and protocol send out for approval of study amendments
Amendment 2: 2006 March: Final Approval of Amendments by Study Committee, R2 Activation and inclusion of infants
Amendment 3: 2007 July: Change from oral Busulfan to I.V. Busulfan (Busilvex®)
Amendment 4: 2009 July: Change and activation of R2 (ch14.18/CHO ± aldesleukin (IL-2) s.c)
Amendment 5: 2011 June: R1 closed in favour of BUMEL and activation of R3 (Rapid COJEC vs. modified N7)

HRNBL1.5/SIOOPEN valid per 01.06.2011
SIOP EUROPE NEUROBLASTOMA (SIOPEN)
EXECUTIVE COMMITTEE

UK / President
SWITZERLAND / Secretary
BELGIUM / Member
FRANCE / Member
ISRAEL / Member
ITALY / Member
NORWAY / Member
SPAIN / Member
UK / Member
AUSTRIA / Advisory Member
FRANCE / Advisory Member
ITALY / Advisory Member
UK / Advisory Member
GREECE / New Country Coordinator

Penelope Brock
Maja Beck-Popovic
Geneviève Laureys
Dominique Valteau-Couanet
Isaac Yaniv
Alberto Garaventa
Klaus Beiske
Adela Cañete
Kate Wheeler
Ruth Ladenstein
Jean Michon
Bruno De Bernardi
Andy Pearson
Vassilios Papadakis

PAEDIATRIC ONCOLOGY
NATIONAL CO-ORDINATORS

AUSTRALIA
AUSTRIA
BELGIUM
CZECH REPUBLIC
DENMARK
FINLAND
FRANCE
GREECE
HUNGARY
IRELAND
ISRAEL
ITALY
NORWAY
POLAND
PORTUGAL
SERBIA
SLOVAKIA
SPAIN
SWEDEN (including ICELAND)
SWITZERLAND
UNITED KINGDOM

Toby Trahair
Ruth Ladenstein
Geneviève Laureys
Josef Malis
Henrik Schroeder
Kim Vettenranta
Dominique Valteau-Couanet
Vassilios Papadakis
Miklós Garami
Anne O’Meara
Isaac Yaniv
Roberto Luksch
Ellen Ruud
Walentyna Balwierz
Ana Forjaz de Lacerda
Dragana Vujic
Pavel Bician
Victoria Castel
Per Kogner
Maja Beck-Popovic
Penelope Brock
## SIOP EUROPE NEUROBLASTOMA (SIOPEN)
### SPECIALITY COMMITTEES AND CHAIRS

<table>
<thead>
<tr>
<th>Speciality Committee</th>
<th>Chair</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BIOLOGY</strong></td>
<td>Peter Ambros</td>
<td>AUSTRIA</td>
</tr>
<tr>
<td><strong>BONE MARROW</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>IC/CG Study Group:</em></td>
<td>Klaus Beiske</td>
<td>NORWAY</td>
</tr>
<tr>
<td></td>
<td>Peter Ambros</td>
<td>AUSTRIA</td>
</tr>
<tr>
<td><strong>Molecular Monitoring Group</strong></td>
<td>Sue Burchill</td>
<td>UK</td>
</tr>
<tr>
<td><strong>IMMUNOTHERAPY</strong></td>
<td>Holger Lode</td>
<td>GERMANY</td>
</tr>
<tr>
<td><strong>NUCLEAR MEDICINE &amp; PHYSICS</strong></td>
<td>Ariane Boubaker</td>
<td>SWITZERLAND</td>
</tr>
<tr>
<td></td>
<td>Zvi Bar-Server</td>
<td>ISRAEL</td>
</tr>
<tr>
<td><strong>PATHOLOGY</strong></td>
<td>Emanuelle D’Amore</td>
<td>ITALY</td>
</tr>
<tr>
<td></td>
<td>Michel Peuchmaur</td>
<td>FRANCE</td>
</tr>
<tr>
<td></td>
<td>Klaus Beiske</td>
<td>NORWAY</td>
</tr>
<tr>
<td><strong>PHARMACOLOGY</strong></td>
<td>Gareth Veal</td>
<td>UK</td>
</tr>
<tr>
<td></td>
<td>Gilles Vassal</td>
<td>FRANCE</td>
</tr>
<tr>
<td><strong>RADIOLOGY</strong></td>
<td>Marcus Hörmann</td>
<td>AUSTRIA</td>
</tr>
<tr>
<td><strong>RADIOThERAPY</strong></td>
<td>Mark Gaze</td>
<td>UK</td>
</tr>
<tr>
<td></td>
<td>Karin Dieckmann</td>
<td>AUSTRIA</td>
</tr>
<tr>
<td></td>
<td>Tom Boterberg</td>
<td>BELGIUM</td>
</tr>
<tr>
<td><strong>SURGERY</strong></td>
<td>Keith Holmes</td>
<td>UK</td>
</tr>
<tr>
<td><strong>STATISTICAL COMMITTEE</strong></td>
<td>Ulrike Poetschger</td>
<td>AUSTRIA</td>
</tr>
<tr>
<td></td>
<td>Véronique Mosseri</td>
<td>FRANCE</td>
</tr>
<tr>
<td></td>
<td>Paolo Bruzzi</td>
<td>ITALY</td>
</tr>
<tr>
<td></td>
<td>Keith Wheatley</td>
<td>UK</td>
</tr>
</tbody>
</table>

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 Paediatric HaematologyOncology
- CRCTU: Cancer Research UK Clinical Trials Unit
- GPOH: German Group of Paediatric HaematologyOncology
- NCRI CCL CSG: Neuroblastoma Group UK
- HSPHO: Hellenic Society of Paediatric 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.
### Synopsis

<table>
<thead>
<tr>
<th>Title</th>
<th>High Risk Neuroblastoma Study 1.5 of SIOP-Europe (SIOPEN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EudraCT number</td>
<td>2006-001489-17</td>
</tr>
<tr>
<td>Disease</td>
<td>Neuroblastoma</td>
</tr>
<tr>
<td>Study design</td>
<td>Open, multicentre randomised Phase III Trial; therapy optimisation study;</td>
</tr>
</tbody>
</table>

**Background**

In this protocol the term high-risk neuroblastoma refers to children with either:
- disseminated disease (INSS stage 4: about 40 to 50% of all neuroblastomas) over the age of one, or
- INSS stage 2 and 3 disease with amplification of the MYCN proto-oncogene (about 3% of all neuroblastomas). Between 10% and 20% of children with stage 3 and occasional patients with stage 2 disease are characterised by amplification of the MYCN gene in their tumours. This biological characteristic has clearly been shown to be associated with a greater risk of relapse and death from disease progression. These patients may benefit from very aggressive treatment and, based on this hypothesis, they are included in this protocol.
- Infants (< 12 months at diagnosis) with MYCN amplified tumours are included. Specific treatment guidelines need to be respected for this age group. Rationale and treatment adaptations for infants are specified in the relevant chapters.
- NOTE: Patients aged 12-18 months at diagnosis, with stage 4 neuroblastoma, no MYCN amplification and without segmental chromosomal alterations (SCA) are thought to have a good prognosis (see section 4.10, page 75) and will stop treatment after induction therapy and surgery to the primary tumour.

Children with this type of presentation and age represent the largest neuroblastoma subgroup. Their prognosis remains poor in most cases and our ability to predict the clinical course and the outcome of the individual patient is modest. Treatment intensification usually included an intensified induction regimen followed by the resection of the primary tumour and a final cycle of MAT combined with stem cell transplantation (SCT). As a result of this change a remarkable progress in therapeutic results was achieved in terms of (a) response rate, (b) 3- and 5- years EFS and OS, and (c) number of potential cures. Early results from UK and USA were confirmed by several reports from France, Italy, Germany, Japan and other national groups. However, the expectation that the refinement of these strategies would progressively improve patients’ outcome was frustrated by a number of trials that failed to increase the 5-year event-free survival above 30%.

**Primary objectives**

- To test the hypothesis that the modified N7 induction regimen will improve the metastatic response rates or event free survival (EFS) as compared to Rapid COJEC (R3 randomisation).
- To test the hypothesis that the addition of subcutaneous aldesleukin (IL-2, (Proleukin®)) to immunotherapy with chimeric 14.18 anti-GD2 monoclonal antibody produced in Chinese hamster ovary (CHO) cells (ch14.18/CHO) in addition to differentiation therapy with isotretinoin (13-cis-RA) following myeloablative therapy (MAT) and autologous SCR, will improve EFS in patients with high-risk neuroblastoma (stage 4 disease or stages 2 and 3 with MYCN amplification, all over the age of one, or infants with MYCN amplification) (R2 randomisation).
<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th><strong>Activated 02/2002</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Study</td>
<td>Established diagnosis of neuroblastoma according to the International Neuroblastoma Staging System (INSS).</td>
</tr>
<tr>
<td></td>
<td>Age below 21 years.</td>
</tr>
<tr>
<td></td>
<td>High risk neuroblastoma defined as either:</td>
</tr>
<tr>
<td></td>
<td>a) INSS stage 2, 3, 4, and 4s with MYCN amplification, or</td>
</tr>
<tr>
<td></td>
<td>b) INSS stage 4 without MYCN amplification aged &gt; 12 months at diagnosis</td>
</tr>
<tr>
<td></td>
<td>Patients who have received no previous chemotherapy except for one cycle of etoposide and carboplatin (VP16/Carbo). In this situation patients will receive Rapid COJEC induction and the first Rapid COJEC cycle may be replaced by the first cycle VP16/Carbo (etoposide / carboplatin).</td>
</tr>
<tr>
<td></td>
<td>Written informed consent, including agreement of parents or legal guardian for minors, to enter a randomised study if the criteria for randomisation are met.</td>
</tr>
<tr>
<td></td>
<td>Tumour cell material available for determination of biological prognostic factors.</td>
</tr>
<tr>
<td></td>
<td>Females of childbearing potential must have a negative pregnancy test.</td>
</tr>
<tr>
<td></td>
<td>Females of childbearing potential must agree to use an effective birth control method.</td>
</tr>
<tr>
<td></td>
<td>Female patients who are lactating must agree to stop breast-feeding.</td>
</tr>
<tr>
<td></td>
<td>Registration of all eligibility criteria with the data centre within 6 weeks from diagnosis.</td>
</tr>
<tr>
<td></td>
<td>Provisional follow up of 5 years.</td>
</tr>
<tr>
<td></td>
<td>National and local ethical committee approval.</td>
</tr>
</tbody>
</table>

**Amended Version 04/2006**

Inclusion of infants with MYCN amplification

<table>
<thead>
<tr>
<th>R0 Randomisation</th>
<th><strong>Closed in 11/2005</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>(Rapid COJEC vs. modified N7)</td>
<td>Following the results of the R0 randomisation, the prophylactic use of G-CSF (filgrastim) is recommended during Rapid COJEC induction treatment.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>R3 Randomisation</th>
<th><strong>Activated 06/2011</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>(Rapid COJEC vs. modified N7)</td>
<td>Diagnosis of neuroblastoma confirmed.</td>
</tr>
<tr>
<td></td>
<td>Stage 4 or Stage 4s with MYCN amplification.</td>
</tr>
<tr>
<td></td>
<td>No prior chemotherapy.</td>
</tr>
<tr>
<td></td>
<td>Written informed consent to participate in R3 randomisation, and for minors an agreement by parents or legal guardian.</td>
</tr>
<tr>
<td></td>
<td>Females of childbearing potential must have a negative pregnancy test. Patients of childbearing potential must agree to use an effective birth control method.</td>
</tr>
<tr>
<td></td>
<td>Female patients who are lactating must agree to stop breast-feeding.</td>
</tr>
</tbody>
</table>

**NOTE:** Patients with MYCN-amplified localised disease (INSS stages 2 and 3) of any age should be enrolled in the trial, but are not eligible for the R3 randomisation and will receive Rapid COJEC induction.

<table>
<thead>
<tr>
<th>R1 Randomisation</th>
<th><strong>Activated 02/2002</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>(BuMel vs. CEM)</td>
<td>R1 closed in 10/2010 following the result of R1 Randomisation showing significant superiority for myeloablative therapy (MAT) with busulfan and melphalan (BuMel) in patients with high risk neuroblastoma over MAT with continuous infusion of carboplatin, etoposide and melphalan (CEM). BuMel is now the standard MAT.</td>
</tr>
</tbody>
</table>
### Inclusion Criteria for BuMel MAT

#### Revised for Amendment Version 1.5 (06/2011)

- Localised patients without evidence of disease progression, must start BuMel MAT no later than day 150 after the start of Rapid COJEC induction.
- Patients in metastatic CR following front-line induction (Rapid COJEC or modified N7) must start BuMel MAT no later than day 150 after the start of induction.
- Patients receiving two courses of TVD to achieve an adequate metastatic response (metastatic CR or PR) must start BuMel MAT no later than day 210 after the start of induction.
  
  **CR or PR at metastatic site:**
  - At least 50% reduction in skeletal mIBG positivity and not more than 3 positive, but improved spots on mIBG.
  - Cytomorphological CR in 2 BM aspirates and no positive BM biopsy.
- Complete restaging of disease as close as possible before the start of BuMel MAT must take place following front-line induction (Rapid COJEC or modified N7) or two courses of TVD.
- Organ functions (liver, kidney, heart, lungs) fulfilling criteria prior to MAT
  - ALT, bilirubin < 3 x normal (see SmPC)
  - **Creatinine clearance and/or GFR ≥ 60 ml/min/1.73m²** and serum creatinine < 1.5mg/dl. Call study co-ordinator for MAT dose modifications if GFR < 60ml/min/1.73m² and serum creatinine ≥ 1.5mg/dl.
  - Shortening fraction ≥ 28%, or ejection fraction ≥ 55%, no clinical congestive heart failure.
  - Normal chest X-ray and oxygen saturation.
- Sufficient stem cells available. Minimum required: 3 x10⁶ CD34 cells/kg body weight, if a BM harvest was unavoidable at least 3 x10⁸ MNC/kg body weight.
- Females of childbearing potential must have a negative pregnancy test. Patients of childbearing potential must agree to use an effective birth control method. Female patients who are lactating must agree to stop breast-feeding.

**NOTE:**

- No more free drug supply for Busilvex since R1 is closed.
- Even patients presenting at diagnosis with a large abdominal or large pulmonary primary should receive BuMel MAT. 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. This should be discussed with the current Radiotherapy Panel.
- Patients aged 12-18 months at diagnosis, with stage 4 neuroblastoma, no MYCN amplification and without segmental chromosomal alterations (SCA) are not eligible for MAT. These patients are thought to have a good prognosis and will stop treatment after induction therapy and surgery to the primary tumour.
### Inclusion Criteria For R2 Randomisation

<table>
<thead>
<tr>
<th>13-cis RA + ch14.18/ CHO vs. 13-cis RA + ch14.18/ CHO + aldesleukin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Activated 12/2006, Amended version 2011</strong></td>
</tr>
<tr>
<td>• All patients must be enrolled on the study and have completed therapy including intensive induction (Rapid COJEC or modified N7) with or without two additional cycles of TVD.</td>
</tr>
<tr>
<td>• BuMel MAT (minor modifications for toxicity concerns allowed) are permitted to render the patient eligible for R2 randomisation, even after more than one line of induction treatment. However, patients must be no more than 9 months from the date of starting the first induction chemotherapy after diagnosis to the date of PBSCR.</td>
</tr>
<tr>
<td>• Complete re-staging, which shows no signs of progression, following recovery from major transplant related toxicities.</td>
</tr>
<tr>
<td>• Stable WBC above 2 x10⁹/L (or stable neutrophil count greater than 0.5 x10⁹/L) in two counts taken 48 hours apart after cessation of G-CSF.</td>
</tr>
<tr>
<td>• Written informed consent to participate in R2 randomisation, and for minors an agreement by parents or legal guardian.</td>
</tr>
<tr>
<td>• Females of childbearing potential must have a negative pregnancy test. Patients of childbearing potential must agree to use an effective birth control method. Female patients who are lactating must agree to stop breast-feeding.</td>
</tr>
<tr>
<td>• Radiotherapy must be scheduled to stop at least 7 days prior to the start of immunotherapy.</td>
</tr>
<tr>
<td>• Immunotherapy (starting with week 1 isotretinoin (13-cis-RA)) must start no later than day 120 post PBSCR.</td>
</tr>
</tbody>
</table>

### Exclusion Criteria

Any negative answer concerning the inclusion criteria of the study, R3 or R2 will render the patient ineligible for the corresponding therapy phase.

### Amendment 1

• The time to randomise for R1 was altered from previously 120 days (from the first day of chemotherapy) to 150 days

### Amendment 2

• 04/2006:
  Inclusion of infants with MYCN amplification

  The time to randomise for R1 was extended to 210 days but only for patients having received 2 cycles of additional TVD for insufficient response and meeting the R1 response criteria after 2 TVD courses.

  • 11/2006:
    Activation of R2 with the use of antibody ch14.18/CHO

### Amendment 3

• 10/2007:
  Change from oral to intravenous Busulfan (Busilvex); for R1 randomised patients Busilvex is free of charge (Please note: Infants were not eligible for R1, but received BUMEL. Busilvex was not free of charge for infants.)

### Amendment 4

• 07/2009:
  Change in the R2 randomisation, new R2 randomisation therapy:
  Arm A: 13-cis RA + ch14.18/CHO
  Arm B: 13-cis RA + ch14.18/CHO + aldesleukin (IL-2) s.c.
  To be eligible for R2 patients were allowed to receive elective MAT or either BUMEL or CEM (with minor adjustments), so long as the time from first induction chemotherapy to PBSCR date was less than 9 months.
Activation of R3: randomisation of two different induction treatments:
- Rapid COJEC
- Modified N7

Patients with MYCN-amplified localised disease are not eligible for R3 randomisation and will receive Rapid COJEC induction.

Recommendation that all patients receive BuMel MAT. Even patients presenting at diagnosis with a large abdominal or large pulmonary primary. 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. This should be discussed with the current Radiotherapy Panel.

Patients aged 12-18 months at diagnosis, with stage 4 neuroblastoma, no MYCN amplification and without segmental chromosomal alterations (SCA) are not eligible for MAT. These patients are thought to have a good prognosis and will stop treatment after induction therapy and surgery to the primary tumour.

With the closure of the R1 randomisation, Busilvex is no longer supplied free of charge.

### Study period
02/02/2002 -2014

<table>
<thead>
<tr>
<th>Exp. patient recruitment</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2230</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Original Protocol</th>
<th>Amended Version</th>
</tr>
</thead>
<tbody>
<tr>
<td>R0</td>
<td>184</td>
<td>Closed with 238 patients</td>
</tr>
<tr>
<td>R1</td>
<td>780</td>
<td>Closed with 598 patients</td>
</tr>
<tr>
<td>R2 (new immunotherapy strategy as of 01.07.2009)</td>
<td>630</td>
<td>400</td>
</tr>
<tr>
<td>R3</td>
<td>630</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Current recruitment 23.05.2011</th>
<th>Total registered patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1670</td>
</tr>
</tbody>
</table>

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>R0</td>
<td>Closed with 239 patients (November 2005)</td>
</tr>
<tr>
<td>R1</td>
<td>closed with 598 patients (October 2010)</td>
</tr>
<tr>
<td>R2</td>
<td>160 patients</td>
</tr>
</tbody>
</table>
| **Study chair international** | Assoc. Prof. Ruth Ladenstein, MD  
St. Anna Children’s Hospital  
Children’s Cancer Research Institute (CCRI)  
Zimmermannplatz 10  
1090 Vienna  
Austria  
Email: ruth.ladenstein@ccri.at  

**CRA:**  
Dr. Ingrid Pribill  
Tel.: +43/(0)1/ 40470-4960  
Fax.: +43/(0)1/40470-7430  
Email: ingrid.pribill@ccri.at |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Participating countries/Subcentres</strong></td>
<td>Australia, Austria, Belgium, Czech Republic, Denmark, Finland, France, Greece, Hungary, Ireland, Israel, Italy, Norway, Poland, Portugal, Serbia, Slovakia, Spain, Sweden (including Iceland), Switzerland, United Kingdom</td>
</tr>
</tbody>
</table>
ABBREVIATIONS USED IN THIS PROTOCOL IN ALPHABETICAL ORDER

ABMT autologous bone marrow transplantation
AIPF automatic immuno-fluorescence plus FISH
ANC absolute neutrophil count
ARDS acute respiratory distress syndrome
ASCT autologous stem cell transplantation
AUC area under the curve
BM bone marrow
BUMEL busulfan and melphalan MAT regimen
CADO French induction regimen consisting of cyclophosphamide, adriamycin, vincristine
carboplatin
CCRI Children’s Cancer Research Institute
CCSG Children’s Cancer Study Group
CCNU CCNitrosourea
carboplatin, etoposide and melphalan MAT regimen
carcinogenic genomic hybridisation
Ch14.18/CHO Chimeric 14.18 anti-GD2 monoclonal antibody produced in Chinese hamster ovary
cells
CI confidence interval
CMV cytomegalovirus
COG Children’s Oncology Group
COJEC rapid, platinum-containing induction schedule (CBDCA, CDDP, CYC, VCR, VP16)
cyclophosphamide
CR complete response
CRP C-reactive protein
CT computer tomography
CTV clinical target volume
CXR Chest x-ray
dose-limiting toxicity
deoxycytidine acid
doxyribonucleic acid
doxorubicin
European Group for Bone Marrow (Stem Cell) Transplantation
Electrocardiograph
event-free survival
European Neuroblastoma Study Group
European Neuroblastoma Group for Quality Assessment of Biological Markers
Fluorescence in situ hybridisation
granulocyte stimulating growth factor
ganglioside
glomerular filtration rate
gastro-intestinal tract
gross tumour volume
Gray
Human anti-chimeric Antibody
herpes simplex virus
homovanillic acid
Immunocytology
determination of GD2 with immunohistochemistry
international commission of radiation units
interleukin-2, aldesleukin, Proleukin®
INCR  International Neuroblastoma Response Criteria
INSS  International Neuroblastoma Staging System
IVIG  Intravenous immune globulin
LDH  lactate dehydrogenase
LMCE  French Paediatric Neuroblastoma Group: Lyon-Marseille- I. Curie(Paris)- East of France
LI  local irradiation
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
MRI  magnetic resonance imaging
MSKCC  Memorial Sloan-Kettering Cancer Centre
MTD  maximum tolerated dose
NB (L)  neuroblastoma
NCA  nurse controlled analgesia
NSE  neuron-specific enolase
OS  overall survival
PBSCH  peripheral blood stem cell harvest
PBSCR  peripheral blood stem cell rescue
PCA  patient controlled analgesia
PCP  pneumocystis carinii pneumonia
PCR  polymerase chain reaction
PD  progressive disease
PEM  MAT regimen using cisplatin, etoposide, melphalan,
PET  Positron emission tomography
PR  partial remission
PTV  planning target volume
QRT-PCR  quantitative reverse transcriptase polymerase chain reaction
RA  13-cis retinoic acid
RNA  ribonucleic acid
R0  randomisation on the use of G-CSF during induction
R1  randomisation of MAT regimen (BuMel vs. CEM)
R2  randomisation of immunotherapy (ch14.18/CHO ± aldesleukin (IL-2))
R3  randomisation of induction regimen (Rapid COJEC vs. modified N7)
SD  stable disease
SIOP  Société Internationale d’Oncologie Pédiatrique
SIOPEN  Société Internationale d’Oncologie Pédiatrique European Neuroblastoma
SFOP  Société Française d’Oncologie Pédiatrique
SmPC  Summary of Product Characteristics
SMZ  sulfamethoxazol
SPECT  single-photon emission computed tomography
TBI  total body irradiation
TMP  trimethoprim
VAMP  MAT regimen including teniposide, adriamycin, melphalan, cisplatin,
VCR  vincristine
VGPR  very good partial remission
VMA  vanillyl mandelic acid
VOD  veno-occlusive disease
VP16  etoposide
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>VZU</td>
<td>varicella-zoster virus</td>
</tr>
<tr>
<td>W</td>
<td>weeks</td>
</tr>
<tr>
<td>WBC</td>
<td>white blood cells</td>
</tr>
</tbody>
</table>
INTERNATIONAL DATA CENTRE

Study Chair / Contact Address:
Ass. Prof. Dr. Ruth Ladenstein
St. Anna Children’s Hospital / CCRI
Studies and Statistics for Integrated Research and Projects (S²IRP)
Zimmermannplatz 10
A-1090 Vienna, AUSTRIA
Phone: 0043-1-40470-4750
Fax: 0043-1-40470-7430
Email address: ruth.ladenstein@ccri.at

International Sponsor
St. Anna Kinderkrebsforschung
Zimmermannplatz 10
1090 Vienna, Austria

IMPORTANT NOTE
This document is intended to describe a collaborative study in High-Risk Neuroblastoma and to provide information for entering patients. The trial committee does not intend it to be used as an aide-memoire or guide for treatment of NON-registered patients. Amendments may be necessary; these will be circulated to known participants of the trial, but institutions entering patients are advised to contact the appropriate study centres to confirm the correctness of the protocol in their possession. Although this protocol was written and checked with care, typing errors may have occurred and the physician in charge therefore has the final responsibility of controlling the drug dosages given to individual patients. Before entering patients clinicians must ensure that the study protocol has been cleared by their ethical committee.

SAE REPORTING
Investigators are responsible for the reporting of SAEs for all patients registered on this trial as per chapter 27; Appendix: Guidelines for reporting toxicity/serious adverse events.
CONTENTS

SYNOPSIS ....................................................................................................................... 4

1 SUMMARY OF APPROVED AMENDMENTS ................................................................. 19
  1.1 AMENDMENT AS OF MARCH 15, 2006 ................................................................. 19
  1.2 AMENDMENT AS OF JULY 31, 2007 ................................................................. 20
  1.3 AMENDMENT AS OF JULY 1, 2009 ................................................................. 22
  1.4 AMENDMENT AS OF JUNE, 2011 ................................................................. 29

2 SPECIFIC AIMS ............................................................................................................. 40
  2.1 PRIMARY AIMS ...................................................................................................... 40
  2.2 SECONDARY AIMS .............................................................................................. 40

3 TRIAL DESIGN ........................................................................................................... 41
  3.1 INDUCTION CHEMOTHERAPY ............................................................................. 42
  3.2 TOPOTECAN, VINCRISTINE AND DOXORUBICIN FOR PATIENTS WITH AN INADEQUATE METASTATIC
      RESPONSE AFTER INDUCTION ................................................................. 46
  3.3 PBSC HARVEST .................................................................................................... 46
  3.4 SURGERY ............................................................................................................. 47
  3.5 MYELOABLATIVE THERAPY AND PERIPHERAL BLOOD STEM CELL RESCUE ..... 47
  3.6 RADIOTHERAPY .................................................................................................. 48
  3.7 DIFFERENTIATION THERAPY ........................................................................... 48
  3.8 IMMUNOTHERAPY APPROACH (SECOND RANDOMISATION - R2 MODIFICATION ACTIVATED JULY 2009) .... 48
  3.9 TREATMENT AND RESPONSE OVERVIEW ..................................................... 50

4 BACKGROUND AND RATIONALE .......................................................................... 51
  4.1 BACKGROUND ....................................................................................................... 51
  4.2 RATIONALE FOR THE VARIOUS THERAPEUTIC ELEMENTS OF THE PROPOSED STUDY ..... 52
  4.3 RATIONALE OF THE SIOPEN PHASE I BRIDGING STUDY ................................ 69
  4.4 RESULTS OF THE SIOPEN PHASE I BRIDGING STUDY ..................................... 70
  4.5 IMMUNOTHERAPY WITH ANTI-GD2 MONOCLONAL ANTIBODY AND CYTOKINES (IL-2 AND GM-CSF) .... 71
  4.6 THE CHILDREN’S ONCOLOGY GROUP STUDY ANBL0032 .................................... 72
  4.7 CH14.18/CHO AND ALDESLEUKIN (IL-2) IN HR-NBL-1.5/SIOPEN ...................... 73
  4.8 BACKGROUND TO INTEGRATE MYCN AMPLIFIED (NMA) INFANTS IN THE CURRENT STUDY: ............ 73
  4.9 RATIONALE FOR TREATMENT REDUCTION IN CHILDREN 12-18 MONTHS AT DIAGNOSIS WITH FAVOURABLE
      BIOLOGY ?

5 ELIGIBILITY AND PATIENT ENTRY CRITERIA ...................................................... 76
  5.1 ELIGIBILITY CRITERIA FOR THE STUDY .......................................................... 76
  5.2 R0 RANDOMISATION CLOSED IN NOVEMBER 2005 ........................................ 76
  5.3 ELIGIBILITY CRITERIA FOR THE R3 RANDOMISATION ..................................... 76
  5.4 ELIGIBILITY CRITERIA FOR BUMEL MAT (R1 RANDOMISATION CLOSED OCTOBER 2010) .................... 77
  5.5 ELIGIBILITY CRITERIA FOR THE R2 RANDOMISATION (2ND REVISION ACTIVATED) .......................... 78

6 ASSESSMENT OF EXTENT OF DISEASE, RESPONSE AND TOXICITY .................... 78
  6.1 PRE-TREATMENT INVESTIGATIONS ................................................................... 78
  6.2 NECESSARY INTERACTIONS TO SECURE TUMOUR AND BIOLOGICAL SAMPLE FLOW ............... 85
  6.3 INVESTIGATIONS AT DIAGNOSIS, DURING RAPID COJEC INDUCTION AND TVD .................... 87
  6.4 INVESTIGATIONS AT DIAGNOSIS, DURING MODIFIED N7 INDUCTION AND TVD .................... 88
  6.5 INVESTIGATIONS AT PRE- AND POST-MAT PHASE ........................................... 89
  6.6 INVESTIGATIONS DURING IMMUNOTHERAPY PHASE UNTIL THE END OF TREATMENT ............. 90
  6.7 FOLLOW UP INVESTIGATIONS ............................................................................ 91

7 SURGERY .................................................................................................................... 92
  7.1 INTRODUCTION ................................................................................................... 92
  7.2 AIMS .................................................................................................................... 93
  7.3 TIMING ............................................................................................................... 93
  7.4 DEFINITION OF PROCEDURES ......................................................................... 93
  7.5 DEFINITION OF MAJOR SURGICAL COMPLICATIONS ....................................... 94
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.13</td>
<td>Biological Review</td>
<td>254</td>
</tr>
<tr>
<td>30.14</td>
<td>BM/PBSC Harvest Review</td>
<td>255</td>
</tr>
<tr>
<td>30.15</td>
<td>MIBG Review</td>
<td>255</td>
</tr>
<tr>
<td>30.16</td>
<td>Radiological Review</td>
<td>255</td>
</tr>
<tr>
<td>30.17</td>
<td>Follow Up Data</td>
<td>255</td>
</tr>
<tr>
<td>30.18</td>
<td>Study Approval</td>
<td>255</td>
</tr>
<tr>
<td>30.19</td>
<td>Institutional/Local Ethical Approval and Patient Consent</td>
<td>255</td>
</tr>
<tr>
<td>30.20</td>
<td>Data Monitoring and Safety Committee (DMSC)</td>
<td>255</td>
</tr>
<tr>
<td>30.21</td>
<td>Toxicity Monitoring</td>
<td>256</td>
</tr>
<tr>
<td>30.22</td>
<td>Treatment Stopping Rules for Individual Patients</td>
<td>256</td>
</tr>
<tr>
<td>31</td>
<td>Appendix: Address List</td>
<td>257</td>
</tr>
<tr>
<td>31.1</td>
<td>SIOP Europe Neuroblastoma Executive Committee</td>
<td>257</td>
</tr>
<tr>
<td>31.2</td>
<td>Data Monitoring</td>
<td>259</td>
</tr>
<tr>
<td>31.3</td>
<td>National Co-ordinators</td>
<td>260</td>
</tr>
<tr>
<td>31.4</td>
<td>Biology Committee</td>
<td>264</td>
</tr>
<tr>
<td>31.5</td>
<td>Bone Marrow ImmunocytoLOGY Studies Committee</td>
<td>268</td>
</tr>
<tr>
<td>31.6</td>
<td>Molecular Monitoring Studies Committee</td>
<td>269</td>
</tr>
<tr>
<td>31.7</td>
<td>Immunotherapy Committee</td>
<td>270</td>
</tr>
<tr>
<td>31.8</td>
<td>Nuclear Medicine and Physics Committee</td>
<td>272</td>
</tr>
<tr>
<td>31.9</td>
<td>Pathology Committee</td>
<td>275</td>
</tr>
<tr>
<td>31.10</td>
<td>Pharmacology Committee</td>
<td>278</td>
</tr>
<tr>
<td>31.11</td>
<td>Radiology Committee</td>
<td>281</td>
</tr>
<tr>
<td>31.12</td>
<td>Radiotherapy Committee</td>
<td>284</td>
</tr>
<tr>
<td>31.13</td>
<td>Statistical Committee</td>
<td>287</td>
</tr>
<tr>
<td>31.14</td>
<td>Stem Cell Committee</td>
<td>288</td>
</tr>
<tr>
<td>31.15</td>
<td>Surgery Committee</td>
<td>290</td>
</tr>
<tr>
<td>31.16</td>
<td>Summary of National Contact Details for Material Transfer</td>
<td>293</td>
</tr>
<tr>
<td>32</td>
<td>Appendix: Declaration of Helsinki</td>
<td>294</td>
</tr>
<tr>
<td>32.1</td>
<td>Introduction</td>
<td>294</td>
</tr>
<tr>
<td>32.2</td>
<td>Principles for All Medical Research</td>
<td>295</td>
</tr>
<tr>
<td>32.3</td>
<td>Additional Principles for Medical Research Combined with Medical Care</td>
<td>297</td>
</tr>
<tr>
<td>33</td>
<td>Appendix: Sample Information Sheet/Consent Forms</td>
<td>299</td>
</tr>
<tr>
<td>33.1</td>
<td>Sample Information Sheet for Study Enrolment and Induction Treatment</td>
<td>299</td>
</tr>
<tr>
<td>33.2</td>
<td>Consent Form for Study Enrolment and Induction Treatment</td>
<td>310</td>
</tr>
<tr>
<td>33.3</td>
<td>Randomisation R3 Concerning the Induction Chemotherapy Regimen</td>
<td>311</td>
</tr>
<tr>
<td>33.4</td>
<td>Consent Form for Induction Randomisation R3</td>
<td>313</td>
</tr>
<tr>
<td>33.5</td>
<td>Immunotherapy Randomisation R2</td>
<td>314</td>
</tr>
<tr>
<td>33.6</td>
<td>Consent Form for Randomisation R2</td>
<td>321</td>
</tr>
<tr>
<td>34</td>
<td>Reference List</td>
<td>322</td>
</tr>
</tbody>
</table>
1 Summary of approved amendments

1.1 Amendment as of March 15, 2006

1.1.1 Changes related to Induction

1.1.1.1 Infants eligibility into the HR-NBL-1/SIOPEN Trial
Infants with MYCN amplified tumours are eligible – task for rapid reference biology!
Rapid COJEC dose modifications for infants and low weight children
Infants are not eligible for R1 (elective BUMEL), but for R2

1.1.1.2 R0 closed
Following the results of the R0 randomisation, the prophylactic use of G-CSF is recommended during COJEC induction treatment

1.1.1.3 Response
Patients with an inadequate metastatic response to allow R1 randomisation of myeloablative therapy (BUMEL compared to CEM) followed by (PBSCR) at the end of the Rapid COJEC induction should receive 2 TVD (Topotecan, Vincristine, and Doxorubicin) cycles.

1.1.2 Surgery

1.1.2.1 Preservation of renal function
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.

1.1.2.2 Postoperative MIBG scan
Improved information on the post-surgical primary tumour status through one additional postoperative MIBG scan prior MAT.

1.1.3 Changes related to MAT

1.1.3.1 R1 (MAT- question) change of eligibility
- If R1 eligibility criteria are met after 2 TVD cycles, patients may be randomised for the R1 question and continue on the High Risk Study.
- Randomisation 1 allowed up to day 150 for patients fulfilling R1 response criteria after Rapid COJEC and up to day 210 for patients achieving documented R1 response criteria after day 210.

1.1.4 CHANGES RELATED TO MRD

1.1.4.1 R2 ACTIVATION

Patients will be randomised for additional immunotherapy with the chimeric 14.18 anti-GD2 monoclonal antibody (mAb) at a dose of 20mg/m²/day over 5 days every 4 weeks for 5 courses. The first course will start three weeks after initiation of 13-cis RA.

Eligibility for R2 includes patients
- with stage 4 neuroblastoma over the age of one year, patients with stage 2 and 3 neuroblastoma with MYCN amplification, and patients with neuroblastoma under the age of 12 months with MYCN amplification.
- having received myeloablative therapy following initial induction COJEC chemotherapy, which may have included topotecan, vincristine and doxorubicin.
- at the time of re-evaluation, have no evidence of progression.

1.1.5 COMMITTEE AMENDMENTS

1.1.5.1 IC/CG GROUP OF THE BONE MARROW AND RT-PCR COMMITTEE

Revised sampling logistics! Crucial improvements needed in reporting BM initial and response data to the study site as well as improvement to organise and perform the BM sampling processes according to guidelines by IC/CG and RT-PCR group throughout the study hallmarks!

1.1.5.2 NUCLEAR MEDICINE AND PHYSICS COMMITTEE

Implement European scoring and enhance SPECT for local tumor issues

1.1.5.3 PHARMACOLOGY COMMITTEE

Pharmacodynamics and pharmacogenetics: Sampling sheets for MAT and MRD

1.1.5.4 NEW MONITORING GUIDELINES FOR OTOTOXICITY

1.1.5.5 IMMUNOTHERAPY WITH Ch14.18/CHO ANTI-GD2 MAB

Logistics and sampling according to guidelines

1.1.5.6 REVISED STATISTICS AND BIOMETRICAL METHODOLOGY

1.2 Amendment as of July 31, 2007

1.2.1 CHANGE TO CHAIRS AND NATIONAL COORDINATORS (PAGE 1)

Chair und Past Chair
National Coordinators: Norway, Greece
1.2.2 Molecular Monitoring Group; merging of RT-PCR Studies and Stem Cell Studies (Page 2)

1.2.3 Members of the committees: Nuclear Medicine & Physics, Pharmacology (Page 2)

1.2.4 Change from Oral to Intravenous Busulfan (Bumel MAT)

Because intravenous Busulfan (Busilvex®) is easier to administer, is already registered even for infants and because it is anticipated that I.V. Busulfan (Busilvex®) will produce lower VOD rates with equivalent efficacy in terms of Event Free Survival and Overall Survival the Study Committee decided the change from oral to intravenous Busulfan during BUMEL MAT. For patients R1 randomised to BUMEL MAT I.V. Busulfan (Busilvex®) will be provided as free of charge. For this reason I.V. Busulfan is not free of charge for infants which will not be R1 randomised, but receive BUMEL MAT.

1.2.5 Correction of Etoposide dose during CEM MAT (Page 20, 89)

Etoposide 350mg/m²/course was replaced by 1352mg/m²/course

1.2.6 Revised Treatment Summary Flow Chart (Page 23)

1.2.7 New Phrasing for Chemotherapy Regimen Details of TVD (Page 30, 69)

1.2.8 Changes to the R1 Elegibility Criteria (Page 44, 70)

New as explicit inclusion criteria: Age > 1 year

Change of ALT, Bilirubin according to SmPC: ALT, bilirubin < 3 x normal replaced by ALT, bilirubin ≤ 2 x normal (see SmPC)

New inclusion criteria: Patients of childbearing potential have been informed that they have to practice an effective method of birth control while participating in this study.

1.2.9 Changes of the Timepoints for GFR EDTA (Page 50)

1.2.10 Serum Sample: Missing Timepoint Included (Page 51)

1.2.11 Southern Blot removed from Biological Studies

Page 59/60, 132-134

1.2.12 Guidelines for Otoacoustic Emissions Included (Page 74/75)
1.2.13 Correction of Melphalan dose during BUMEL MAT in case of body weight below 12kg (Page 86)

Melphalan dose 40mg/kg was replaced by 4mg/kg.

1.2.14 Inclusion of “PAIN” in Toxicity Grading (Page 212)

1.2.15 Modification of the International version of the Patient Information Sheet (Page 261-270)

Close of R0 randomisation
Change from oral to intravenous Busulfan

1.3 Amendment as of July 1, 2009

A copy of the protocol with tracked changes is available on request.

N.B. Page numbers reflect those applicable to this amended version of the protocol.

1.3.1 Typing and English Corrections
Multiple corrections made throughout the protocol for English and typing errors. These changes are not detailed below.

1.3.2 Changes/Additions for Clarity and Consistency
Multiple corrections made throughout the protocol for consistency and clarification of various items. These changes are not detailed below.

1.3.3 ESIOP replaced with SIOPEN
For accuracy ESIOP has been replaced with SIOPEN throughout the protocol where appropriate.

1.3.4 Documentation of the Antibody – ch14.18/CHO
For consistency mention of the antibody is now ch14.18/CHO throughout the protocol where appropriate.

1.3.5 Addition of New Countries with respective National Coordinators and Trial Groups (Pages 1-2)

Australia:
Dr Toby Trahair
ANZCHOG – Australia and New Zealand Haematology/Oncology Group

Ireland:
Dr Anne O’Meara
ICORG – The All Ireland Cooperative Oncology Research Group

Serbia:
Dr Dragana Vujic

Members of the Executive Committee updated.

Replaced UKCCSG with CCLG – Children’s Cancer and Leukaemia Group
1.3.6 **Addition of Synopsis (Pages 3-6)**
Added a synopsis of the trial.

1.3.7 **Update of Abbreviations Section (Pages 7-8)**
Added the following abbreviations:
- ASCT – autologous stem cell transplantation
- Ch14.18/CHO – Chimeric 14.18 anti-GD2 monoclonal antibody produced in Chinese hamster ovary cells
- CXR – Chest x-ray
- ECG – electrocardiograph
- IVIG - Intravenous immune globulin
- SIOPEN – Société Internationale d’Oncologie Pédiatrique European Neuroblastoma

Updated the following abbreviation:
- Added “aldesleukin” and “Proleukin®” to IL-2 abbreviation explanation

1.3.8 **SAE Reporting (Page 9)**
Information regarding SSAR and SUSAR reporting deleted and replaced with a more general statement; “Investigators are responsible for the reporting of SAEs for all patients registered on this trial as per chapter 27; Appendix: Guidelines for reporting toxicity/serious adverse events”.

1.3.9 **Contents (Pages 10-14)**
Contents list updated to reflect changes and additions of sections and page numbers.

1.3.10 **Summary of Amendment as of July 1, 2009 (Pages 18-24)**
Details of changes and additions of the 4th amendment documented.

1.3.11 **Section 2, Aims (Page 25)**
- The primary aim relating to the R2 randomisation has been updated to reflect the new treatment. i.e. R2 randomisation is now: 13-cis retinoic acid + ch14.18/CHO vs. 13-cis retinoic acid + ch14.18/CHO + aldesleukin (IL-2).
- The secondary aim relating to the post MAT phase with regards to 13-cis retinoic acid has been updated to reflect the new treatment.

1.3.12 **Section 3, Trial Design (Page 26)**
The new R2 treatment and wider eligibility criteria for R2 have been added. Now any patient enrolled on HR-NBL-1 and receiving R1 randomised MAT or elective MAT (CEM or BUMEL, minor modifications are acceptable) is eligible for the R2 randomisation.

1.3.13 **Section 3.9, Immunotherapy Approach (Page 31)**
This whole section has been updated to reflect the new R2 treatment.

1.3.14 **Section 3.10, Revised Treatment Summary Flow Chart (Page 32)**
Diagram and legend updated to reflect the new R2 treatment.

1.3.15 **Section 4.2, Rationale for the Various Therapeutic Elements of the Proposed Study (Page 34)**
Therapeutic choice “f” has been updated to reflect the new R2 treatment. Reference 72 added to therapeutic choice “a”.

HRNBL1.5/SIOPEN valid per 01.06.2011
1.3.16 **SECTION 4.2.1, FIRST TREATMENT MODALITY: COJEC AS INDUCTION REGIMEN (PAGES 35-37)**

This section has been updated to reflect the newest results from the ENSG-5 study that have been published in Lancet Oncology. Reference 72 added where appropriate.

1.3.17 **SECTION 4.2.2, TVD RESCUE STRATEGY (PAGE 38)**

Information relating to treatment deleted. Now only rationale information appears.

1.3.18 **SECTION 4.2.6, FIFTH THERAPEUTIC CHOICE (PAGE 43)**

Title changed to; “Fifth therapeutic choice: for patients not randomised in R2, differentiation therapy with 13-cis-retinoic acid alone”.

1.3.19 **SECTION 4.2.7, SIXTH THERAPEUTIC CHOICE (PAGE 43-44)**

- Title changed to; “Sixth therapeutic choice: for patients randomised in R2, randomised immunotherapy with ch14.18/CHO with or without aldesleukin (IL-2)”.
- Information added to the first part of this section regarding the background and rationale for this therapeutic choice.

1.3.20 **SECTION 4.3, PRELIMINARY DATA (PAGE 44)**

Title changed to; “Preliminary data for ch14.18 anti-GD₂ antibody”.

1.3.21 **SECTION 4.5, RESULTS OF THE SIOPEN PHASE I BRIDGING STUDY (PAGES 49)**

Toxicity information added (taken from the IMPD).

1.3.22 **SECTION 4.6, CURRENT CHILDREN’S ONCOLOGY GROUP STUDY (PAGE 49-50)**

- Section numbering updated; in the previous version of the protocol this was section 4.5.1.1.
- Information in this section has been replaced with the information regarding the current Children’s Oncology Group (COG) study, ANBL0032.

1.3.23 **SECTION 4.7, CH14.18/CHO AND ALDESLEUKIN (IL-2) IN HR-NBL-1/SIOPEN (PAGE 50)**

- Section numbering updated; in the previous version of the protocol this was section 4.5.1.2.
- The title and information in this section has been updated to reflect the use of aldesleukin (IL-2) in the new R2 treatment.

1.3.24 **SECTION 4.8, ASPECTS OF COMBINING 13-CIS RA AND CH14.18/CHO (PAGE 50)**

Section numbering updated; in the previous version of the protocol this was section 4.5.1.3.

1.3.25 **SECTION 4.9, ASPECTS OF ADDING ALDESLEUKIN (IL-2) TO THE IMMUNOTHERAPY (PAGE 50-51)**

New section containing information regarding the background and rationale of aldesleukin (IL-2) treatment.
1.3.26 **SECTION 4.10, BACKGROUND TO INTEGRATE MYCN AMPLIFIED (NMA) INFANTS IN THE CURRENT STUDY (PAGE 51)**

Section numbering updated; in the previous version of the protocol this was section 4.5.2.

1.3.27 **SECTION 5.3, ELIGIBILITY CRITERIA FOR THE R1 RANDOMISATION (PAGE 54)**

Criteria for patients of child bearing potential changed (for consistency) to; “Females of childbearing potential must have a negative pregnancy test. Patients of childbearing potential must agree to use an effective birth control method. Female patients who are lactating must agree to stop breast-feeding”.

1.3.28 **SECTION 5.4, ELIGIBILITY CRITERIA FOR THE R2 RANDOMISATION (PAGES 54-55)**

The title and information in this section has been updated to reflect the new R2 treatment.

1.3.29 **SECTION 6.1.9, BIOLOGY STUDIES (PAGE 58)**

“If not supplied contact your National Coordinator for details” added under “Address of your national reference laboratory”.

1.3.30 **SECTION 6.3, INVESTIGATIONS AT DIAGNOSIS, DURING INDUCTION AND PRE-MAT PHASE (PAGE 60)**

Physical examination, ECG, and pulmonary function tests added to list of tests. mIBG removed from primary tumour scans as it appears on its own.

1.3.31 **SECTION 6.4.1, FLOW SHEET 2 FOR INVESTIGATIONS AFTER MAT AND PRIOR TO LOCAL IRRADIATION, DURING MRD TREATMENT PHASE AND END OF TREATMENT (PAGE 61)**

Height, physical examination, blood pressure, CRP, electrolytes, CXR, ECG, pulmonary function tests and NK added to list of tests. Serum sample updated to reflect that now all R2 randomised patients need these samples taken. 
A note has been added about the use of radiographic contrast in conjunction with aldesleukin (IL-2).

1.3.32 **SECTION 6.5, FOLLOW UP INVESTIGATIONS (PAGES 62-63)**

- Imaging requirements for the primary site have been updated (section 6.5.1.1, page 62).
- Bone marrow aspirates have been added to the metastatic assessment (section 6.5.1.2, page 62).
- Urine catecholamine assessment has been added for patients with positive urine catecholamines at diagnosis (section 6.5.1.3, page 62).
- General assessment (physical examination, height, weight, and full blood count) has been added (section 6.5.2.1, page 62).
- Age for audiometry testing changed from 4 years to 3.5 years (section 6.5.2.3, page 62).
- Cardiac follow-up changed to include patients receiving TVD (section 6.5.2.4, page 63).
- Pulmonary follow-up added details added (section 6.5.2.5, page 63).

1.3.33 **SECTION 8.4, FURTHER IMPORTANT ASPECTS (PAGE 69)**

“If not supplied contact your National Coordinator for details” add under “Address of your national reference laboratory”.
1.3.34 **Section 9.1, Type of Biology Studies (Page 69)**
“If not supplied contact your National Coordinator for details” add under “Address of your national reference laboratory”.

1.3.35 **Section 11.1.1, Topotecan, Vincristine and Doxorubicin Rescue Strategy (Page 79-80)**
Details added regarding rescue strategy for patients once the TVD trial closes. Rationale section deleted. TVD treatment summary flow chart added.

1.3.36 **Section 11.2, Check List Prior to MAT (Page 81)**
“Females of childbearing potential must have a negative pregnancy test. Patients of childbearing potential must agree to use an effective birth control method. Female patients who are lactating must agree to stop breast-feeding” added.

1.3.37 **Section 11.3, Monitoring for Ototoxicity (Page 82)**
Age for audiometry testing changed from 3 years to 3.5 years.

1.3.38 **Section 13, 13-cis Retinoic Acid Therapy (Page 106)**
Title changed to “13-cis retinoic acid therapy for patients not randomised in R2”.

1.3.39 **Section 13.2, Suggested Supportive Care (Page 106)**
Title changed to “Suggested supportive care for 13-cis RA”.

1.3.40 **Section 13.3, Criteria Prior to Each Cycle of 13-cis RA (Page 107)**
Added criteria for: bilirubin, veno-occlusive disease, serum calcium, creatinine clearance/GFR, seizure disorder and CNS toxicity.

1.3.41 **Section 13.4, Dose Modifications (Pages 107-108)**
Title changed to “Dose modifications for 13-cis RA”.

1.3.42 **Section 14, Immunotherapy (Page 108)**
Title changed to “Immunotherapy with Ch14.18/CHO with or without aldesleukin (IL-2) for patients randomised in R2”.

1.3.43 **Section 14.1, Indication (Page 108)**
Added information about aldesleukin (IL-2).

1.3.44 **Section 14.2, Treatment Schedule (Pages 108-109)**
Title changed to “Treatment schedule for patients randomised to ch14.18/CHO alone”. Information regarding the use IVIG in conjunction with ch14.18/CHO added.

1.3.45 **Section 14.3, Mode of Action (Page 109)**
Title changed to “Mode of action for ch14.18/CHO”.

1.3.46 **Section 14.5, Toxicity (Page 109)**
Title changed to “Toxicity for ch14.18/CHO”.

HRNBL1.5/SIOPEN valid per 01.06.2011
1.3.47 **SECTION 14.7, TREATMENT SCHEDULE FOR PATIENTS RANDOMISED TO CH14.18/CHO AND SUBCUTANEOUS ALDESLEUKIN (IL-2) (PAGES 111-112)**
New section added with information regarding the R2 treatment arm with ch14.18/CHO and aldesleukin (IL-2).

1.3.48 **SECTION 14.8, CRITERIA PRIOR TO EVERY CYCLE OF ALDESLEUKIN (IL-2) (PAGE 112)**
New section added with information regarding criteria requirements prior to each cycle of aldesleukin (IL-2).

1.3.49 **SECTION 14.9, DOSE MODIFICATIONS FOR ALDESLEUKIN (IL-2) (PAGE 112)**
New section added with information regarding dose modifications for aldesleukin (IL-2).

1.3.50 **SECTION 14.10, DOSE MODIFICATIONS FOR CH14.18/CHO WITH ALDESLEUKIN (IL-2) (PAGE 112)**
New section added with information regarding toxicities that do and do not require dose modifications for ch14.18/CHO with aldesleukin (IL-2).

1.3.51 **SECTION 14.10.1, EXPECTED TOXICITIES THAT DO NOT REQUIRE CH14.18/CHO DOSE MODIFICATION (PAGE 113)**
New section added with information regarding ch14.18/CHO toxicities that do not require dose modifications.

1.3.52 **SECTION 14.10.2, DOSE MODIFICATION OF CH14.18/CHO FOR HYPERSENSITIVITY REACTIONS (PAGE 113)**
New section added with information regarding dose modification for ch14.18/CHO hypersensitivity reactions.

1.3.53 **SECTION 14.10.3, CRITERIA FOR DOSE MODIFICATION OF CH14.18/CHO WITH ALDESLEUKIN (IL-2) (PAGES 113-114)**
New section added with information regarding dose modification for ch14.18/CHO with aldesleukin (IL-2).

1.3.54 **SECTION 14.10.4, CRITERIA FOR STOPPPING CH14.18/CHO (PAGE 114-115)**
New section added with information regarding criteria for stopping treatment with ch14.18/CHO and aldesleukin (IL-2).

1.3.55 **SECTION 14.11, PHARMACOKINETICS (PAGES 115)**
- Section numbering updated; in the previous version of the protocol this was section 4.7.
- Title changed to “Pharmacokinetics for ch14.18/CHO”.
- Sixty patients (previously thirty) will now take part in the pharmacokinetic studies.
- Figure number corrected to “Figure 2”.

1.3.56 **SECTION 14.12, IMMUNOLOGICAL MONITORING (PAGES 116-117)**
- Section numbering updated; in the previous version of the protocol this was section 4.8.
- Title changed to “Immunological monitoring for ch14.18/CHO”.
- Sixty patients (previously thirty) will now take part in the immunological monitoring studies.
- Table labelled as “Table 5”.
- Figure number corrected to “Figure 3”.

HRNBL1.5/SIOPEN valid per 01.06.2011
1.3.57 **SECTION 15, USE OF GCSF (PAGES 118-119)**
The information in this section has been updated to reflect the information in the paper being prepared on the R0 randomisation for submission to the JCO. Reference 122 added.

1.3.58 **SECTION 17.1.1, PRIMARY ENDPOINTS (PAGE 126)**
The R2 aim has been updated to; “The aim is to test the effect of aldesleukin (IL-2) on 3-year survival”.

1.3.59 **SECTION 17.4.2, RANDOMISATION R2: IMMUNOTHERAPY QUESTION (PAGE 128)**
Information updated to reflect the new R2 randomisation.

1.3.60 **SECTION 17.5.1.2, RANDOMISATION R2: IMMUNOTHERAPY QUESTION (CH14.18/CHO WITH OR WITHOUT ALDESLEUKIN (IL-2) (PAGE 128)**
Information updated to reflect the new R2 randomisation.

1.3.61 **SECTION 17.7.1, RELATED TO INDUCTION PHASE (PAGE 130)**
Deleted “1) Toxic deaths occurring during induction treatment”.

1.3.62 **SECTION 17.7.2, RELATED TO SURGERY (PAGE 130)**
Deleted “2) Related to surgery” and new section 17.7.2 created.

1.3.63 **SECTION 17.7.3, RELATED TO MAT PHASE (PAGE 130)**
Deleted “3) Related to MAT” and new section 17.7.3 created.

1.3.64 **SECTION 19, APPENDIX: PATHOLOGY AND BIOLOGY GUIDELINES (PAGES 135-148)**
Figure and table numbers corrected (pages: 135-137, 142-145, and 147-148).

1.3.65 **SECTION 20, APPENDIX: 123I MIBG IMAGING PROTOCOL (PAGES 153-156)**
Entire section, including score sheet, deleted and updated.

1.3.66 **SECTION 21, APPENDIX: MONITORING FOR OTOTOXICITY (PAGES 160-165)**
- References to attached protocols that are not included deleted (pages: 160, 164, and 165).
- The two standard of procedure diagrams have been replaced with diagrams showing correct arrow placement (pages: 161 and 162).

1.3.67 **SECTION 23, APPENDIX: DRUG INFORMATION (PAGES 172-201)**
- The toxicity frequency tables for the following drug have been updated: carboplatin, etoposide, cyclophosphamide, vincristine, GCSF, 13-cis RA, ch14.18.
- Toxicity frequency tables have been added for topotecan and doxorubicin.
- References and information not applicable to this trial from the drug information for IV Busulfan have been deleted or referenced to the correct page (pages: 179-182, 187-189, and 191)
- Section 23.10 “Distribution Logistics” now section 23.9.1 (page 193). The drugs following this have had their sections numbers updated. i.e. Melphalan now section 23.10 (page 196), GCSF now section 23.11 (page 196), 13-cis RA now section 23.12 (page 198), and ch14.18 now section 23.13 (page 200).
• Section 23.14 (page 200-202), drug information and toxicity frequencies for aldesleukin (IL-2) has been added. References 175-177 added.
• Ingrid Pribill’s contact details have replaced Sabine Rau’s (pages: 194-195)

1.3.68 SECTION 26, APPENDIX: PERFORMANCE SCALES (PAGE 217)
Age information added.

1.3.69 SECTION 27, APPENDIX: GUIDELINES FOR REPORTING TOXICITY/SERIOUS ADVERSE EVENTS (PAGES 218-222)
• The information in this section has been mostly rewritten.
• Section 27.1 “Definitions and reporting methods” added.
• Section 27.2 “Profile of expected toxicities in this trial” created, the list that appears here was under II.d. in the previous version.

1.3.70 SECTION 31, APPENDIX: ADDRESS LIST (PAGES 231-250)
Committee members and details updated where appropriate (pages: 231-237 and 250).

1.3.71 SECTION 31, APPENDIX: DECLARATION OF HELSINKI (PAGES 269-272)
Replaced with most recent Declaration (Oct 2008)

1.3.72 SECTION 33.1, INFORMATION SHEET (PAGES 273-280)
• Title changed to “Information sheet for study enrolment and induction treatment”
• Information relating to R2 randomisation has been updated to reflect the new R2 treatment (pages: 274-275, 277, and 279).
• The information given under “Side effects to chemotherapy drugs” on page 280 as been rewritten.

1.3.73 SECTION 33.2, APPENDIX: CONSENT FORM (PAGE 283)
• Title changed to “Consent form for study enrolment and induction treatment”.
• Information added relating to consent for use of medical information including tissue samples.

1.3.74 SECTION 33.5, IMMUNOTHERAPY RANDOMISATION R2 (PAGES 288-294)
The majority of this information sheet has been changed to reflect the new R2 treatment.

1.3.75 SECTION 33.6, CONSENT FORM FOR RANDOMISATION R2 (PAGE 295)
This consent form has been updated to reflect the new R2 treatment.

1.4 Amendment as of June, 2011

A copy of the protocol with tracked changes is available on request.

N.B. Page numbers reflect those applicable to this amended version of the protocol.

1.4.1 Typing and English corrections
Multiple corrections made throughout the protocol for English and typing errors. These changes are not detailed below.
1.4.2 CHANGES/ADDITIONS FOR CLARITY AND CONSISTENCY
Multiple corrections made throughout the protocol for consistency and clarification of various items. These changes are not detailed below.

1.4.3 HR-NBL-1 CHANGED TO HR-NBL-1.5
Reference to HR-NBL-1 changed throughout to HR-NBL-1.5 to reflect the new version of the protocol.

1.4.4 RAPID COJEC
For accuracy reference to COJEC changed throughout to Rapid COJEC.

1.4.5 13-CIS RETINOIC ACID
For accuracy reference to 13-cis retinoic acid changed throughout to isotretinoin (13-cis-RA).

1.4.6 AUTOLOGOUS STEM CELL TRANSPLANT
Reference to autologous stem cell transplant and peripheral blood stem cell transplant have all been changed to peripheral blood stem cell rescue (PBSCR) for consistency (references to published material excluded).

1.4.7 DIFFERENTIATION THERAPY AND MRD TREATMENT
Reference to differentiation therapy and MRD treatment have all been changed to immunotherapy for consistency (references to published material excluded).

1.4.8 SIOP EUROPE NEUROBLASTOMA (SIOPEN) EXECUTIVE COMMITTEE (PAGES 1 & 2)
The list with SIOPEN executive members and the national coordinators from the respective countries has been updated and moved from page 1 to page 2.

1.4.9 NATIONAL COORDINATORS (PAGE 2)
The list of national coordinators has been moved from page 1 to page 2. Finland, under the coordination of Dr. Kim Vettenranta, has been added to the list.

1.4.10 SIOP EUROPE NEUROBLASTOMA (SIOPEN) SPECIALITY COMMITTEES AND CHAIRS (PAGE 3)
The list with special committees and chairs has been updated.

• The RT-PCR studies and stem cell group is replaced by the Molecular Monitoring Group with Sue Burchill as chair.
• The new chair of the nuclear medicine and physics committee is Arianne Boubaker from Switzerland.
• Keith Wheatley replaces David Machin as the statistician from the UK. David has retired.

1.4.11 GROUPS ADDED (PAGE 3)
The following groups were added:

• CRCTU Cancer Research UK Clinical Trials Unit
• NCRI CCL CSG Neuroblastoma Group UK

HRNBL1.5/SIOPEN valid per 01.06.2011 30
1.4.12 SYNOPSIS (PAGES 4-9)

- The following statement has been added to the background “NOTE: Patients aged 12-18 months at diagnosis, with stage 4 neuroblastoma, no MYCN amplification and without segmental chromosomal alterations (SCA) are thought to have a good prognosis (see section 4.10, page 75) and will stop treatment after induction therapy and surgery to the primary tumour.”
- Primary objectives have been adapted to reflect the changes with a new randomisation arm (R3) and the closure of R1.
- Study Inclusion criteria have been adapted to give a clearer definition of high-risk neuroblastoma.
- New R3 inclusion criteria have been added.
- Note added regarding closure of R1 randomisation. BuMel is now the standard MAT.
- Inclusion criteria for BuMel MAT added. Also, notes regarding:
  - no more free drug supply for Busilvex
  - patients presenting at diagnosis with a large abdominal or large pulmonary mass
  - patients aged 12-18 months at diagnosis with favourable biology.
- For consistency, inclusion criteria for R2 were adapted to reflect criteria in section 5.5.
- Major changes for HR-NBL-1.5 included under amendment 5.
- Study period and patient numbers updated.

1.4.13 ABBREVIATION LIST (PAGES 10-12)
The following abbreviations were added:
- AIPF - automatic immuno-fluorescence plus FISH
- Doxo - doxorubicin
- FISH - Fluorescence in situ hybridisation
- HaChA - Human anti-chimeric Antibody
- IC - Immunocytology
- ICH-GD2 - determination of GD2 with immunohistochemistry
- MLPA - multiplex ligation-dependent probe amplification
- MNA - MYCN amplified
- MR - mixed response
- NCA - nurse controlled analgesia
- PCA - patient controlled analgesia
- PET - Positron emission tomography
- QRT-PCR - quantitative reverse transcriptase polymerase chain reaction
- R3 - randomisation of induction regimen (Rapid COJEC vs. modified N7)
- SmPC - Summary of Product Characteristics
- SPECT - single-photon emission computed tomography

1.4.14 CONTENT (PAGES 14-18)
Content list was updated to reflect the changes and addition/deletions of sections and pages.

1.4.15 SUMMARY OF AMENDMENT AS OF JUNE, 2011 (PAGES 29-39)
Content list was updated to reflect the changes and addition/deletions of sections and pages.

1.4.16 SECTION 2, SPECIFIC AIDS (PAGE 40)
The primary and secondary aims have been changed in order to reflect the opening of the R3 randomisation and the closure of the R1 randomisation.
1.4.17 **SECTION 3, TRIAL DESIGN (PAGE 41)**
The new R3 randomisation, the changes in the consolidation phase after R1 closure and an update of the immunotherapy are described.

1.4.18 **SECTION 3.1, INDUCTION CHEMOTHERAPY (PAGE 42)**
New inclusion criteria for induction chemotherapy have been added.

1.4.19 **SECTION 3.1.3 & 3.1.4, MODIFIED N7 INDUCTION (PAGE 44)**
New section with detailed information on the modified N7 treatment option has been added, including dose modifications for infants and low weight children.

1.4.20 **SECTION 3.2, TOPOTECAN, VINCRISTINE AND DOXORUBICIN FOR PATIENTS WITH AN INADEQUATE METASTATIC RESPONSE AFTER INDUCTION (PAGE 46)**
Information included regarding treatment of patient after TVD if response criteria are not met. Also, dosing for infants and low weight children added.

1.4.21 **SECTION 3.5, MYELOABLATIVE THERAPY AND PERIPHERAL BLOOD STEM CELL RESCUE (PAGE 47)**
Information updated to reflect the closure of the R1 randomisation and the use of BuMel MAT.

1.4.22 **SECTION 3.7, DIFFERENTIATION THERAPY, (PAGE 48)**
Information added stating that patients ≤12 kg should not receive a dose reduction of isotretinoin (13-cis-RA).

1.4.23 **SECTION 3.8, IMMUNOTHERAPY APPROACH (SECOND RANDOMISATION - R2 MODIFICATION ACTIVATED JULY 2009) (PAGE 48)**
Dosing for infants and low weight children added for ch14.18/CHO and aldesleukin (Il-2).

1.4.24 **SECTION 3.9, TREATMENT AND RESPONSE OVERVIEW (PAGE 50)**
Flow chart has been adapted according to the new R3 randomisation. New table with diagnostic and response evaluation was added.

1.4.25 **SECTION 4, BACKGROUND AND RATIONALE (PAGE 51)**
The following statement has been added to the background introduction “NOTE: Patients aged 12-18 months at diagnosis, with stage 4 neuroblastoma, no MYCN amplification and without segmental chromosomal alterations (SCA) are thought to have a good prognosis (see section 4.10, page 75) and will stop treatment after induction therapy and surgery to the primary tumour.”

1.4.26 **SECTION 4.2, RATIONALE FOR THE VARIOUS THERAPEUTIC ELEMENTS OF THE PROPOSED STUDY (PAGE 52)**
The rationale and the treatment options have been changed in order to reflect the opening of the R3 randomisation and the closure of R1.

1.4.27 **SECTION 4.2.1, FIRST TREATMENT MODALITY: COMPARISON OF TWO INDUCTION REGIMENS RAPID COJEC VS. MODIFIED N7 (PAGE 52)**
The title was changed from “COJEC as Induction Regimen” to “Comparison of two induction regimens (Rapid COJEC versus modified N7)”
1.4.28 **SECTION 4.2.1.1, RAPID COJEC REGIMEN (PAGE 53)**
New section added to contain the information relating to the rapid COJEC regimen.

1.4.29 **SECTION 4.2.1.2, MODIFIED N7 REGIMEN (PAGE 57)**
New section with background information on the modified N7 therapy was added.

1.4.30 **SECTION 4.2.1.3, RESPONSE IN THE RANDOMISED INDUCTION PHASE (PAGE 59)**
Section added.

1.4.31 **SECTION 4.2.2, TVD (TOPOTECAN-VINCRISTINE-DOXORUBICIN) RESCUE STRATEGY (PAGE 59)**
Information regarding implications of receiving TVD following Rapid COJEC or modified N7 added.

1.4.32 **SECTION 4.2.4, THIRD TREATMENT MODALITY: BU-MEL MAT REGIMEN (PAGE 60)**
This section was updated with the R1 randomisation results of the HR-NBL-1/SIOOPEN study. BuMel is now recommended as standard treatment in the HR-NBL-1.5/SIOOPEN trial. All background information relating to CEM has been removed. Also added information regarding BuMel and radiation in patients presenting at diagnosis with a large abdominal or large pulmonary mass.

1.4.33 **SECTION 4.2.5, FOURTH TREATMENT MODALITY: RADIOTHERAPY, (PAGE 63)**
Information added regarding BuMel and radiation in patients presenting at diagnosis with a large abdominal or large pulmonary mass.

1.4.34 **SECTION 4.2.6.4, RATIONALE FOR NO ISOTRETINOIN (13-CIS-RA) DOSE REDUCTION IN CHILDREN ≤ 12 KG (PAGE 64)**
Section added.

1.4.35 **SECTION 4.6, IMMUNOTHERAPY WITH ANTI-GD2 MONOCLONAL ANTIBODY AND CYTOKINES (IL-2 AND GM-CSF) PAGE 71**
New section has been added to give more background information on immunotherapy with anti-GD2 monoclonal antibody and cytokines.

1.4.36 **SECTION 4.7, THE CHILDREN’S ONCOLOGY GROUP STUDY ANBL0032 (PAGE 72)**
Section updated to reflect the latest results from The Children’s Oncology Group Study ANBL0032.

1.4.37 **SECTIONS 4.8 AND 4.9 (PREVIOUS VERSION), (PAGE 72)**
Sections deleted, because information now contained within the new section 4.6.
1.4.38 **SECTION 4.10, RATIONALE FOR TREATMENT REDUCTION IN CHILDREN 12-18 MONTHS AT DIAGNOSIS WITH FAVOURABLE BIOLOGY (PAGE 72)**

Section added.

1.4.39 **SECTION 5.1, ELIGIBILITY CRITERIA FOR THE STUDY (PAGE 76)**

Study Inclusion criteria have been adapted to give a clearer definition of high-risk neuroblastoma. Also, the first chemotherapy cycle for localised patients has been changed to “1 cycle of etoposide and carboplatin (VP/Carbo).”

1.4.40 **SECTION 5.3, ELIGIBILITY CRITERIA FOR THE R3 RANDOMISATION (PAGE 76)**

Section added for the new induction randomisation (R3).

1.4.41 **SECTION 5.4, ELIGIBILITY CRITERIA FOR BuMel MAT (R1 RANDOMISATION CLOSED OCTOBER 2010) (PAGE 77)**

Section updated to reflect the closure of the R1 randomisation and recommendation that all patients should now receive BuMel MAT, except for patients between 12 and 18 months at diagnosis with favourable biology.

1.4.42 **SECTION 5.5, ELIGIBILITY CRITERIA FOR THE R2 RANDOMISATION (2ND REVISION ACTIVATED) (PAGE 78)**

Changes made to reflect the opening of the R3 randomisation and the closure of the R2 randomisation. Also clarifications made regarding the timing of radiotherapy, the delay from PBSCR and timing of the randomisation.

1.4.43 **SECTION 6.1.7, BONE MARROW, APHAERETIC PRODUCT AND PERIPHERAL BLOOD SAMPLING AND EVALUATION (PAGE 79)**

The entire section has been updated. Sample procedures and time points are explained in more detail and Figure 10 with sampling procedures has been added.

1.4.44 **SECTION 6.2, NECESSARY INTERACTIONS TO SECURE TUMOUR AND BIOLOGICAL SAMPLE FLOW (PAGE 85)**

Figure deleted (information now appears in Figure 10). Further information added to pathologist, biologist and cytologist roles. Immunocytologist and Molecular Monitoring Group details added.

1.4.45 **SECTION 6.3, INVESTIGATIONS AT DIAGNOSIS, DURING RAPID COJEC INDUCTION AND TVD (PAGE 87)**

Updated chart and footnotes added.

1.4.46 **SECTION 6.4, INVESTIGATIONS AT DIAGNOSIS, DURING MODIFIED N7 INDUCTION AND TVD (PAGE 88)**

New chart and footnotes added.

1.4.47 **SECTION 6.5, INVESTIGATIONS AT PRE- AND POST-MAT PHASE (PAGE 89)**

New chart and footnotes added.
1.4.48 Section 6.6, Investigations during Immunotherapy Phase until the End of Treatment (Page 90)
Updated chart and footnotes added.

1.4.49 Section 6.7.2.4, Cardiac Follow-Up (Page 92)
Details of cardiac follow-up updated to reflect addition of modified N7 treatment.

1.4.50 Section 7.3, Timing (Page 93)
A more detailed definition of partial response has been added.

1.4.51 Section 8.2, Recommendations for Handling of Tumour Material (Page 96)
A caution has been added regarding recommendations for open biopsies, tru cut biopsies and fine needle aspirations (moved from previous section 8.4).

1.4.52 Section 8.4 (Previous Version), (Page 97)
Section deleted (caution now appears in section 8.2).

1.4.53 Section 9, Biological Studies (Page 98)
Changes made to reflect new biology structure for HR-NBL-1.5/SIOPEN.

1.4.54 Section 10.2, Timing of Peripheral Stem Cell Harvest (Page 99)
Changes made to reflect the addition of modified N7 treatment.

1.4.55 Section 10.3, Recommended Procedure for PBSC Mobilisation and Day of Collection (Page 100)
Changes made to reflect the addition of modified N7 treatment.

1.4.56 Section 11, Chemotherapy Regimen Details (Page 100)
The following paragraph has been added to the start of this section. “The administration regimens that appear below are guidelines only. In cases where national and/or local institutional guidelines differ to these, the local/national guidelines may be used; this is at the discretion of the local/national investigators.”

1.4.57 Section 11.2, Modified N7 Induction (Page 105)
Section added with detailed information about the modified N7 regimen.

1.4.58 Section 11.3, Topotecan, Vincristine and Doxorubicin (TVD) Strategy (Page 109)
Information about TVD regimen updated to reflect closure of TVD study and the use of TVD in the HR-NB1-1.5/SIOPEN study.

1.4.59 Section 11.3.1, Chemotherapy Regimen Details of TVD (Page 109)
Dose adjustments for infants and low weight children added.

1.4.60 Section 11.4, Check List Prior to MAT (Page 110)
Section updated to reflect the closure of the R1 randomisation and recommendation that all patients should now receive BuMel MAT, except for patients between 12 and 18 months at diagnosis with favourable biology.
1.4.61 Section 11.6, Monitoring of Renal Function (Page 115)
Sections relating to CEM deleted.

1.4.62 Section 11.7, Bumel Mat Regimen (Page 115)
Introduction text updated to reflect the closure of the R1 randomisation. Also, the use of oral busulfan is permitted if i.v. busulfan is not available, although the use of oral busulfan is not recommended.

1.4.63 Sections relating to CEM – MAT regimen (Page 118)
All sections relating to the CEM – MAT regimen have been deleted (i.e. sections 11.6, 11.7 and 11.8 of the previous version).

1.4.64 Section 11.7.3, Drug Sampling (Page 118)
Section on PK sampling for BuMel MAT regimen added.

1.4.65 Section 12, Radiotherapy (Page 119)
The entire section has been updated. In particular, a prospective review of radiotherapy plans and doses is required in the HR-NBL-1.5/SIOPEN study.

1.4.66 Section 14.2, Treatment Schedule for Patients Randomised to Ch14.18/CHO alone (Page 124)
Dose adjustments for infants and low weight children added for ch14.18/CHO. Low weight children receiving isotretinoin (13-cis-RA) should not receive a dose reduction.

1.4.67 Section 14.4, Mode of Administration of Ch14.18/CHO (Page 125)
Additional information added regarding premedication and the administration of ch14.18/CHO. Dose reduction for infants and low weight children added.

1.4.68 Section 14.5, Toxicity of Ch14.18/CHO (Page 125)
The following paragraph was added to the start of this section. “Close monitoring of the patient will be required during the administration of ch14.18/CHO. Each cycle requires adequate nursing support during the first 2 hours of each infusion.”

1.4.69 Section 14.6.1, Mandatory Medication for Pain during Ch14.18/CHO Infusion (Page 126)
Two pain medication schedules are now described – the original one that has been used since the ch14.18/CHO antibody treatment was included into the protocol and another one using NCA and PCA pumps. Centres can choose according to their local resources.

1.4.70 Section 14.6.2, Prohibited Treatments (Page 128)
New section with information on prohibited treatments was added.

1.4.71 Section 14.6.3, Mandatory Surveillance and Monitoring during Antibody Infusion (Page 128)
Information on surveillance during antibody infusion has been updated.
1.4.72 **SECTION 14.7, TREATMENT SCHEDULE FOR PATIENTS RANDOMISED TO CH14.18/CHO AND SUBCUTANEOUS ALDESLEUKIN (IL-2) (PAGE 129)**
Updated information on timing of aldesleukin (IL-2) treatment added. Also, dose reductions for infants and low weight children added.

1.4.73 **SECTION 14.8, CRITERIA PRIOR TO IMMUNOTHERAPY (PAGE 130)**
Section changed from “criteria prior to every cycle of aldesleukin (IL-2)” to “criteria prior to immunotherapy”. The information within the section has been updated accordingly.

1.4.74 **SECTION 14.9, DOSE MODIFICATIONS FOR PATIENTS RANDOMISED TO CH14.18/CHO ALONE (PAGE 130)**
Entire section (and sub-sections) modified to clarify when dose modifications for patients randomised to receive ch14.18/CHO alone are needed.

1.4.75 **SECTION 14.10, DOSE MODIFICATIONS FOR PATIENTS RANDOMISED TO CH14.18/CHO PLUS ALDESLEUKIN (IL-2) (PAGE 132)**
Entire section (and sub-sections) modified to clarify when dose modifications for patients randomised to receive ch14.18/CHO plus aldesleukin (IL-2) are needed.

1.4.76 **SECTION 14.11, PHARMACOKINETICS FOR CH14.18/CHO (PAGES 133)**
The entire section has been updated and new information regarding PK sampling added. New sampling sheets are provided.

1.4.77 **SECTION 14.12, IMMUNOLOGICAL MONITORING FOR CH14.18/CHO (PAGE 137)**
The entire section has been updated and new information regarding immunological sampling added. New sampling sheets are provided.

1.4.78 **SECTION 16, SUPPORTIVE CARE GUIDELINES (PAGE 143)**
The following paragraph has been added. “The supportive care details that appear below are guidelines only. In cases where local institutional guidelines differ to these, the local guidelines maybe used; this is at the discretion of the local investigator.”

1.4.79 **SECTION 16.9.1, PROPHYLAXIS OF HVOD (PAGE 146)**
No prophylaxis is recommend for HOVD in the HR-NBL-1.5/SIOPEN study. Therefore, information relating to the prophylactic use of ursodiol has been deleted.

1.4.80 **SECTION 16.9.3, MANAGEMENT OF HVOD (PAGE 147)**
Defibrotide at the earliest sign of HVOD is strongly encouraged. Defibrotide guidelines are added.

1.4.81 **SECTION 17.1, END POINTS (PAGE 149)**
Primary and secondary endpoints updated to reflect the closure of the R1 randomisation and the opening of the R3 randomisation.

1.4.82 **SECTION 17.2, RANDOMISATION (PAGE 151)**
Randomisation information updated to reflect the closure of the R1 randomisation and the opening of the R3 randomisation.
1.4.83 **SECTION 17.4, POWER CONSIDERATIONS (PAGE 152)**
Power consideration information updated to reflect the closure of the R1 randomisation and the opening of the R3 randomisation.

1.4.84 **SECTION 17.5, ANALYSIS (PAGE 153)**
Analysis information updated to reflect the closure of the R1 randomisation and the opening of the R3 randomisation.

1.4.85 **SECTION 17.7.1, RELATED TO INDUCTION PHASE (PAGE 155)**
Monitoring guidelines for severe toxicities during the induction phase updated to reflect the opening of the R3 randomisation.

1.4.86 **SECTION 18.5, TRIAL SPECIFIC RESPONSE DEFINITIONS (PAGE 158)**
New chart with trial related response criteria added.

1.4.87 **SECTION 19, APPENDIX: PATHOLOGY AND BIOLOGY GUIDELINES FOR RESECTABLE AND UNRESECTABLE NEUROBLASTIC TUMOURS AND BONE MARROW EXAMINATION GUIDELINES (PAGE 159)**
The list of co-operating partners has been updated.

1.4.88 **SECTION 19.1, GENERAL REMARKS (PAGE 159)**
The section has been updated with organisational updates.

1.4.89 **SECTION 19.3.3.2, SEGMENTAL CHROMOSOMAL ALTERATIONS (SCA) (PAGE 168)**
Former section “Chromosome 1p36.3 status” was replaced by new section “Segmental Chromosomal Alterations (SCA)”.

1.4.90 **SECTION 19.3.4.1, EXPRESSION STUDIES (PAGE 168)**
Former section “Other Chromosomal regions of potential interest” replaced by new section “Expression studies”.

1.4.91 **SECTION 19.4, BONE MARROW EXAMINATION GUIDELINES (PAGE 169)**
The entire section has been updated and more detailed information is given.

1.4.92 **SECTION 19.5, HANDLING OF THE BONE MARROW CELLS IN THE LABORATORY (PAGE 170)**
The entire section has been updated.

1.4.93 **SECTION 19.6, FORM B1. QRT-PCR IN PERIPHERAL BLOOD, BONE MARROW AND PBSC HARVEST REQUEST FORM (PAGE 171)**
New sampling sheet QRT-PCR added.

1.4.94 **SECTION 19.7, APHAERETIC PRODUCTS (PAGE 172)**
New section added with information on handling aphaeretic products.
1.4.95 **SECTION 20, APPENDIX: \(^{123}\text{I} \text{mIBG Imaging Protocol (Page 172)}**
Changes made throughout section for clarity, including a new section on quality control and a new mIBG score sheet.

1.4.96 **SECTION 23, APPENDIX : DRUG INFORMATION (PAGE 195)**
Sections 23.1-23.6, 23.8-23.10, 23.12 and 23.14: added that all drugs are commercially available.

1.4.97 **SECTION 23.9.1, I.V. BUSULFAN (BUSILVEX ®) (PAGE 201)**
Since the closure of the R1 randomisation Busilvex® is no longer supplied by the Sponsor. The section on distribution logistics has been changed accordingly.

1.4.98 **SECTION 23.9.2, ORAL BUSULFAN (PAGE 216)**
Information on drug and adverse reactions for children of oral busulfan added.

1.4.99 **SECTION 23.13, CHIMERIC 14.18 ANTI-GD2 MONOCLONAL ANTIBODY (CH 14.18) (PAGE 221)**
Entire section updated. This section now includes details on: preparation, method of administration, instructions for handling and disposal, distribution logistics and drug accountability.

1.4.100 **SECTION 24, APPENDIX: PHARMACOLOGY STUDIES (PAGE 231)**
The sending of samples for busulfan, melphalan and isotretinoin (13-cis-RA) PK studies have been changed for clarity. New sampling sheets are provided. Information regarding PK studies for CEM has been deleted.

1.4.101 **SECTION 28, APPENDIX: TOXICITY GRADING AND PAIN ASSESSMENT (PAGE 247)**
Pain assessment tools added.

1.4.102 **SECTION 31, APPENDIX: ADDRESS LIST (PAGE 257)**
Committee members and contact details updated where appropriate
- 31.5 - Title change to “Bone Marrow Immunocytology Studies Committee”
- 31.6 - New section Molecular Monitoring Studies Committee was added
- 31.16 - New section added “Summary of National Contact Details for Material Transfer”

1.4.103 **SECTION 33, APPENDIX: SAMPLE INFORMATION SHEET/CONSENT FORMS (PAGE 299)**
Sample information sheets and consent forms updated to reflect the closure of the R1 randomisation and the opening of the R3 randomisation.

1.4.104 **SECTION 34, REFERENCE LIST (PAGE 322)**
The reference list was updated accordingly.
2 Specific Aims

2.1 Primary Aims

- To test the hypothesis that the modified N7 induction regimen will improve the metastatic response rates or event free survival (EFS) as compared to Rapid COJEC.
- To test the hypothesis that the addition of subcutaneous aldesleukin (IL-2, (Proleukin®)) to immunotherapy with chimeric 14.18 anti-GD2 monoclonal antibody produced in Chinese hamster ovary (CHO) cells (ch14.18/CHO) in addition to differentiation therapy with isotretinoin (13-cis-RA) following myeloablative therapy (MAT) and autologous SCR, will improve EFS in patients with high-risk neuroblastoma (stage 4 disease or stages 2 and 3 with MYCN amplification all over the age of one, or infants with MYCN amplification).
- To test the hypothesis that MAT with intravenous (I.V.) formulation of Busulfan (BU, Busilvex®) and melphalan (BuMel) in patients with high risk neuroblastoma (stage 4 disease or stages 2 and 3 disease with MYCN amplification, all above one year of age at diagnosis) will result in a superior, 3-year, event free survival (EFS) than MAT with continuous infusion carboplatin, etoposide and melphalan (CEM). (AIM achieved – R1 closed in favour of BuMel).

2.2 Secondary Aims

Related to diagnosis and induction phase
- To evaluate BM response to Rapid COJEC (after the fourth and eight cycles) and modified N7 (after the third and fifth cycles) with ICH-GD2, AIPF and QRT-PCR.
- To evaluate response to Rapid COJEC and modified N7 induction therapies with mIBG for standardised scoring of skeletal response.
- To determine the effect of response of metastatic disease to induction therapy on EFS and overall survival (OS).
- To investigate the relationship between complete surgical resection of the primary tumour and OS.
- To collect data on selected, validated biological features and to determine the effect of these on EFS, the incidence of relapse/progression and OS.
- To compare the toxicity, in particular episodes of febrile neutropenia and grade 3-4 infection, associated with induction therapy with Rapid COJEC and modified N7.
- To compare the toxicity and, in particular, episodes of febrile neutropenia associated with induction therapy with a rapid schedule platinum regimen (COJEC) when administered with and without G-CSF. (AIM achieved-R0 closed).
- To determine the effect of elective haemopoietic support with G-CSF during induction therapy on the success of peripheral blood stem cell harvesting (PBSCH) after induction therapy. (AIM achieved- R0 closed).

Related to MAT phase
- To determine the effect of MAT with BUMEL or CEM on 5-year EFS, 3- and 5-year PFS and 3- and 5-year OS. (AIM achieved - R1 closed).
- To determine and compare the acute and long term toxicities of BUMEL and CEM used as MAT. (AIM for acute toxicities achieved - R1 closed).
- To sample PK data to calculate the effect of AUC as achieved according to dosing guidelines. AUC will be correlated with EFS after adjustment to factors such as
immunotherapy (if relevant by study results). The aim is to investigate if there is an ideal narrow window for outcome prediction in balance with toxicity observed.

- To monitor drug levels of MAT (in particular of busulfan and melphalan) and to relate them to patients outcome and toxicity.
- To collect data on selected, validated biological features and to determine the effect of these on EFS and OS.

Related to post MAT phase

- To investigate the relationship between the extent of radiotherapy at the primary site (the pre-surgical tumour volume) and EFS, incidence of relapse/progression and overall survival.
- To determine the tolerance of isotretinoin (13-cis-RA) following MAT with the addition of immunotherapy with ch14.18/CHO with or without aldesleukin (IL-2).
- To collect data on selected, validated biological features and to determine the effect of these on EFS and OS.

3 Trial Design

This is a randomised study of the European SIOP Neuroblastoma (SIOPEN) Group in high-risk neuroblastoma (stages 2, 3, 4 and 4s MYCN-amplified neuroblastoma, stage 4 MYCN non-amplified ≥ 12 months at diagnosis).

Induction

In the induction phase, all patients with stage 4 neuroblastoma and those with stage 4s MYCN-amplified neuroblastoma will be randomised (R3) to Rapid COJEC or modified N7; localised patients will receive Rapid COJEC.

Arm A is the rapid, dose intensive induction chemotherapy (Rapid COJEC) with the recommended prophylactic use of G-CSF (following the results of the R0 randomisation) as in the HR-NBL-1 protocol [1]. Arm B uses a modified version of the Memorial Sloan-Kettering (MSK) regimen N7 using five cycles as recently published [2, 3]. Following induction treatment peripheral blood stem cell harvest (PBSCH) will be performed and complete excision of the primary tumour will be attempted.

Patients with an inadequate metastatic response to allow BuMel MAT followed by PBSCR at the end of induction should receive 2 TVD (Topotecan, Vincristine, Doxorubicin) cycles. If an adequate metastatic response (negative bone marrow evaluation and ≤ 3 MIBG bone spots) is achieved after 2 TVD cycles they may continue on the High Risk Study.

After Rapid COJEC induction, localised patients will proceed to consolidation.

Patients aged 12-18 months at diagnosis, with stage 4 neuroblastoma, no MYCN amplification and without segmental chromosomal alterations (SCA) are thought to have a good prognosis (see section 4.10, page 75) and will stop treatment after induction therapy and surgery to the primary tumour.

Consolidation

Consolidation will consist of BuMel MAT (following the results of the R1 randomisation) followed by peripheral blood stem cell rescue (PBSR) and radiotherapy to the site of the primary tumour.

Immunotherapy

During the immunotherapy phase, patients will be randomised (R2) to immunotherapy with isotretinoin (13-cis-RA) and ch14.18/CHO, with or without aldesleukin (IL-2).

Any patient registered on the HR-NBL-1/SIOOPEN study who started treatment according to the protocol and ultimately receives BuMel MAT (minor modifications for toxicity concerns are
allowed) followed by autologous PBSCR is eligible for randomization in R2 for the immunotherapy question. However, patients must be no more than 9 months from the date of starting the first induction chemotherapy after diagnosis to the date of PBSCR.

The treatment of infants with MYCN amplification is an integral part of this protocol. Rationale and treatment adaptations for infants are specified in the relevant chapters.

3.1 Induction Chemotherapy

Only patients with stage 4 neuroblastoma or stage 4s MYCN-amplified neuroblastoma will be randomised to Rapid COJEC vs. modified N7. Patients with localised disease (stage 2 and 3 MYCN-amplified) will receive Rapid COJEC.

3.1.1 THE ‘RAPID COJEC SCHEDULE

The ‘Rapid COJEC Schedule’ lasts ten weeks and proceeds regardless of the neutrophil or platelet count and controlled infections. Rapid COJEC consists of three different courses of chemotherapy: Course A, Course B and Course C. One of these courses is given every 10 days. Drug doses used in each of these are summarised here as cumulative dose per course. Course A consists of carboplatin (CBDCA) 750mg/m²/course, etoposide (Vp16) 350mg/m²/course in two divided doses and vincristine (VCR) 1.5mg/m²/course (maximum dose of 2 mg). The doses of drugs used in Course B are: cisplatin (CDDP) 80mg/m²/course as continuous intravenous infusion and vincristine (VCR) 1.5mg/m²/course (maximum dose of 2mg). Course C consists of etoposide 350mg/m²/course, cyclophosphamide (CYC) 2.1g/m²/course and vincristine 1.5mg/m²/course (maximum dose 2 mg). Course A starts on days 0 and 40, B on days 10, 30, 50 and 70 and C on days 20 and 60. Daily doses and detailed infusion schedules are given in detail in Section 11.1, page 101.

In the initial phase of the study a randomised supportive care question (R0), on the use of G-CSF during the induction period, was carried out. This R0 randomisation closed in November 2005 and following the results of this analysis the use of prophylactic G-CSF is now recommended for all patients [1] (Details Section 15).

<table>
<thead>
<tr>
<th>R0</th>
<th>G-CSF</th>
<th>Dose/day</th>
<th>days</th>
<th>days</th>
<th>days</th>
<th>days</th>
<th>days</th>
<th>days</th>
<th>days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5µg/kg</td>
<td>3-8</td>
<td>12-18</td>
<td>23-28</td>
<td>32-38</td>
<td>43-48</td>
<td>52-58</td>
<td>63-68</td>
</tr>
<tr>
<td></td>
<td>CBDCA</td>
<td>750mg/m²</td>
<td>⚠️</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vp16</td>
<td>175mg/m²</td>
<td></td>
<td>⚠️</td>
<td>⚠️</td>
<td>⚠️</td>
<td>⚠️</td>
<td>⚠️</td>
<td>⚠️</td>
</tr>
<tr>
<td></td>
<td>VCR</td>
<td>1.5mg/m²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CDDP</td>
<td>80mg/m²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ctn</td>
<td>ctn</td>
<td>ctn</td>
</tr>
<tr>
<td></td>
<td>CYC</td>
<td>1050mg/m²</td>
<td></td>
<td></td>
<td>⚠️</td>
<td>⚠️</td>
<td>⚠️</td>
<td>⚠️</td>
<td>⚠️</td>
</tr>
</tbody>
</table>

DAY: 0 10 20 30 40 50 60 70 80 90 100 110

COURSE: A B C B A B C B

CYCLE: 1 2 3 4 5 6 7 8

HARVEST: H ↔ H ↔ H ↔

SURGERY: Sx ↔

STAGING: ◆ ◆ ◆ ◆ ■

◆ Primary site MRI or CT, ultrasound and mIBG (skeletal and local) and Bone Marrow: 2 aspirates/ 2 biopsies;
◆ Bone Marrow: 2 aspirates, primary site ultrasound
■ Primary site: control of surgical success (method MRI, CT: 2 weeks after surgery; an additional postoperative mIBG Scan prior to MAT is mandatory)
### 3.1.2 The ‘Rapid COJEC Schedule’ for Infants and Low Weight Children:

Infants and children with a body weight below 12 kg should be dosed according to their weight in kg instead of their body surface area (m²) according to the known formula of 30 kg = 1 m². In infants weighing < or equal to 5 kg, a further 1/3 dose reduction is advised.

**Monitoring and supportive care during induction chemotherapy:**

- Informed consent will follow the same procedure as for older children.
- Data will be collected on the RDE system of the HRNBL-1.5/SIOPEN Study – necessary basic modification available by April 4th, 2006.
- Two special monitoring time-points - ® - during induction chemotherapy have been introduced to monitor for early progressions.
- Special attention should be taken to ensure adequate infection management according to accepted standards of antibiotic policies in neutropenic patients.
- Special emphasis should be given to timely standard antifungal treatment
- Special care should be taken in the management of central venous lines
- Special emphasis should be given to adequate nutritional support.

It is strongly recommended that those patients who may develop more toxicity than the older population be referred for treatment to a highly experienced Pediatric Oncology Unit in their respective country (according to SIOP recommendations with an annual recruitment of at least 50 new patients/year).

<table>
<thead>
<tr>
<th></th>
<th>G-CSF</th>
<th>CBDCa</th>
<th>VP16</th>
<th>VCR</th>
<th>CDDP</th>
<th>CYC</th>
<th>Day</th>
<th>Course</th>
<th>Cycle</th>
<th>Monitor Toxicity</th>
<th>Harvest</th>
<th>Surgery</th>
<th>Response</th>
<th>Staging</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose/day</td>
<td>5µg/kg</td>
<td>25 mg/kg</td>
<td>5.8 mg/kg</td>
<td>0.05 mg/kg</td>
<td>2.6 mg/kg</td>
<td>35 mg/kg</td>
<td>0</td>
<td>A</td>
<td>1</td>
<td>X</td>
<td>X</td>
<td>Sx</td>
<td>®</td>
<td>◆</td>
</tr>
<tr>
<td>Days</td>
<td>3-8</td>
<td>12-18</td>
<td>23-28</td>
<td>32-38</td>
<td>43-48</td>
<td>52-58</td>
<td>63-68</td>
<td>72 till harvest</td>
<td>10</td>
<td>AUD</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harvest</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>X</td>
<td>Sx</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cycle</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>B</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>
3.1.3 MODIFIED N7 INDUCTION

The modified N7 induction is a dose intensive induction chemotherapy regimen including two putatively non cross-resistant drug combinations: high-dose cyclophosphamide plus doxorubicin/vincristine (CAV) and high-dose cisplatin/etoposide (P/E).

1. The original regimen with 7 cycles was modified reducing the number of cycles to 5, with a lower dosage of VCR and using G-CSF [3].

2. Cycles 1, 2, and 4 consist of CAV: Cyclophosphamide (70 mg/kg) is infused intravenously (i.v.) for 6 hours on days 0 and 1 (140 mg/kg/cycle). Beginning on day 0, doxorubicin (75 mg/m² over three days, i.e. 25 mg/m² per day) and vincristine (0.067 mg/kg over three days, i.e. 0.022 mg/kg per day, up to a maximum dose of 2.0 mg per course) are given by 72-hour i.v. infusion. For further information and details of hydration see section 11.2, page 105.

3. Cycles 3 and 5 consist of P/E, with 1-hour i.v. infusions of 50 mg/m² of cisplatin on days 0 to 3 (200 mg/m²/cycle), and 2-hour i.v. infusions of 200 mg/m² of etoposide on days 0 to 2 (600 mg/m²/cycle). For further information and details of hydration see section 11.2, page 105.

4. G-CSF 5 µg/kg is administered for every cycle. Administration is started 72 hours after the end of chemotherapy and given until the absolute neutrophil count (ANC) is > 1.0 International Units.

5. Cycles of chemotherapy are started on count recovery (ANC ≥ 500/uL and platelets ≥ 75,000/uL). The patient should have been off G-CSF for at least 48 hours. It is expected that courses will be delivered approximately every three weeks. If there are concerns regarding delays to chemotherapy, because of protracted haematological recovery contact your National Coordinator to discuss the possibility of a dose reduction.

6. The cardioprotectant dexrazoxane should NOT be administered [4].

<table>
<thead>
<tr>
<th>G-CSF</th>
<th>5µg/kg</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CYC</td>
<td>70mg/kg</td>
<td></td>
</tr>
<tr>
<td>DOXO</td>
<td>25mg/m²</td>
<td></td>
</tr>
<tr>
<td>VCR</td>
<td>0.022 mg/kg</td>
<td></td>
</tr>
<tr>
<td>CDDP</td>
<td>50mg/m²</td>
<td></td>
</tr>
<tr>
<td>Vp16</td>
<td>200mg/m²</td>
<td></td>
</tr>
<tr>
<td>DAY(approx)</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>COURSE</td>
<td>CAV</td>
<td>CAV</td>
</tr>
<tr>
<td>CYCLE</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>HARVEST</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SURGERY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STAGING</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* GSCF is started 72 hours after last chemo and given until ANC is > 1.0 International Units.

◆ Primary site MRI or CT, ultrasound and mIBG (skeletal and local) and Bone Marrow: 2 aspirates/2 biopsies;

◆ Bone Marrow: 2 aspirates, primary site ultrasound

■ Primary site: control of surgical success (method MRI, CT: 2 weeks after surgery; an additional postoperative mIBG Scan prior to MAT is mandatory)
### Modified N7 for Infants and Low Weight Children:

Infants and children with a body weight below 12 kg should be dosed according to their weight in kg instead of their body surface area (m²) according to the known formula of 30 kg = 1 m²; drugs already prescribed using mg/kg (CYC and VCR) will be prescribed at 2/3 of the original dose. In infants weighing ≤ 5 kg, a further 1/3 dose reduction is advised.

<table>
<thead>
<tr>
<th>Dose/day</th>
<th>5µg/kg</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>G-CSF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYC</td>
<td>46.7mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOXO</td>
<td>0.83mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VCR</td>
<td>0.015mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDDP</td>
<td>1.7mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vp16</td>
<td>6.7mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DAY (approx)</strong></td>
<td>0</td>
<td>6</td>
<td>21</td>
<td>27</td>
<td>42</td>
<td>49</td>
<td>63</td>
<td>69</td>
<td>84</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>COURSE</strong></th>
<th><strong>CAV</strong></th>
<th><strong>CAV</strong></th>
<th><strong>P/E</strong></th>
<th><strong>CAV</strong></th>
<th><strong>P/E</strong></th>
</tr>
</thead>
</table>

| **CYCLE** | 1 | 2 | 3 | 4 | 5 |

<table>
<thead>
<tr>
<th><strong>MONITOR TOXICITY</strong></th>
<th>AUD</th>
<th>X</th>
<th>AUD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HARVEST</strong></td>
<td>H</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td><strong>SURGERY</strong></td>
<td>Sx</td>
<td></td>
<td>MAT</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>RESPONSE</strong></th>
<th>®</th>
<th></th>
<th>®</th>
</tr>
</thead>
</table>

| **STAGING** | ◆ |  |  |  |  |

* GSCF is started 72 hours after last chemo and given until ANC is > 1.0 International Units.

◆ Primary site MRI or CT, ultrasound and mIBG (skeletal and local) and Bone Marrow: 2 aspirates/2 biopsies;
◆ Bone Marrow: 2 aspirates, primary site ultrasound
◆ Primary site: control of surgical success (method MRI, CT: 2 weeks after surgery; an additional postoperative mIBG Scan prior to MAT is mandatory)

® Special monitoring point for early progression

**AUD** Audiometry

---

**Monitoring and supportive care during induction chemotherapy:**

- Informed consent will follow the same procedure as for older children.
- Data will be collected on the RDE system of the HRNBL-1.5/SIOPEN Study – necessary basic modification available by April 4th, 2006.
- Two special monitoring time-points - ® - during induction chemotherapy have been introduced to monitor for early progressions.
- Special attention should be taken to ensure adequate infection management according to accepted standards of antibiotic policies in neutropenic patients.
- Special emphasis should be given to timely standard antifungal treatment
- Special care should be taken in the management of central venous lines
- Special emphasis should be given to adequate nutritional support.

*It is strongly recommended that those patients who may develop more toxicity than the older population be referred for treatment to a highly experienced Pediatric Oncology Unit in their respective country (according to SIOP recommendations with an annual recruitment of at least 50 new patients/year).*
3.2 Topotecan, vincristine and doxorubicin for patients with an inadequate metastatic response after induction

Patients who are not in metastatic CR following induction should receive two cycles of Topotecan-Vincristine-Doxorubicin (TVD). This treatment option also includes the infant population with insufficient response or early progression during induction.

3.2.1 CHEMOTHERAPY REGIMEN DETAILS OF TVD

**Topotecan** To be administered i.v. in the morning, as a 30 minute infusion in saline 100 ml/m² at a dose of 1.5 mg/m²/day for 5 consecutive days (days 1-5).

**Vincristine** To be administered as a 48-hour continuous infusion at a dose of 1 mg/m²/day in 50 ml/m²/day 0.9% saline (maximum dose 1mg/day), starting one hour after the final topotecan infusion (days 5-6).

**Doxorubicin.** To be administered simultaneously with the vincristine as a 48-hour continuous infusion at a dose of 22.5 mg/m²/day in 50 ml/m²/day of 0.9% saline solution (days 5-6).

Anti-emetic therapy should be given according to the clinical condition and institutional policies. G-CSF, 5 μg/kg/day either subcutaneously or intravenously, should be started 72 hours after the conclusion of the vincristine and doxorubicin infusion and continued until neutrophil recovery (ANC >1.0 in International Units). A second cycle will be administered at the same dose 21-28 days from the start of the first cycle, provided that certain conditions are met (see section 11.3.1, page 109). The patient should have been off G-CSF for at least 48 hours. A limited disease evaluation after the first cycle of TVD should include ultrasound of the primary tumour, 24hr urinary catecholamine estimation and plasma LDH. Full tumour re-evaluation must be performed after two cycles of TVD to evaluate whether or not the patient has achieved a sufficient metastatic response (negative bone marrow evaluation, ≤ 3 MIBG bone spots) to re-enter the HR-NBL-1.5/SIOPEN Study. Otherwise the patient is off the HR-NBL1.5 study, but may be eligible for a open phase I or II study. If in doubt, check with your National Coordinator for treatment recommendations.

NOTE: Infants and children with a body weight below 12kg should be dosed according to their weight in kg instead of their body surface area (m²) according to the known formula of 30 kg = 1m².

**Topotecan** 0.05 mg/kg/day

**Vincristine** 0.033 mg/kg/day

**Doxorubicin** 0.75 mg/kg/day

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

3.3 PBSC Harvest

Patients achieving a complete response (CR) or partial response (PR) at metastatic sites on mIBG scanning, with no evidence of disease from two bone marrow aspirates on cytomorphological evaluation and no positive BM biopsy, will undergo harvest of peripheral blood stem cells (PBSC). Harvest should be performed after a full re-staging of disease status ONLY following stimulation with G-CSF. Harvest may be scheduled either after the last chemotherapy cycle or out of steady state mobilisation prior or after surgery. Granulocyte colony stimulating factor (G-CSF) is given daily for 5 days in a dose of 10 micrograms/kg/day. Infants should only undergo PBSC harvest in highly experienced centres.
and transfer for this procedure needs to be considered in time. For children weighing less than 10 kg, it is recommended that the cell separator is primed with irradiated, white cell depleted CMV negative, packed red cells resuspended in 5% albumin and diluted with saline to match the patient's haematocrit. Primary bone marrow harvest is an option for infants below or equal 9kg and should be discussed with the apheresis team.

The aim is to obtain a total CD34 harvest of at least \( 3 \times 10^6 \)/kg cells. It is not recommended that in vitro purging of the cells is carried out. The stem cells are to be returned after MAT has been administered.

### 3.4 Surgery

Complete surgical excision of the primary tumour should be attempted after full recovery from induction treatment, TVD or MAT with the major aim of the study to achieve complete resection and to improve local control.

### 3.5 Myeloablative Therapy and Peripheral Blood Stem Cell Rescue

Patients with localised disease may proceed to BuMel MAT following Rapid COJEC induction provided that there is no evidence of progression and the other eligibility criteria are met (see section 5.4). Stage 4 and stage 4s \( MYCN \)-amplified patients may proceed to BuMel MAT after front-line induction (Rapid COJEC or modified N7) provided that a metastatic CR has been achieved and the other eligibility criteria are met.

Patients with metastatic disease not fulfilling the above response criteria after induction should receive two cycles of Topotecan-Vincristine-Doxorubicin (TVD) (see sections 3.2 and 11.3). **If adequate response** (negative bone marrow evaluation, \( \leq 3 \) MIBG bone spots) **is achieved after two cycles of TVD patients may proceed to BuMel MAT regimen, but no later than day 210.** If adequate response after 2 cycles is not achieved patients may be eligible for an open phase I or II study. If in doubt, check with your National Coordinator for treatment recommendations.

The current protocol amendment highly recommends the use of intravenous busulfan. The BuMel MAT regimen consists of intravenous administration of busulfan (Busilvex®) as a two-hour infusion every 6 hours over 4 (or 5) consecutive days through a central venous catheter (dosage according to BW, see page 116) and the short I.V. infusion of melphalan (140 mg/m²). Since the use of oral busulfan is related to a very large therapeutic window, with an important percentage of patients outside the target AUC of 900 – 1400 units (personal communication G. Vassal, April 2011) and a significantly higher VOD rate of up to 30% [5], its use is discouraged. The prophylactic use of defibrotide is not encouraged, because the rate of VOD grade 3 (severe) is documented with 3% within this trial while the complete rate of VOD (Grade 1 and 2 included) is 18%.

For further details of BuMel MAT see Section 11.7

Even patients presenting at diagnosis with a large abdominal or large pulmonary primary should receive BuMel MAT. However, the radiation field and dose should be discussed with the current Radiotherapy Panel (see page 121 for a list of members).

Melphalan (140mg/m²/day) is administered as a 15 minute short infusion at least 24h after the last busulfan dose. Peripheral blood stem cells are reinfused 24 hours after melphalan administration.
3.6 Radiotherapy

Radiation treatment, to the pre-operative extension of the primary tumour, will be given to all patients after MAT and prior to differentiation and immunotherapy. A total volume of 21 Gy will be delivered to the target volume at the primary tumour site in daily fractions of 1.5 Gy starting as close as possible to day 60 after PBSCR and no later than day 90. A longer interval is only acceptable in case of severe transplant related toxicities (i.e. VOD, ARDS, renal impairment, uncontrolled infection).

3.7 Differentiation Therapy

All patients (including those refusing R2 randomisation) will receive 6 cycles of isotretinoin (13-cis-RA). Six cycles of isotretinoin (13-cis-RA) given by mouth should be administered at a dose of 160mg/m²/day divided into two equal doses over 14 days every 4 weeks and should be started after completion of local irradiation on day 90 post PBSCR, and certainly no later than by day 120 post PBSCR.

NOTE: Patients ≤ 12kg should not receive a dose reduction of isotretinoin (13-cis-RA); the rationale for this is outlined in section 4.2.6.4 (page 64).

Treatment Schedule for isotretinoin (13-cis-RA):

<table>
<thead>
<tr>
<th>W1</th>
<th>W2</th>
<th>W3</th>
<th>W4</th>
<th>W5</th>
<th>W6</th>
<th>W7</th>
<th>W8</th>
<th>W9</th>
<th>W10</th>
<th>W11</th>
<th>W12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>rest</td>
<td>rest</td>
<td></td>
<td></td>
<td></td>
<td>rest</td>
<td>rest</td>
<td></td>
<td></td>
<td>rest</td>
</tr>
<tr>
<td>RA</td>
<td>RA</td>
<td>RA</td>
<td>RA</td>
<td>RA</td>
<td>RA</td>
<td>RA</td>
<td>RA</td>
<td>RA</td>
<td>RA</td>
<td>RA</td>
<td>RA</td>
</tr>
</tbody>
</table>

W: weeks related to start of isotretinoin (13-cis-RA) treatment

3.8 Immunotherapy Approach (Second Randomisation - R2 modification activated July 2009)

Patients randomised for R2 will all receive ch14.18/CHO with or without aldesleukin (IL-2) subcutaneously.

The ch14.18/CHO will be given at a dose of 20mg/m²/day over five days every four weeks for five courses. The first course will start in the fourth week after initiation of isotretinoin (13-cis-RA).

Those patients randomised to receive aldesleukin (IL-2) will receive the following administration schedule:

- During weeks 3, 7, 11, 15 and 19 aldesleukin (IL-2) will be given at a dose of 6 MIU/m²/day over five days subcutaneously (Monday-Friday).
During weeks 4, 8, 12, 16 and 20 aldesleukin (IL-2) will be given two hours after the stop of the ch14.18/CHO infusion at a dose of 6 MIU/m²/day over five days subcutaneously (Monday-Friday).

Treatment schedule for isotretinoin (13-cis-RA), ch14.18/CHO with or without aldesleukin (IL-2) for patients elected by randomisation in R2

<table>
<thead>
<tr>
<th>W1</th>
<th>W2</th>
<th>W3</th>
<th>W4</th>
<th>W5</th>
<th>W6</th>
<th>W7</th>
<th>W8</th>
<th>W9</th>
<th>W10</th>
<th>W11</th>
<th>W12</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>RA</td>
<td>IL-2</td>
<td>IL-2</td>
<td>RA</td>
<td>RA</td>
<td>IL-2</td>
<td>IL-2</td>
<td>RA</td>
<td>RA</td>
<td>IL-2</td>
<td>IL-2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>W13</th>
<th>W14</th>
<th>W15</th>
<th>W16</th>
<th>W17</th>
<th>W18</th>
<th>W19</th>
<th>W20</th>
<th>W21</th>
<th>W22</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>RA</td>
<td>IL-2</td>
<td>IL-2</td>
<td>RA</td>
<td>RA</td>
<td>IL-2</td>
<td>IL-2</td>
<td>RA</td>
<td>RA</td>
</tr>
</tbody>
</table>

W: weeks related to start of isotretinoin (13-cis-RA) treatment
GD2: ch14.18/CHO

Dosing of ch14.18/CHO and aldesleukin (IL-2) for infants and children with a body weight below 12kg should be according to their weight in kg instead of their body surface area (m²) according to the known formula of 30 kg = 1 m². In infants weighing ≤ to 5 kg, a further 1/3 dose reduction is advised.

Ch14.18/CHO will be dosed at 0.67 mg/kg/day and aldesleukin (IL-2) will be dosed at 0.2 MIU/kg/day.

As mentioned in section 3.7, patients should not receive a dose reduction for isotretinoin (13-cis-RA).

The guidelines for pain during ch14.18/CHO treatment must be strictly followed (see section 14.6, page 126).
3.9  Treatment and response overview

Figure 1: Treatment Summary Flow Chart HR-NBL1.5/SIOPEN

Table 1: Diagnostic and Response Evaluations

<table>
<thead>
<tr>
<th></th>
<th>Induction Phase</th>
<th>MAT Phase</th>
<th>MRD Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DE</td>
<td>RE 1</td>
<td>RE 2a</td>
</tr>
<tr>
<td><strong>Metastatic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BM trephines</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>BM aspirates</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Peripheral Blood</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Aphaeretic product</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Skeleton mIBG</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>PET (optional)</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td><strong>Local</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumour Biopsy</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Primary Site MRI/CT</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>mIBG (± SPECT)</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>PET (optional)</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>US</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
</tbody>
</table>
4 Background and Rationale

In this protocol the term **high-risk neuroblastoma** refers to children with either:

- disseminated disease (INSS stage 4: about 40 to 50% of all neuroblastomas) over the age of one, or
- INSS stage 2 and 3 disease with amplification of the *MYCN* proto-oncogene (about 3% of all neuroblastomas). Between 10% and 20% of children with stage 3 and occasionally patients with stage 2 disease are characterised by amplification of the *MYCN* gene in their tumours. This biological characteristic has clearly been shown to be associated with a greater risk of relapse and death from disease progression. These patients may benefit from very aggressive treatment and, based on this hypothesis, they are included in this protocol.
- Infants (< 12 months at diagnosis) with *MYCN* amplified tumours are included. Specific treatment guidelines need to be respected for this age group.

**NOTE:** Patients aged 12-18 months at diagnosis, with stage 4 neuroblastoma, no *MYCN* amplification and without segmental chromosomal alterations (SCA) are thought to have a good prognosis (see section 4.10, page 75) and will stop treatment after induction therapy and surgery to the primary tumour.

Since the literature related to high-risk neuroblastoma mainly focuses on disseminated disease most of the background will refer to stage 4.

4.1 Background

Children with this type of presentation and age represent the largest neuroblastoma subgroup. Their prognosis remains poor in most cases and our ability to predict the clinical course and the outcome of the individual patient is modest. With conventional (i.e. non-intensive) chemotherapy long-term survival of these children used to be sporadic (< 5% of the cases)[6-17]. The great improvement in supportive care, which occurred in the early 80’s, led to the widespread use of dose-intensive regimens [18-26]. Treatment intensification usually included an intensified induction regimen followed by the resection of the primary tumour and a final cycle of MAT combined with stem cell transplantation (SCT) [27-33]. As a result of this change a remarkable progress in therapeutic results was achieved in terms of (a) response rate, (b) 3- and 5-years EFS and OS, and (c) number of potential cures. Early results from UK and USA [34-38] were confirmed by several reports from France, Italy, Germany, Japan and other national groups [28, 39-47]. However, the expectation that the refinement of these strategies would progressively improve patients’ outcome was frustrated by a number of trials that failed to increase the 5-year event-free survival above 30% [23, 47-50]. In addition, even 5-year survivors showed only an 80% chance of continued remission [51-53]. A few reports on smaller patient series claiming superior results appeared most likely to be related to limited statistical power due to small numbers and, possibly, patient selection [54].

Few clinical factors seem to have prognostic significance in children treated with modern high-dose protocols. The most consistent one is the disappearance of all detectable metastatic disease.[53, 55, 56]. However, the lack of uniform criteria to document the regression of skeletal and bone marrow disease (the most common sites of metastatic disease) have made it difficult to
compare results.\textsuperscript{[57-61]} The advantage of having a few large co-operative groups investigating major therapeutic questions related to high-risk neuroblastoma appears obvious. Biological studies \textsuperscript{[62-73]} as well as some serologic factors, i.e. LDH, ferritin and NSE \textsuperscript{[74-76]}, which are of great prognostic value for localised disease, were reported to be less indicative for high-risk patients. However, this may be related to the inconsistent evaluation and data sets of these factors in most studies reporting on their prognostic value. In summary, even the currently used highly intensive chemotherapy regimens, although superior to the conventional ones, are ineffective in more than half of the children with stage 4 neuroblastoma older than one year of age at diagnosis. Therefore, new additional approaches are needed.

\textbf{4.2 Rationale for the Various Therapeutic Elements of the Proposed Study}

The protocol proposed in the following sections will have the largest European patient subset of any previous European Neuroblastoma protocol. This current study is expected to make an important contribution towards the understanding of the many issues which remain unresolved in disseminated aggressive neuroblastoma due to its recruitment of a large number of patients in a relatively short period of time.

Based on the above considerations the present protocol has been designed with the following goals:

\begin{itemize}
  \item[a)] To find an induction regimen capable of rapidly and effectively achieving a metastatic response or improving EFS.
  \item[b)] To reduce the incidence of local relapse.
  \item[c)] To promote the eradication of the minimal residual disease.
\end{itemize}

To realise these goals five major therapeutic choices were included in this protocol:

\begin{itemize}
  \item[a)] To investigate in a randomised fashion which induction schedule (Rapid COJEC or modified N7) will be able to induce a better response or EFS.
  \item[b)] To encourage extensive surgical removal of the primary tumour at the end of induction.
  \item[c)] To administer local irradiation to all patients after MAT.
  \item[d)] To administer differentiation therapy (isotretinoin (13-cis-RA)) after MAT and irradiation \textsuperscript{[77-80].}
  \item[e)] To investigate in a randomised fashion the potential of immunotherapy, in addition to isotretinoin (13-cis-RA), to eradicate minimal residual disease by comparing ch14.18/CHO (an anti-neuroblastoma monoclonal antibody \textsuperscript{[81, 82]}) alone and in combination with subcutaneous aldesleukin (IL-2).
\end{itemize}

The rationale of the above choices are summarised in the next sections.

\textbf{4.2.1 FIRST TREATMENT MODALITY: COMPARISON OF TWO INDUCTION REGIMENS RAPID COJEC VS. MODIFIED N7}

Many different induction regimens for high-risk neuroblastoma have been described in the literature. Most regimens utilise cisplatin and/or carboplatin, cyclophosphamide, etoposide and vincristine, with some including doxorubicin. No regimen has been proven to be conclusively superior, as there has been no randomised study. It is difficult to compare event free survival (EFS), progression free survival (PFS) and overall survival (OS) rates between different
published induction regimens as the influence of MAT, local treatment and differentiation therapy have to be considered. In addition, variable definitions and the inconsistent use of mIBG scanning rates following induction therapy hamper the comparison of response data.

4.2.1.1 RAPID COJEC REGIMEN
In Europe no clearly superior induction regimen is evident from the literature (Table 1). The NB 87 regimen of SFOP, comprising high dose cisplatin and etoposide, alternating with cyclophosphamide, doxorubicin and vincristine (CADO) has been most extensively used for over 10 years [20]. Again, the exact effect of this regimen on EFS and OS is unclear as post-induction therapy varied according to the degree of initial response - further chemotherapy with carboplatin and etoposide; single MAT with vincristine, total body radiation, melphalan or two MAT procedures with the second utilising CCNU and etoposide (LMCE1, LMCE3) [43, 83].

Table 2: Comparison of European Neuroblastoma Studies

<table>
<thead>
<tr>
<th>National Groups</th>
<th>Protocol</th>
<th>No. of Cases</th>
<th>Event-free Survival</th>
<th>Overall Survival</th>
<th>Journal</th>
</tr>
</thead>
<tbody>
<tr>
<td>France</td>
<td>LMCE1</td>
<td>72</td>
<td>8%</td>
<td>51% (4yrs)</td>
<td>J. Clin. Oncol. 1991</td>
</tr>
<tr>
<td></td>
<td>F-NB97</td>
<td>47</td>
<td>38% (4yrs)</td>
<td></td>
<td>J. Clin. Oncol. 2005</td>
</tr>
<tr>
<td>Germany</td>
<td>NB85</td>
<td>135</td>
<td></td>
<td>20%</td>
<td>Klin.Pädiatrie 1990</td>
</tr>
<tr>
<td></td>
<td>NB90</td>
<td>206</td>
<td></td>
<td>32%</td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>NB-85</td>
<td>106</td>
<td>18%</td>
<td>27%</td>
<td>J. Clin. Oncol. 2003</td>
</tr>
<tr>
<td></td>
<td>NB-89</td>
<td>76</td>
<td>17%</td>
<td>26%</td>
<td>(B. DeBernardi)</td>
</tr>
<tr>
<td></td>
<td>NB-92</td>
<td>170</td>
<td>16%</td>
<td>28%</td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>N-I-87</td>
<td>60</td>
<td></td>
<td>24%</td>
<td>Eur. J. Cancer 1995</td>
</tr>
<tr>
<td></td>
<td>N-II-92</td>
<td>72</td>
<td></td>
<td>30% (4yrs)</td>
<td>Med and Ped. Oncol 2001</td>
</tr>
<tr>
<td>UK</td>
<td>ENSG5    -OPEC/OJEC</td>
<td>130</td>
<td>17.7%</td>
<td>18.6%</td>
<td>Lancet Oncol 2008</td>
</tr>
<tr>
<td></td>
<td>-COJEC</td>
<td>125</td>
<td>31.3%</td>
<td>39.6%</td>
<td></td>
</tr>
</tbody>
</table>

A retrospective review of the therapy of over 700 patients with stage 4 neuroblastoma throughout Europe between 1990 and 1994 by Dr Valteau-Couanet, on behalf of the SIOP Europe Neuroblastoma group indicated a high (40%) local relapse rate, but no conclusive superiority of any induction regimen used during this period [84]. Between 1990 and 1999 the European Neuroblastoma Study Group (ENSG) conducted a randomised study (ENSG5) to investigate the effect of dose intensity of induction therapy on EFS for stage 4 neuroblastoma over the age of one. The results have been published in Lancet Oncology [85]. Two hundred and sixty-two patients were randomised to receive one of two induction regimens - COJEC (rapid) or OPEC/OJEC (standard). Each regimen utilised the same drugs - cisplatin, carboplatin, etoposide, cyclophosphamide and vincristine - in the same dose, but the dose intensity (in mg/m² per week) of COJEC was 1.8 fold higher (Table 3) [62-66].
Therapy in the COJEC arm was administered every 10 days, regardless of neutrophil and platelet counts and controlled infection, whilst it was delivered every 21 days in the OPEC/OJEC arm if there was haematological recovery (absolute neutrophil count greater than $1 \times 10^9/L$ and platelet count $100 \times 10^9/L$). In those patients who were responding after induction therapy and had achieved a bone marrow complete response (no evidence of detectable disease after cytomorphological evaluation of two bone marrow aspirates and two trephines), attempted surgical excision of the primary tumour was undertaken, followed by MAT with single agent melphalan at a dose of $180 \text{ mg/m}^2$, and haemopoietic stem cell rescue in the form of autologous bone marrow. Median time elapsing between the last course of chemotherapy and MAT was about 3 months. No radiation therapy was administered. Those patients who were within six months of myeloablative therapy in 1999 received six months of isotretinoin (13-cis-RA), as a result of the superior EFS published by the CCG [23]. With a median follow-up of 12.5 years for alive patients, the 5-year EFS was higher for those patients treated with COJEC (30.2%) than with OPEC/OJEC (18.2%), p = 0.022. In particular, of patients in whom overall response could be assessed, CR and VGPR responses were achieved in 58 of 109 (53%) patients assigned standard treatment and in 61 of 82 (74%) patients assigned rapid treatment, p=0.002 (Table 4) [85]. However, 10-year EFS was 18.2% for patients receiving OPEC/OJEC and 27.1% for patients receiving COJEC, p=0.085.

### Table 3: Comparison of the total Doses and Dose Intensity in OPEC/OJEC and COJEC

<table>
<thead>
<tr>
<th>DRUG</th>
<th>TOTAL DOSE</th>
<th>DOSE-INTENSITY</th>
<th>FACTOR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OPEC/OJEC</td>
<td>COJEC</td>
<td>OPEC/OJEC</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>320</td>
<td>320</td>
<td>17.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carboplatin</td>
<td>1500</td>
<td>1500</td>
<td>83.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclo</td>
<td>4200</td>
<td>4200</td>
<td>233</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Etoposide</td>
<td>1400</td>
<td>1400</td>
<td>77.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vincristine</td>
<td>10.5</td>
<td>12.0</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4: Response Rates for OPEC/OJEC (standard) and COJEC (rapid) Treatment (updated as per ENSG5 paper)

<table>
<thead>
<tr>
<th></th>
<th>Standard</th>
<th>Rapid</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>41 (38)</td>
<td>36 (44)</td>
<td>77</td>
</tr>
<tr>
<td>VGPR</td>
<td>17 (16)</td>
<td>25 (31)</td>
<td>42</td>
</tr>
<tr>
<td>PR</td>
<td>22 (20)</td>
<td>8 (10)</td>
<td>30</td>
</tr>
<tr>
<td>MR</td>
<td>7 (6)</td>
<td>7 (6)</td>
<td>14</td>
</tr>
<tr>
<td>NR</td>
<td>12 (11)</td>
<td>4 (4)</td>
<td>16</td>
</tr>
<tr>
<td>PD</td>
<td>10 (9)</td>
<td>2 (2)</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>109 (100)</td>
<td>82 (100)</td>
<td>191</td>
</tr>
<tr>
<td>Not evaluable</td>
<td>5</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Patients not formally assessed</td>
<td>18</td>
<td>44</td>
<td>61</td>
</tr>
<tr>
<td>Total</td>
<td>132</td>
<td>130</td>
<td>262</td>
</tr>
</tbody>
</table>

CR=complete response, VGPR=very good partial response, PR=partial response, MR=mixed response, NR=no response, PD=progressive disease.
The preliminary analysis of the results of Rapid COJEC in the present HR-NBL-1 protocol shows similar results in terms of activity and safety [1].

Table 5: Comparison of the toxicity of OPEC/OJEC (standard) and COJEC (rapid) Treatment (updated as per ENSG5 paper)

<table>
<thead>
<tr>
<th>Courses of chemotherapy</th>
<th>Standard (S)</th>
<th>Rapid (R)</th>
<th>R-S (R-S)</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients died, n (%)</td>
<td>4 (3.2)</td>
<td>5 (4.1)</td>
<td>0.8</td>
<td>0.5-6.3</td>
<td>0.73</td>
</tr>
<tr>
<td>Median time in hospital, days (range)</td>
<td>37 (3-117)</td>
<td>59 (4-178)</td>
<td>22.0</td>
<td>18-26</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Two or more episodes of febrile neutropenia, n (%)</td>
<td>96 (77)</td>
<td>116 (94)</td>
<td>16.9</td>
<td>8.3-25.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>One or more episodes of sepsicaemia, n (%)</td>
<td>35 (28)</td>
<td>55 (45)</td>
<td>16.5</td>
<td>4.5-27.9</td>
<td>0.0063</td>
</tr>
<tr>
<td>Proven fungal infections</td>
<td>1 (1)</td>
<td>2 (2)</td>
<td>0.2</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Patients who received antibiotics, n (%)</td>
<td>110 (89)</td>
<td>118 (96)</td>
<td>7.2</td>
<td>0.5-14.4</td>
<td>0.031</td>
</tr>
<tr>
<td>Mean time on antibiotics, days (range)</td>
<td>19 (3-103)</td>
<td>31 (0-87)</td>
<td>0.8</td>
<td>0.5-6.3</td>
<td>0.73</td>
</tr>
<tr>
<td>Patients who received antifungal treatment, n (%)</td>
<td>19 (15)</td>
<td>51 (42)</td>
<td>27.0</td>
<td>15-37.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Median time on antifungal treatment, days (range)</td>
<td>15 (3-103)</td>
<td>19 (4-69)</td>
<td>0.8</td>
<td>0.5-6.3</td>
<td>0.73</td>
</tr>
<tr>
<td>Grade 3 or 4 gastrointestinal toxic effects, n (%)</td>
<td>31 (25)</td>
<td>43 (35)</td>
<td>10.0</td>
<td>1.5-21.1</td>
<td>0.086</td>
</tr>
<tr>
<td>Renal toxic effects, where GFR&lt;80 ml/min per body surface area of 1.73m², n (%)</td>
<td>21 (17)</td>
<td>46 (37)</td>
<td>20.5</td>
<td>9.4-30.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Grade 3 or 4 neurological toxic effects, n (%)</td>
<td>2 (1.6)</td>
<td>6 (4.9)</td>
<td>3.3</td>
<td>1.6-8.8</td>
<td>0.15</td>
</tr>
<tr>
<td>Obetoxicity (Brick 2-4), n (%)</td>
<td>4/72 (5.6)</td>
<td>4/69 (5.8)</td>
<td>0.2</td>
<td>0.8-4.9</td>
<td>0.95</td>
</tr>
</tbody>
</table>

An analysis has been undertaken, in 2002, comparing the EFS and OS of NB 87 (used as induction chemotherapy in the LMCE 3 regimen), OPEC/OJEC and COJEC (Pearson ADJ, Frappaz D, Phillip T, Imeson J). There was no significant difference in EFS or OS between LMCE 3 and COJEC; however, LMCE 3 had a superior EFS and OS compared to OJEC/OPEC.
It is noteworthy to state that the ENSG5 study used only melphalan as post-induction consolidation either after OPEC/OJEC or COJEC, while a more intense MAT approach was used within LMCE 3. It is further important to note the LMCE 3 (Lyon-Marseille-Curie East of France) strategy: After induction with the French Society of Pediatric Oncology NB87 regimen and surgery, patients having achieved complete remission immediately proceeded to consolidation therapy with vincristine, melphalan, and fractionated total-body irradiation (VMT). All other patients underwent a post-induction strategy before VMT, either an additional megatherapy regimen or further chemotherapy with etoposide/carboplatin. The progression-free survival (PFS) is 29% at 7 years from diagnosis.

Based upon the above data, COJEC will be used as Arm A of the induction therapy for the current study as:

1. There is no significant difference in EFS and OS between different European induction regimens [84].
2. The EFS and OS are very similar following NB 87 (LMCE 3) and COJEC. MAT intensity was greater after LMCE3 induction than after COJEC induction, i.e. melphalan alone in the latter.
3. The response rates with COJEC are similar to those obtained with NB 87/LMCE 3. However, induction therapy is completed within 10 weeks with COJEC, in contrast to 18 weeks with NB 87. No doxorubicin is utilised in the COJEC regimen.
4. In over 95% of patients there is no significant impairment of glomerular filtration rate (GFR) (GFR < 80 ml/min/1.7m²) with the COJEC regimen.
4.2.1.2 MODIFIED N7 REGIMEN

The decision for the randomised induction arm was based on the comparison of recent front-line induction studies (Table 5) and the European experience with N7 [2].

Table 6: Summary and comparison of four front-line neuroblastoma phase III studies indicating cumulative drug dose

<table>
<thead>
<tr>
<th>Cancer Centre</th>
<th>Duration</th>
<th>CR-VGPR</th>
<th>CR-PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSK N7</td>
<td>140 days</td>
<td>83%</td>
<td>93%</td>
</tr>
<tr>
<td>COG</td>
<td>170 days</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>ENSG 5</td>
<td>100 days</td>
<td>75%</td>
<td>85%</td>
</tr>
<tr>
<td>SIOPEN</td>
<td>150 days</td>
<td>56%</td>
<td>75%</td>
</tr>
</tbody>
</table>

In 1994, the Memorial Sloan Kettering Cancer Center (MSKCC) described the anti-tumour and toxic effects of a dose-intensive induction chemotherapy regimen used in 24 children with newly diagnosed high-risk neuroblastoma [86]. Because of favourable effects against metastatic disease and primary tumours [86, 87], and in the absence of clearly better results with other induction regimens in use in the 1990s [88-92], they have continued to use the same induction chemotherapy in successor MSKCC multimodality protocols [3, 93, 94].

Rapid responses were evident such that after 3-5 cycles, 91% of patients had complete remission in the bone marrow and 86% had >50% shrinkage of primary tumours; the overall CR/VGPR after 5 cycles was 83%. Five cycles of this induction regimen, plus surgery, were sufficient to achieve CR/VGPR in ≈ 80% of children with high-risk neuroblastoma reducing acute and long-term toxicities in particular regarding the risk of secondary leukaemia/myelodisplastic syndrome [94].
Established regimens utilised in North America, e.g. CCG 3891 [23], have not been demonstrated to be superior. However the effect of the N7 regimen was confounded by the influence of surgical resection of the primary tumour and the use of immunotherapy with an anti-GD2 antibody.

The results from the N7 regimen as developed by Kushner and colleagues at the MSKCC show superior response rates [3, 93]. Recent results from two European groups (SFOP (Figures 4 and 5) and Austria (Figure 3)) have failed to confirm the high response rates reported for this regimen [2, 95].

Figure 4: RESULTS OF THE NB94 AUSTRIAN PROTOCOL

<table>
<thead>
<tr>
<th>Stage</th>
<th>n</th>
<th>Events</th>
<th>5-years pSU</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>63</td>
<td>1</td>
<td>0.96±0.02</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>0</td>
<td>1.00±0.00</td>
</tr>
<tr>
<td>3</td>
<td>26</td>
<td>0</td>
<td>1.00±0.00</td>
</tr>
<tr>
<td>4</td>
<td>46</td>
<td>22</td>
<td>0.54±0.07</td>
</tr>
<tr>
<td>4s</td>
<td>10</td>
<td>1</td>
<td>0.90±0.09</td>
</tr>
</tbody>
</table>

p<0.0001

Figure 5 and 6: OUTCOME DATA FROM THE NB97 FRENCH PROTOCOL

RESULTS OF NB 97 SFOP PROTOCOL IN CHILDREN OVER 1 YEAR WITH STAGE 4 NEUROBLASTOMA
D. Valteau-Couanet, et al. Not yet published

CR= 45%
R1 criteria= 79%
However, they both report that patients achieving CR show a high and long-term stable EFS. For this reason, we believe that a high quality of control of minimal disease with a “superior quality of CR” with this type of induction is possible. Based on the above data, 5 cycles of the MSKCC N7 protocol (modified N7) will be used as Arm B of induction therapy for the current study as it appears to be the superior in terms of percentage of CR and long term results for patients achieving CR.

4.2.1.3 RESPONSE IN THE RANDOMISED INDUCTION PHASE

A careful evaluation of the response during and at the end of the two induction regimens will be performed to investigate which of the two regimens (COJEC or modified N7) will be able to induce a better response. The aim is to identify a regimen for the induction of the future high-risk neuroblastoma protocol on which to add new molecules to improve response. In addition to CT-MRI of the primary, mIBG scintigraphy, and 2 bone marrow aspirates and biopsies all the patients should be evaluated by ICH-GD2, AIPF and PCR at diagnosis at day 40 of COJEC or before the 4th cycle of modified N7 and at the end of induction (on peripheral blood and on the pool of 2 bone marrow aspirates from opposite sites).

4.2.2 TVD (TOPOTECAN-VINCRISTINE-DOXORUBICIN) RESCUE STRATEGY

Approximately one third of children with high-risk neuroblastoma do not respond to first-line therapy. Topotecan is a specific topoisomerase-I inhibitor that has demonstrated good activity against neuroblastoma in vitro and in vivo, both alone and in combination with other antitumour compounds, such as etoposide, cisplatin and cyclophosphamide. In a Phase II study carried out by the Italian Neuroblastoma Group, topotecan was given for 5 consecutive days prior to vincristine and doxorubicin administered simultaneously in a 48-hour continuous infusion. Of 25 patients so treated, 14 achieved PR after 2 cycles with a 56% major response rate. Toxicity was mainly myelosuppression with no toxic deaths recorded. Preliminary data from the TVD European Study in the HR-NBL-1/SIOPEN protocol for patients that had not achieved the response required for the R1 randomisation after rapid COJEC induction regimen show similar results in terms of activity and safety.
Topotecan is a new drug for both Arm A and Arm B. Doxorubicin is also a new drug for patients earlier treated with the Rapid COJEC induction regimen. Patients treated with modified N7 previously received a total doxorubicin dose of 225 mg/m². These patients could not be considered as resistant to doxorubicin as they obtained a partial response with modified N7. With the addition of two TVD cycles they will reach a total dose of 315 mg/m² with an intensification of this drug that resulted in an increase of response in the Ewing model.

4.2.3 SECOND TREATMENT MODALITY: SURGERY

This study aims to achieve complete primary tumour excision prior to MAT to improve local control. Surgical issues are discussed in detail in section 7.

4.2.4 THIRD TREATMENT MODALITY: BuMel MAT REGIMEN

A very large number of MAT regimens have been employed since this therapeutic modality was introduced in the early 80’s. Their large variety, and consequently the limited number of patients treated with each individual regimen has hindered the ability to reach firm conclusions on their respective antitumour effect and toxicity. Most investigators however, currently agree on the following statements:

- Children should be spared total body irradiation, given its severe toxicity on organ development and risk of secondary cancer [53].
- Peripheral blood progenitor cells are preferable to bone marrow progenitors, particularly because of the rapid recovery of the peripheral blood count they induce which lowers the procedure related morbidity [96]. There is data to support the use of myeloablative therapy [97, 98].
- The most encouraging one is the CCG 3891 protocol randomising MAT followed by autologous purged bone marrow against further intensive chemotherapy without stem cell support [23, 99].

In the HR-NBL-1/SIOPEN study two MAT regimens (BuMel vs. CEM) were compared in a randomised fashion. CEM was considered as the US standard MAT regimen and BuMel the European regimen. The hypothesis was that BUMEL will provide an improvement in the 3-year EFS. The results of an interim analysis showed that BuMel was superior based on 3-year EFS and 3-year OS and the DMC recommended that the randomisation be stopped and all patients receive BuMel. The R1 randomisation was closed on the 11th October 2010. Of 1483 patients, 584 were being randomised for the high dose question at data lock. A significant difference in event free survival (3-year EFS 49% vs. 33%, p<0.001 (Figure 6)) and overall survival (3-year OS 61% vs. 48%, p=0.003) favouring the BuMel regimen was shown. The relapse/progression rate was significantly higher after CEM (0.60±0.03) than after BuMel (0.48±0.03)(p<0.001). Toxicity data had reached 80% completeness at last analysis. The severe toxicity rate up to day 100 (ICU and toxic deaths) was below 10%, but was significantly higher for CEM (p= 0.014). The acute toxic death rate was 3% for BuMel and 5% for CEM (NS). The acute HDT toxicity profile favours the BuMel regimen in spite of a total VOD incidence of 18% (grade 3: 5%).

The Peto rule of P<0.001 at interim analysis on the primary endpoint, EFS was met. Hence randomisation was stopped and BuMel is now recommended as standard treatment in the HR-NBL-1.5/SIOPEN trial.
Figure 7: Event-Free Survival by randomised arm

<table>
<thead>
<tr>
<th>Patients</th>
<th>Events</th>
<th>3-yrs. pEFS</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUMEL</td>
<td>263</td>
<td>119</td>
<td>0.49±0.04</td>
</tr>
<tr>
<td>CEM</td>
<td>262</td>
<td>160</td>
<td>0.33±0.03</td>
</tr>
</tbody>
</table>

Institut Gustave Roussy experience has shown that patients presenting at diagnosis with a large abdominal or large pulmonary mass receiving BuMel MAT still may receive radiation to the primary tumour (personal communication D. Valteau-Couanet). 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.

Experience with BuMel is largely European and derives from studies performed in neuroblastoma and Ewing’s sarcoma.

- **Institut Gustave Roussy Data [5]**

A multivariate analysis on prognostic factors was performed in data sets of 218 metastatic neuroblastoma patients over one year of age [55]. Skeletal disease was present in 79% of cases and bone marrow involvement in 93%. MYCN oncogene amplification was found in 27% of the patients studied. The probability of EFS at 5 years post-diagnosis was 29% in this series. Three major favourable prognostic factors were significant and independent in the multivariate analysis: age under 2 years at diagnosis (P<0.01), absence of bone marrow metastases at diagnosis (p<0.04) and the MAT regimen containing the busulfan /melphalan combination (p = 0.001). The quality of response to conventional primary chemotherapy was close to significance (p = 0.053). Thus factors related to the patient (age) and extent of disease were predictive of outcome in neuroblastoma patients treated with conventional chemotherapy, surgical excision of the primary and consolidation with MAT/SCT. The type of conditioning regimen appeared to be the most important prognostic factor.
• **EBMT Data**

Equally the EBMT registry was able to depict in the year 2000 analysis, a significant advantage for the BUMEL/MAT regimen in high-risk neuroblastoma patients [100]. Patients after the BUMEL/MAT regimen had an overall survival of 51% at 5 years (p=0.033) in comparison with other MAT regimens used Europe-wide.

**Figure 8: EBMT 2000 analysis: 1011 NBL patients with single MAT**

![Graph showing EBMT 2000 analysis: 1011 NBL patients with single MAT](image)

- **Busulfan/Melphalan without CYC-TTP:** n=133, pSU at 5 years=0.51±0.06
- **Busulfan/Melphalan+CYC-TTP:** n=121, pSU at 5 years=0.34±0.05
- **Melphalan alone:** n=186, pSU at 5 years=0.37±0.04
- **Melphalan+:** n=206, pSU at 5 years=0.43±0.04
- **others:** n=40, pSU at 5 years=0.37±0.10
- **TBI:** n=325, pSU at 5 years=0.32±0.03

• **European Experience with I.V. BU**

Clinical results of a new dosing strategy of IV busulfan as part of BuMel preparative regimen before autologous transplantation in children with high-risk solid tumours: reduced regimen-related toxicity and veno-occlusive disease


Oral busulfan (Bu) and melphalan (Mel) has been extensively used as a high-dose chemotherapy (HDC) regimen followed by autologous hematopoietic stem cell transplantation (ASCT) in pediatric patients (pts) with high-risk (HR) solid tumors. With the availability of IV Bu, a new dosing strategy based on body weight (BW) was defined [101]. We assessed prospectively this approach. Pharmacokinetic results were reported in children and adolescents [102] and the final clinical results based on 31 children (17 boys/14 girls), median age 4 y (range 0.7 to 14.9 y) and weight 14.5 kg (range 7.2 to 62.5), are presented here. Pts received IV Bu (Busilvex®) over 2 h at a dose of 1.0 mg/kg, 1.2 mg/kg, 1.1 mg/kg, 0.95 mg/kg or 0.8 mg/kg for <9 kg, 9- to < 16 kg, 16-23 kg, >23-34 kg, and > 34 kg BW, respectively. Mel 140 mg/m² was then administered followed by HSCT. Clonazepam was given as seizures prophylaxis. Indications for HSCT were: HR neuroblastoma (NB), n = 27: 9 CR1/ CR2, 11 VGPR, 7 PR1/PR2, Ewing sarcoma (EW), n = 4: 2 CR1, 2 PR1. Regimen-related toxicity (RRT) was graded according to NCI-CTC 2.0. Kaplan-Meier EFS and OS were calculated. No adverse effect was observed during IV Bu administration. Pts received 5.8 x10^6 CD34+/kg (range 3-34.8) with post transplant G-CSF in
27/31. Neutrophils (> 0.5 x10^9/L) and platelets (> 50.0 x10^9/L) recovery occurred at day 11 (range 10-15) and day 34 (range 11-133), respectively. Digestive toxicity (mucositis) was the main RRT: grade I-II and grade III occurred in 24 and 14 pts, respectively. Four pts (13%) had hepatic veno-occlusive disease (VOD) but none was severe. No early or late regimen-related death occurred. After a median time of 9 months (2.6–40.4), 15/31 pts had disease relapse/progression and 11/31 pts had died. With a median follow-up of 41.2 months (range 3.2-52.2) EFS and OS rates were as follows: 42% +/-22% and 57% +/-20%, for HR-NB, respectively; it was 38% and 67% for HR-EW, respectively. Oral BuMel was the standard HDC regimen in pts with HR-NB but its use was limited by the toxicity, especially VOD (up to 40%). The current results suggest that the IVBu has similar efficacy within the BuMel regimen but with reduced toxicity, especially VOD (13%). Therefore, the IVBu schedule was introduced in the HR-NBL-1/SIOOPEN study.

4.2.5 **FOURTH TREATMENT MODALITY: RADIOTHERAPY**

All patients will receive local irradiation following BuMel MAT/PSCR according to the pre-surgical tumour volume at the primary site even if complete excision was achieved. This includes patients presenting at diagnosis with a large abdominal or large pulmonary mass receiving BuMel MAT. 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. According to the CCG data, consistent local radiation should be well tolerated without the use of TBI and may improve the local control rate at the primary site.

- This radiation has been piloted in a MSKCC protocol and in a limited institution study [97].
- Matthay et al. reported relapse at the primary site in 32 of 68 (47%) patients with disease progression following BMT on the CCG-321P3 study, in which 1000-2000 cGy were given (as 150 cGy BID for seven days) ONLY if disease was present at these sites immediately prior to BMT at primary and metastatic sites [49].
- Also in the retrospective European analysis of stage 4 patients over the age of one treated in Europe between 1990 and 1994, undertaken by Valteau et al on behalf of the SIOP Europe Neuroblastoma Group, a 40% local failure rate was detected (oral communication).
- In the non-TBI regimen utilised by Kushner et al, only one in ten (10%) relapses involved the primary site [36]. These patients were irradiated with 21 Gy on a fractionated schedule at the site of primary disease and regional lymph nodes, as defined at diagnosis.
- Kremens et al, also utilised 21Gy (given as 150 cGy BID for 7 days) to the primary site and BMT in 26 children with neuroblastoma, and observed 4/14 (29%) relapses involving the primary site [45].
- Mugishima et al. transplanted 36 children with advanced neuroblastoma. No relapses occurred at the primary site if intraoperative radiation was given [42].
- A trend for improved five year PFS and for less failure in sites of disease irradiated prior to BMT (doses of 8-24 Gy) was found in a review of 26 patients with advanced neuroblastoma transplanted with a variety of chemotherapy/TBI regimens [103].
- In the pilot study using CEM-LI (91LA6), 56 patients were treated prior to disease progression. Of 16 reviewed relapses only 3 involved the primary site [97]. This is considerably better than the local control rate seen with single daily fractions and lower dose irradiation used in CCG-321P3, where the primary tumour was the site of relapse in more than 50% of the patients [104].
- **In summary, the above data suggests that improved local control may be achieved with local radiation given to the primary tumour site.**

Radiotherapy guidelines are given in detail in section 12.
4.2.6 FIFTH THERAPEUTIC CHOICE: FOR PATIENTS NOT RANDOMISED IN R2 DIFFERENTIATION THERAPY WITH ISOTRETINOIN (13-cis-RA) ALONE

4.2.6.1 Rationale for using isotretinoin (13-cis-RA) in Neuroblastoma
The rationale for using isotretinoin (13-cis-RA) as adjuvant treatment is based on numerous reports that isotretinoin (13-cis-RA) is effective in inducing neuroblastoma differentiation and apoptosis in vitro. This results in a marked decrease in neuroblastoma cell proliferation which has also been shown to be effective in cell lines that are multi-drug resistant to conventional chemotherapeutic drugs [77, 78]. Based on these pre-clinical evaluations, a Phase I study was initiated to evaluate the maximum tolerated dose and toxicity profile of isotretinoin (13-cis-RA) in children with stage 4 neuroblastoma [80].

4.2.6.2 Phase I Study with isotretinoin (13-cis-RA)
This trial revealed minimal toxicity of isotretinoin (13-cis-RA) following BMT. Interestingly, the treatment induced clearing of bone marrow from neuroblastoma cells in 3 of 10 treated patients as determined by histology. Treatment was well tolerated with mild toxicity primarily consisting of cheilitis, dry skin and hypertriglyceridemia [105]. Hypercalcaemia has also been reported, albeit only at higher doses [106].

4.2.6.3 Prospective randomised Multi-Centre Trial with isotretinoin (13-cis-RA)
These promising results led to a prospective randomised multi-centre trial to address the question of whether there was a benefit for patients receiving adjuvant treatment with isotretinoin (13-cis-RA) [23, 79]. In this trial, all patients who completed cytotoxic therapy (either chemotherapy or ABMT consolidation) without disease progression were randomly assigned at week 34 of therapy to receive no further treatment or treatment with isotretinoin (13-cis-RA) for 6 months. Since toxicity tends to increase with repetitive daily dosage, the schedule was 2 weeks of treatment, followed by 2 weeks of rest. This regimen was well tolerated in both the phase I trial [78] and this study. Specifically, patients in the isotretinoin (13-cis-RA) arm received six cycles of isotretinoin (13-cis-RA) (160 mg/m² per day) administered orally in two doses for 14 consecutive days in a 28 day cycle. The result of this trial revealed a clear increase in the survival rate three years after the randomisation among the 130 patients who were assigned to receive isotretinoin (13-cis-RA) in contrast to the 129 patients receiving no further therapy (46 ± 6 % versus 29 ± 5 %, p=0.027). Looking at MYCN-amplified patients only, the isotretinoin (13-cis-RA) group had a survival of 40%, and those not having received isotretinoin (13-cis-RA) had a survival of only 20%. This result also was significant. This advantage for EFS was also demonstrated in subgroups. The highest overall EFS was observed for patients treated with MAT/ABMT and isotretinoin (13-cis-RA) (55 ± 10 %), followed by patients with MAT/ABMT but no isotretinoin (13-cis-RA) (EFS 39%), then patients with chemotherapy consolidation and isotretinoin (13-cis-RA) (EFS 32%), and finally the cohort of patients with chemotherapy and no isotretinoin (13-cis-RA) (EFS18% ± 6 %).

4.2.6.4 Rationale for no isotretinoin (13-cis-RA) dose reduction in children ≤ 12 kg
Recent clinical pharmacology studies carried out by the Newcastle CCLG Pharmacology Studies Group in the UK have investigated the level of inter-patient variability in isotretinoin (13-cis-RA) pharmacokinetics in children with high-risk neuroblastoma. Preliminary data from these studies have indicated that patients ≤12kg, receiving a reduced dose of 5.33 mg/kg, achieve markedly lower isotretinoin (13-cis-RA) plasma concentrations than children receiving a dose of 160 mg/m². Dose increases in patients ≤12kg, to an equivalent dose of 160 mg/m², were well tolerated and resulted in isotretinoin (13-cis-RA) plasma concentrations comparable to those
determined for children >12kg. These preliminary data suggest that reduced dosing should not be implemented for children ≤12kg [107].

Based on these findings, the current protocol includes 160 mg/m² isotretinoin (13-cis-RA) as differentiation therapy following local radiotherapy after MAT/PBSCR.

4.2.7 SIXTH THERAPEUTIC CHOICE: FOR PATIENTS RANDOMISED IN R2 RANDOMISED IMMUNOTHERAPY WITH CH14.18/CHO WITH OR WITHOUT ALDESLEUKIN (IL-2)

Opening of R2 was postponed due to the delay in production of the antibody, including a production failure necessitating a successful recloning in February 2004 to achieve better production yields. Further subcloning, productivity and binding testing confirmed a fourth generation sub-clone with superior productivity and resulted in a new master cell bank. A new large scale production was completed in January 2005. The IMPD is available for National Coordinators submitting to Ethics and Competent Authorities including safety and stability testing.

The phase I study using ch14.18/CHO antibody in children with refractory neuroblastoma (an ESIOP Neuroblastoma Protocol: Final Protocol Version as of 15.03.2005, EudraCT Number: 2005-001267-63, Sponsor's Protocol Code Number: SIOPENNET001) had achieved Ethical Committees approval in three European Countries (Austria, Germany and Italy) and was closed in December 2005. This limited phase I clinical study for equivalence data was activated in June 2005 in three centres to confirm the toxicity profile and pharmacokinetics of the new antibody production. The original protocol as well as a detailed report of these data is available in the IMPD; no SUSARS occurred and the toxicity profile was very much in line with previously published data.

A phase III randomised trial of the chimeric anti-GD2 antibody ch14.18 with GM-CSF and aldesleukin (IL-2) as immunotherapy following dose intensive chemotherapy for high-risk neuroblastoma (ANBL0032) conducted by the Children’s Oncology Group (COG) had randomisation stopped early due to an improvement in event-free survival (EFS) in the immunotherapy patient group. As of the 12th January 2009, 226 eligible patients were randomised: 113 to standard therapy (isotretinoin (13-cis-RA) alone) and 113 to immunotherapy (isotretinoin (13-cis-RA) + ch14.18 + aldesleukin (IL-2) + GM-CSF). Standard therapy was well tolerated while immunotherapy was associated with grade ≥3 pain (21% of 670 cycles); vascular leak syndrome (7.3%); and allergic reactions (7.2%). With median follow-up of 2.1 years after randomisation, EFS was significantly higher for patients randomised to immunotherapy (p=0.0115), with 2-year estimates of 66%±5% vs 46%±5% (83 events observed; 61% of expected information). Preliminary overall survival (OS) was significantly higher for the immunotherapy group (p=0.0223; 86%±4% vs 75%±5% at 2 years). The interim monitoring boundary for large early benefit of immunotherapy was met and randomisation stopped.

4.2.7.1 Background of Immunotherapy with ch14.18 antiGD2 mAb

One concept in the treatment of neuroblastoma is the application of monoclonal antibodies directed against neuroblastoma cells. The chimeric monoclonal antibody (mAb) ch14.18, which recognizes the ganglioside GD2 on neuroblastoma cells, is expressed by virtually all neuroblastoma cells. This antibody could therefore have an important role in the treatment of neuroblastoma. This antibody is called chimeric, because it was genetically engineered to consist of 30% mouse-protein (variable domain of the protein) and of 70% human protein (constant domains of human IgG1). Furthermore, it has been shown that this antibody induces killing of
tumour cells in vitro by both antibody dependent cellular cytotoxicity (ADCC) and complement dependent cellular cytotoxicity (CDC) [108].

The original IgG3 murine anti-GD2 mAb 14.18 was developed by Dr. Ralph Reisfeld [109]. An IgG2a class switch variant of 14.18, called 14.G2a, was prepared in an attempt to mediate enhanced ADCC. This antibody has been tested as a single agent and in combination with IL-2 in clinical trials. When anti-tumour activity was noted, a human/murine chimeric mAb,[110] ch14.18, was formed using the murine variable genes of 14.18 and the human constant IgG1 and \(\kappa\) genes (known to be effective at CDC and ADCC). This was an attempt to improve the clinical utility of the antibody by reducing its immunogenicity and prolonging its half-life. This ch14.18 antibody was tested in phase I clinical trials and the ch14.18 antibody has been combined with IL-2 treatment for patients with melanoma.

4.3 Preliminary Data for ch14.18 anti-GD2 antibody

The following section first reviews past clinical testing with the murine 14.G2a, the chimeric ch14.18 mAb produced in SP2/0 (ch14.17/SP2/0) cells and then summarizes preclinical data in the development of ch14.18 mAb produced in CHO cells (ch14.18/CHO).

4.3.1 Phase I Testing of 14.G2a

In a trial conducted at the M.D. Anderson Cancer Centre, adults with melanoma or neuroblastoma received the murine anti-GD2 mAb 14.G2a by 120-hour continuous intravenous infusion. Dose-limiting toxicity of fever, intractable diarrhea, and nausea and vomiting were seen at 40 mg of 14.G2a/m\(^2\)/d x 5 days. The maximum tolerated dose (MTD) of the antibody on this trial was 30 mg/m\(^2\)/d x 5. At the 40 mg/m\(^2\)/d level, two patients developed slowly reversible hyponatraemia and postural hypotension. In both of these cases, evaluation of the hyponatraemia could not distinguish between salt-losing nephropathy and the syndrome of inappropriate anti-diuretic hormone (SIADH). The hypotension was attributed to autonomic dysfunction in both cases; other objective neurologic abnormalities were not described. In this trial, one partial response was seen in a patient with neuroblastoma, treated below the MTD.

In a separate trial conducted at the University of Alabama, adults with melanoma or children with neuroblastoma received the murine anti-GD2 mAb 14.G2a by a one-hour intravenous infusion on Days 1, 3, 5, and 8. Patients tolerated total doses of 10 mg of 14.G2a without significant side effects. However, toxicity was more severe among ten patients treated with total doses of 60 mg or 120 mg. All ten of these patients experienced abdominal/pelvic pain requiring intravenous morphine infusion during the time of 14.G2a infusion. Five of these 10 patients had delayed neuropathic pain in the extremities. One patient developed confusion, and two developed hyponatraemia consistent with SIADH (one complicated by seizures during hyponatraemia); hyponatraemia in these patients fully resolved within one week of cessation of 14.G2a. Three of the 10 patients receiving 60-120 mg of 14.G2a developed sensorimotor neuropathy. Nerve conduction studies in these patients were consistent with demyelination. Sural nerve biopsy was performed on one patient and demonstrated demyelination. Neuropathy persisted for several weeks after antibody administration, but fully resolved in two patients, with persistent dysesthesia remaining in the third patient. The onset of the neuropathy was typically within 48-72 hours after the Day eight antibody dose.

Laboratory monitoring performed with this trial demonstrated that murine 14.G2a is highly immunogenic in man. Patients developed high human anti-mouse antibody (HAMA) titers at
treatment Day 10, associated with disappearance of 14.G2a from the serum. A trend toward higher HAMA titers in the patients with greater toxicity was observed. It is uncertain why the M.D. Anderson patients tolerated higher doses of 14.G2a than did the Alabama patients. One hypothesis is that the Day eight dose of 14.G2a in the Alabama patients allowed greater binding of 14.G2a to nerve tissue concurrent with the onset of the HAMA response, leading to greater neurotoxicity at lower doses.

Overall for this trial, one mixed and one partial response (at 60 and 120 mg total antibody dose, respectively) were seen in melanoma patients. As some of the toxicity noted in these two trials potentially related to allergic or HAMA reactivity, subsequent testing of the ch14.18 chimeric antibody was initiated.

4.3.2 Phase I Testing of CH14.18/SP2/0

In a phase I trial conducted at the University of Alabama, adults with metastatic melanoma were treated with ch14.18 produced in SP2/0 cells (ch14.18/SP2/0). The first 13 patients received a single treatment with ch14.18, administered as either a single dose of 5-45 mg (administered IV over four hours), or as two daily doses of 50 mg each (100 mg total). Fifty mg was chosen as the maximum daily dose, because abdominal/pelvic pain during antibody infusion precluded administering higher doses. This pain was severe in three of four patients receiving 100 mg of antibody, but was also observed in patients receiving 15 or 45 mg of antibody. No other neurologic side effects occurred, and no other severe toxicity was noted. Eight of 13 patients developed antibodies to ch14.18, but the observed titers were only about one-tenth as great as those detected in the trials of murine 14.G2a. Anti-idiotype and anti-isotype antibodies were detected. Although no anti-tumour responses occurred, antibody was detected on tumour cells by fluorescence-activated cell sorter analysis in some patients treated with ≥ 45 mg of ch14.18. Data on these 13 patients have been published [111].

In a separate phase I trial conducted in Tübingen, Germany, nine patients (ages 2-10) with neuroblastoma received up to 50 mg/m² of ch14.18 daily for five days. All patients had been heavily pre-treated with chemotherapy, including prior high-dose therapy and autologous bone marrow transplantation in some. Six of the nine patients experienced partial tumour responses. Optic nerve atrophy (manifested by decreased visual acuity, and pupillary atony in one patient) was observed in two patients. In both cases, prior radiotherapy had been administered and was implicated in the adverse event. This toxic effect gradually resolved in the six months following therapy with ch14.18. Patients experienced pain during antibody infusion, but no other serious toxicity occurred [81]. More recently, this regimen has been modified to five days of ch14.18 treatment at 20 mg/m²/d, given every six weeks. Thirty-five courses have been given with no neurotoxicity and few side effects. All pain was transient and well controlled with morphine. Anti-tumour responses have been observed.

A third phase I trial with ch14.18 produced in SP2/0 cells was conducted at the University of California San Diego (UCSD) to evaluate the toxicity, immunogenicity, and pharmacokinetics and to obtain preliminary information on its clinical efficacy. Ten patients with refractory neuroblastoma and one patient with osteosarcoma were treated. They received 20 courses of mAb ch14.18 at dose levels of 10, 20, 50, 100, and 200 mg/m². Dose escalation was performed in cohorts of three patients; intrapatient dose escalation was also permitted. Toxicity consisted of pain, tachycardia, hypertension, fever, and urticaria. Most of these toxicities were dose-dependent and rarely noted at dosages of 20 mg/m² and less. Although the maximum-tolerated dose was not reached in this study, clinical responses were observed. These included one partial (PR) and four mixed responses (MRs) and one stable disease (SD) among 10 assessable patients.
Biologic activity of ch14.18 in vivo was shown by binding of ch14.18 to tumour cells and complement-dependent cytotoxicity of post treatment sera against tumour target cells. An anti-ch14.18 immune response was detectable in seven of 10 patients studied. In summary, with the dose schedule used, ch14.18 appears to be clinically safe and effective, and repeated mAb administration was not associated with increased toxicities [82].

Data on the pharmacokinetic were obtained along this trial and reported separately [112]. The plasma clearance of ch14.18 was biphasic. Following the first course of treatment t1/2, alpha was 3.4 +/- 3.1 h and t1/2, beta 66.6 +/- 27.4 h in 9/10 children. The t1/2, beta values were significantly less than those of 181 +/- 73 h previously reported in adult melanoma patients (P < 0.001), and 147.5 h in the adult osteosarcoma patient in our trial. The latter suggests different pharmacokinetics of mAb ch14.18 in children and adults. After a second course of treatment, administered to 5/10 children, t1/2, beta decreased significantly from 72.9 +/- 19.8 h to 31.7 +/- 18.4 h (P = 0.015). Therefore it was concluded that the elimination kinetics of mAbs ch14.18 in children and adults are different, and furthermore that repeated administration of mAb ch14.18 to children with neuroblastoma leads to accelerated antibody clearance.

4.3.3 PHASE II TESTING OF CH14.18/SP2/0

In a recent study, the effect of treatment with chimeric anti-GD2-antibody ch14.18 on the consolidation of children older than 1 year with metastatic neuroblastoma was reported [113]. Patients with metastatic neuroblastoma older than 1 year who completed initial treatment without events were eligible for the application of ch14.18 mAb produced in SP2/0 cells. Ch14.18 was scheduled in a dose of 20 mg/m²/d during 5 days in six cycles every 2 months. Of 334 assessable patients, 166 received ch14.18/SP2/0, 99 received a 12-month low-dose maintenance chemotherapy instead, and 69 had no additional treatment. Interestingly, the three year overall survival in the group treated with ch14.18 (3-year OS, 68.5% +/- 3.9%) was superior to those receiving maintenance chemotherapy (3-year OS, 56.6% +/- 5.0%) or no additional therapy (3-year OS, 46.8% +/- 6.2%; log-rank test, P = 0.18). However, univariate analysis revealed similar event-free survival rates (EFS) for the three groups. From these data, the authors conclude that consolidation treatment with ch14.18/SP2/0 had no clear impact on the outcome of neuroblastoma patients. However, the trial design did not include a randomisation of the ch14.18 antibody, and the number of 166 patients treated with ch14.18/CHO may not be sufficient to demonstrate a survival benefit. Furthermore, no data were reported on immunological monitoring of patients in this trial defining the immune status which critically impacts on the efficacy of passive immunotherapy. Based on these considerations, the conclusion of this study that immunotherapy with ch14.18 has no clear impact on the outcome of patients with metastatic neuroblastoma can be challenged. Therefore, a larger cohort of metastatic neuroblastoma patients will be treated in a European trial (HR-NBL-1/SIOPEN) following a randomisation design for the ch14.18 antibody.

4.3.4 PRECLINICAL TESTING OF CH14.18/CHO

Based on the results summarized above, the European HR-NBL-1/SIOPEN study group decided to test the hypothesis that adjuvant immunotherapy with anti-GD\textsubscript{2} mAb ch14.18 provides a survival benefit for stage 4 neuroblastoma patients. For this purpose, a large quantity of ch14.18 antibody was produced following GMP production standards. The antibody producing cell lines used to generate recombinant ch14.18 so far are Sp2/0 and NS0 cells, which are both non-secreting murine myeloma cells commonly used for antibody production. However, these murine cell lines carry murine xenotropic retrovirus, in contrast to cells of hamster origin [114]. Therefore, it was decided to switch the production cell
line from SP2/0 to Chinese hamster ovary (CHO) cells, in order to avoid extensive viral clearance studies of murine xenotropic retrovirus during the downstream purification process. Recloning of the plasmid driving the expression of ch14.18 antibody was successfully accomplished in CHO cells. Ch14.18 protein (ch14.18/CHO) was purified from stably producing cell lines and a master working cell bank was generated for subsequent large-scale production under GMP conditions (Polymun).

With this new ch14.18/CHO product Holger Lode and colleagues [115] demonstrated identical binding of ch14.18/CHO to the nominal antigen disialoganglioside GD2 in vitro compared to ch14.18/SP2/0 and ch14.18/ NS0. Binding was GD2 specific, since all precursor- and metabolite-gangliosides of GD2 tested were not recognized by ch14.18/CHO. Second, the functional properties of ch14.18/CHO were determined in complement-dependent cytotoxicity (CDC) and antibody dependent cellular cytotoxicity (ADCC) reactions against GD2 positive neuroectodermal tumour cell lines in vitro. There was no difference in CDC mediated specific tumour cell lysis among the three different ch14.18 antibody preparations. Interestingly, ch14.18/CHO showed superior ADCC activity at low antibody concentrations. Third, the efficacy of ch14.18/CHO was evaluated in the NXS2 neuroblastoma model in vivo. Importantly, the ch14.18/CHO preparation was effective in suppression of experimental liver metastasis in this model. In vivo depletion of NK cells completely abrogated this effect, suggesting that the mechanism involved in the ch14.18/CHO induced anti-neuroblastoma effect is mediated by NK-dependent ADCC.

4.4 Rationale of the SIOPEN Phase I Bridging study

The ch14.18 antibody consists of the murine variable regions of murine anti-ganglioside GD2 antibody 14G2a. A human mouse chimeric antibody ch14.18 was generated by genetically engineering. Specifically, the murine variable regions of 14G2a were cloned into the frame work of a human IgG1 molecule and expressed in SP2/0 cells. Ch14.18 antibody recognizes the disialoganglioside GD2 on several human cancer types, including melanoma, neuroblastoma, small cell lung carcinoma, osteosarcoma, and soft-tissue sarcoma. Therefore, the chimeric 14.18 antibody produced in SP2/0 cells (ch14.18/SP2/0) has been tested as an investigational therapeutic in phase I/II clinical trials, alone and combined with other treatments including IL-2, and has shown anti-tumour effects in these studies.

This ch14.18 antibody product used for previous clinical studies so far was generated in non-secreting murine myeloma cells SP2/0. However, the use of SP2/0 as production cells has important safety limitations, since they do contain murine retroviruses. Therefore, we decided to have the antibody recloned into CHO cells by a company called Polymun. CHO cells are virus free and are considered to be an up to date standard for the production of recombinant protein for clinical trials.

Although the antibody-gene transfer into CHO and SP2/0 was done with exactly the same plasmid assuring an identical protein sequence, changes in the glycosylation of the final protein product may occur since the glycosylation pattern varies between different production cell lines. Glycosylation is important for the immunological effector function of the antibody and the pharmacokinetics in patients. Therefore, this change is considered to be a major change in production requiring bridging studies according to the guidelines provided by the “Committee for Proprietary Medicinal Products” (CPMP) of the “European Agency for the Evaluation of Medicinal Products” (EMEA) (Document Number CPMP/BWP/3207/00) in order to reassess the new ch14.18/CHO product in a Phase I clinical trial.
Based on the commitment of SIOPEN to test ch14.18 in a phase III trial (HR-NBL-1 SIOPEN) aiming at the determination of a role for anti-GD2 immunotherapy in neuroblastoma with chimeric anti-GD2 antibody ch14.18, it appears necessary to re-establish the safety for the recloned ch14.18 antibody in CHO cells.

Preclinical results comparing ch14.18 produced from CHO cells with ch14.18 produced from SP2/0 and NS0 cells suggest that the binding and complement dependent cytotoxicity are independent of the production cell lines used. Ch14.18/CHO mediated antibody dependent cellular cytotoxicity (ADCC) was increased at low antibody concentrations over ch14.18/SP2/0 and ch14.18/NS0. Firstly, identical binding of ch14.18/CHO to the nominal antigen disialoganglioside GD2 in vitro was demonstrated compared to ch14.18/SP2/0 and ch14.18/NS0. Secondly, the functional properties of ch14.18/CHO were determined in complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC) reactions against GD2 positive neuroectodermal tumor cell lines in vitro. There was no difference in CDC mediated specific tumor cell lysis among the 3 ch14.18 antibody preparations. Ch14.18/CHO showed superior ADCC activity at low antibody concentrations. Thirdly, the efficacy of ch14.18/CHO was evaluated in the NXS2 neuroblastoma model in vivo. Importantly, the ch14.18/CHO preparation was effective in suppression of experimental liver metastasis in this model. In vivo depletion of NK-cells completely abrogated this effect, suggesting that the mechanism involved in the ch14.18/CHO induced anti-neuroblastoma effect is mediated by NK-dependent ADCC [115].

4.5 Results of the SIOPEN Phase I Bridging study

The plasmid encoding for ch14.18 mAb was recloned into CHO cells (ch14.18/CHO) and was compared to ch14.18 manufactured from SP2/0 (ch14.18/SP2/0) and NS0 cells (ch14.18/NS0). As the glycosylation pattern varies between the different production cell lines and is considered important for the immunological effector function of the antibody in patients, this change was considered to be a major one in production requiring the reassessment of the new product in a Phase I clinical trial:

Phase I BRIDGING STUDY using ch14.18/CHO antibody IN CHILDREN WITH REFRACTORY NEUROBLASTOMA (SIOPEN Neuroblastoma Protocol)

Final Protocol Version as of 15.03.2005
EudraCT Number: 2005-001267-63, Sponsor's Protocol Code Number: SIOPRENET001

Thus, between August to November 2005, 16 high risk neuroblastoma patients (9 in relapse and 7 poor responders) were entered in the clinical bridging study in 3 European centres with a median age of 7.6 years. The safe dose was confirmed at 20 mg/m²/day over 5 days every 4 to 6 weeks. Supportive care followed written standards, in particular expected visceral pain was controlled through intravenous morphine continuous infusion. A total of 41 cycles (10x3 cycles, 5x2 cycles, 1x1 cycle) were given: There were no treatment related deaths and all toxicities were reversible (see table below for frequency of toxicities). This toxicity profile is very much in line with reports on the previous ch14.18 product manufactured from SP2/0 (ch14.18/SP2/0) [113]. Thus good tolerance and potentially increased effectiveness is expected from the ch14.18/CHO production.

Frequency of toxicity symptoms observed during the use of ch14.18/CHO in 16 patients with refractory neuroblastoma is shown in Table 7.
### Table 7: Toxicities observed in the Bridging Study

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Total number of courses</th>
<th>n courses with toxicity</th>
<th>Percent courses with toxicity</th>
<th>Total number of patients</th>
<th>n patients with toxicity</th>
<th>Percent patients with toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>41</td>
<td>31</td>
<td>76%</td>
<td>16</td>
<td>15</td>
<td>94%</td>
</tr>
<tr>
<td>Elevated C-reactive proteins</td>
<td>40</td>
<td>20</td>
<td>50%</td>
<td>16</td>
<td>8</td>
<td>50%</td>
</tr>
<tr>
<td>Pain despite analgesic therapy</td>
<td>41</td>
<td>12</td>
<td>29%</td>
<td>16</td>
<td>10</td>
<td>63%</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>23</td>
<td>5</td>
<td>22%</td>
<td>10</td>
<td>3</td>
<td>30%</td>
</tr>
<tr>
<td>SGOT/SGPT</td>
<td>26</td>
<td>13</td>
<td>50%</td>
<td>14</td>
<td>11</td>
<td>79%</td>
</tr>
<tr>
<td>Nausea or vomiting</td>
<td>41</td>
<td>14</td>
<td>34%</td>
<td>16</td>
<td>7</td>
<td>44%</td>
</tr>
<tr>
<td>Acute allergic reaction*</td>
<td>41</td>
<td>18</td>
<td>44%</td>
<td>16</td>
<td>7</td>
<td>44%</td>
</tr>
<tr>
<td>Symptoms of serum sickness</td>
<td>41</td>
<td>0</td>
<td>0%</td>
<td>16</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Urinary retention</td>
<td>41</td>
<td>14</td>
<td>34%</td>
<td>16</td>
<td>8</td>
<td>50%</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>41</td>
<td>2</td>
<td>5%</td>
<td>16</td>
<td>2</td>
<td>13%</td>
</tr>
<tr>
<td>Ocular symptoms</td>
<td>17</td>
<td>0</td>
<td>0%</td>
<td>8</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Tachycardia</td>
<td>41</td>
<td>21</td>
<td>51%</td>
<td>16</td>
<td>8</td>
<td>50%</td>
</tr>
<tr>
<td>Hypertension</td>
<td>40</td>
<td>0</td>
<td>0%</td>
<td>16</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Renal Toxicity</td>
<td>40</td>
<td>0</td>
<td>0%</td>
<td>16</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>41</td>
<td>31</td>
<td>76%</td>
<td>16</td>
<td>14</td>
<td>88%</td>
</tr>
<tr>
<td>Thrombopenia</td>
<td>41</td>
<td>17</td>
<td>41%</td>
<td>16</td>
<td>10</td>
<td>63%</td>
</tr>
<tr>
<td>Anemia</td>
<td>41</td>
<td>36</td>
<td>88%</td>
<td>16</td>
<td>15</td>
<td>94%</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>41</td>
<td>29</td>
<td>71%</td>
<td>16</td>
<td>13</td>
<td>81%</td>
</tr>
<tr>
<td>Infection</td>
<td>41</td>
<td>7</td>
<td>17%</td>
<td>16</td>
<td>5</td>
<td>31%</td>
</tr>
</tbody>
</table>

* Including cough, rash, pruritus, hypotension, urticaria and pulmonary obstruction/stridor.

During the infusion intensive visceral pain and pain in the extremities was anticipated. Therefore concomitant morphine treatment was a necessity. If a patient still experienced pain, the ch14.18/CHO infusion was stopped for 30 minutes and a non-opioid analgesic is given. The standardised treatment of anaphylactic reactions includes anti-histamines and, if necessary, corticosteroids. The high prevalence of hematologic abnormalities including leukopenia, thrombopenia, anemia, neutropenia and infection has not been reported in other trials. These findings were not associated with the treatment with ch14.18/CHO. They were all related to advanced stage neuroblastoma also including bone marrow metastases.

### 4.6 Immunotherapy with anti-GD2 monoclonal antibody and cytokines (IL-2 and GM-CSF)

Antibody-dependent cellular cytotoxicity (ADCC) plays an important role in immunotherapy and aldesleukin (IL-2) has been shown to augment natural killer cell-mediated ADCC in vitro and in vivo [116-120]. In a phase I trial, CCG-0901, 33 paediatric patients received escalating doses of the murine mAb 14G2a combined with a fixed dose of aldesleukin (IL-2). The MTD was determined to be 15 mg/m²/d x 5d when combined with 3 x 10⁶ IU/m²/d of aldesleukin (IL-2) for 4 days as continuous intravenous infusion each week for 3 weeks. Aldesleukin (IL-2) toxicities were similar to those toxicities seen with aldesleukin (IL-2) alone. Two patients had a measurable clinical response (1 CR and 1 PR). Three patients had a transient decrease in neuroblastoma involvement of the marrow [121]. A separate study has been completed in adults, combining ch14.18 (2-10 mg/m²/d) x 5 days with aldesleukin (IL-2) (1.5 x 10⁶ IU/m²/d for 4 days continuous intravenous infusion each week, for 3 weeks). Twenty-four patients were treated. Allergic reactions and pain were considered dose limiting toxicities. One patient showed a PR and one a CR [122]. Enhancement of natural killer cell activity by aldesleukin (IL-2) [122, 123] have also been observed in some patients treated with the combination of ch14.18 and aldesleukin (IL-2).
To explore the feasibility of giving ch14.18 in the early post-transplant period, a phase I study of ch14.18 + GM-CSF was conducted (CCG-0935) [124]. The MTD of ch14.18 was determined to be 40 mg/m²/day x 4 days. This study was amended (CCG-0935A) to substitute aldesleukin (IL-2) given as intravenous infusion for GM-CSF in alternate cycles. After engraftment, patients were given ch14.18 at 40 mg/m² with GM-CSF (Courses 1 and 3) or 20 mg/m² with aldesleukin (IL-2) (Course 2). However, more toxicities were observed with both GM-CSF and aldesleukin (IL-2) in combination with ch14.18. These included pain, fever, capillary leak, O₂ requirement due to capillary leak, hypotension, mild reversible increased hepatic transaminases, and infection. There was also a transient thrombocytopenia with GM-CSF and aldesleukin (IL-2) cycles. Subsequently, the antibody dose was changed to 25 mg/m² for both GM-CSF and aldesleukin (IL-2) cycles and the aldesleukin (IL-2) dose was reduced in an attempt to decrease capillary leak. Patients who have undergone high-dose chemotherapy with autologous stem cell rescue for neuroblastoma will receive ch14.18 with GM-CSF (courses 1, 3 and 5) or aldesleukin (IL-2) (Courses 2 and 4) after engraftment.

The use of aldesleukin (IL-2) alone causes activation of natural killer (NK) cells, generation of lymphokine-activated killer (LAK) cells, and augments ADCC [117, 125-127]. Aldesleukin (IL-2) has been effective at inducing measurable anti-tumour responses in patients with renal cell carcinoma and melanoma [128, 129]. In a CCG phase II trial, aldesleukin (IL-2) was administered to children with refractory solid tumours [130]. No anti-tumour effects were observed in children with sarcomas or neuroblastomas, whereas one of five children with renal cell carcinoma had a complete response. Two prospective randomized trials in adult patients with metastatic melanoma failed to show any benefit of aldesleukin (IL-2) administered along with interferon following standard chemotherapy [131, 132]. Aldesleukin (IL-2) has been administered post-autologous BMT in a variety of diseases such as AML, lymphoma, breast cancer and neuroblastoma to eradicate the residual malignant cells via immune activation [133-140]. All the reports in patients with neuroblastoma were feasibility and toxicity studies in the post-ABMT setting, and they were not designed to answer anti-tumour efficacy question [138, 140]. Therefore, it is not possible to know the exact anti-tumour efficacy of aldesleukin (IL-2) alone in neuroblastoma patients with minimal residual disease.

The application of aldesleukin (IL-2) by subcutaneous injection was determined in a multi-centre, observer-blind, randomized phase II parallel study comparing three doses of subcutaneous aldesleukin (IL-2) in stage 4 neuroblastoma. In this study, a safe dose of subcutaneous aldesleukin (IL-2) was established with a sustained increase of natural killer cells (NKC) in an outpatient setting for stage 4 neuroblastoma patients. For this purpose, 33 patients with stage 4 neuroblastoma from 6 countries were entered into the study. Dose levels of 3, 6 and 9x10⁶ IU rIL-2/m² were given s.c. in six 5-day cycles every 2 weeks. Repeated increase of NKC was achieved in 89% of courses with >100% over baseline and/or >1000 NKC/mcL in 58%. On the basis of outpatient limiting dose toxicity at dose level 3, dose level 2 was chosen for the confirmation phase. At dose level 2 the median increase of absolute NKC was 1180 cells/mcL of all 83 cycles, corresponding to a median relative NKC increase over baseline of 711%. Fever was frequent, but controllable with adequate supportive care. 6.5% of patients were hospitalised. Localised pain was moderate and acceptable. Event free and overall survival rates at 5 years were 45% (±9) and 48% (±9) respectively. In conclusion, the low toxicity profile and ability to sustain an increase in NKC of IL-2 at 6x10⁶ IU/m² s.c allows its integration in an outpatient setting [141].

4.7 The Children’s Oncology Group Study ANBL0032
Anti-GD2 monoclonal antibody ch14.18/SP2/0 has shown preclinical and early phase clinical activity against neuroblastoma (NB), with enhanced preclinical efficacy when combined with
GM-CSF or aldesleukin (IL-2). ANBL0032 was a Phase 3 study designed to determine if adding ch14.18/SP2/0 + GM-CSF + IL-2 to standard therapy of isotretinoin (13-cis-RA) given in CR or VGPR after intensive induction and consolidation improved outcome for high-risk NB patients.

This trial included newly diagnosed high-risk neuroblastoma patients who had achieved a CR or PR to induction therapy and had received myeloablative consolidation with stem cell rescue. Patients were randomized to isotretinoin (13-cis-RA) x 6 cycles (standard) or isotretinoin (13-cis-RA) x 6 with 5 concomitant cycles of ch14.18/SP2/0 combined with GM-CSF or aldesleukin (IL-2) in alternating cycles (ImmRx). Interim monitoring of event-free survival (EFS) was performed using a 1-sided log rank test for EFS and overall survival (OS).

As of 12 January 2009, 226 eligible patients were randomized: 113 to standard therapy and 113 to ImmRx. Standard therapy was well tolerated while ImmRx was associated with grade ≥3 pain (21% of 670 cycles); vascular leak syndrome (7.3%); and allergic reactions (7.2%). With median follow-up of 2.1 yrs after randomization, EFS was significantly higher for patients randomized to ImmRx (p=0.0115), with 2-yr estimates of 66%±5% vs. 46%±5% (83 events observed; 61% of expected information). Preliminary OS was also significantly higher for ImmRx (p=0.0223; 86%±4% vs. 75%±5% at 2 yrs). The interim monitoring boundary for large early benefit of ImmRx was met and randomization stopped.

This study suggests that this immunotherapy regimen could be advantageous for patients with high-risk neuroblastoma, although it is desirable to find less toxic ways to simulate immune response [142].

4.8 Ch14.18/CHO and aldesleukin (IL-2) in HR-NBL-1.5/SIOOPEN

Based on the above experience, it is clear that patients receiving adjuvant immunotherapy with ch14.18 have a survival benefit. The HR-NBL-1.5/SIOOPEN trial aims to clarify the benefit of adding subcutaneous aldesleukin (IL-2) to ch14.18/CHO. The intravenous use of aldesleukin (IL-2) in the current COG immunotherapy arm is toxic. In addition, GM-CSF is not commercially available on the European market. Therefore, the SIOOPEN group decided to build on the previous SIOOPEN experience with subcutaneous aldesleukin (IL-2), thus mimicking the COG schedule of intravenous aldesleukin (IL-2).

4.9 Background to integrate MYCN amplified (NMA) infants in the current study:

Infants with neuroblastoma with NMA have a dismal prognosis (29% 2 year-survival in infants with NMA (from data collected by the INES participant countries and analysed by M. Gerrard prior to start of current INES trial). The INES 99.4 trial (European Infants Neuroblastoma Study) tried to improve their outcomes by a common therapeutic strategy for all participating countries: induction chemotherapy consisted of four courses of conventional chemotherapy (Carbo-VP/CADO every 3 weeks), followed by delayed surgery if metastatic CR was obtained with induction chemotherapy. Two post-surgical courses prior to megatherapy were administered (1 each). High dose chemotherapy with BUMEL with peripheral stem cell support was carried out once recovery from the post-surgical CADO course had occurred. Leucapheresis should have been performed prior to surgery proven a clear bone marrow. Local radiotherapy and retinoic acid were administered later.

The principal endpoint of the study was to achieve a 2-year-survival rate around 50%, with two stopping rules: the first one based on toxicity (5%) and the second one based on the 12-month event rate.
The study began in 1999 and 46 patients were registered (median age 8 months). 83% had metastatic disease. Median follow-up is 12 months (range: 0.01-53). Overall survival and event free survival for 42 patients with adequate follow-up is 43% (SD: 0.12), with median survival time of 37 months and 21 deaths due to disease. Treatment was well tolerated with no toxic deaths or severe toxicity. Five patients had tumors with heterogenous NMA and all of them are alive. They have been excluded from the survival analysis, as heterogenous NMA is a recent biological phenomenon under investigation. Thirty eight patients showed homogeneous NMA (centrally reviewed) and only 28% are alive (OS) with a median survival time of 15 months. 95% had metastases at diagnosis. In spite of protocol adherence 10 out of 36 patients evaluable for response to induction chemotherapy progressed or did not respond at the end of it. 42% of the patients did not reach megatherapy as they either progressed/did not respond initially (n=10), relapsed locally before surgery (n=1) or had a relapse just before megatherapy (n=4). Finally, only 19 patients received megatherapy and 63% of them are alive. Univariate analysis shows that stage and type of metastases are important for survival: Stage 4s patients had a better outcome than stage 4 (P= 0.027). A better outcome was also documented for patients with liver metastases (P=0.036). Patients with pleural/lung metastases had a significantly worse outcome (P=0.000). Recruitment on trial was as expected. Although treatment was well tolerated, survival is dismal in this very small and unfavourable group of children (28%) with this approach. Even more, induction chemotherapy did not produce a rapid and satisfactory response to allow for the possibility of megatherapy to be given and despite adherence to protocol in most of the cases, 28% of failures occurred during the initial part of treatment and there have been 6 relapses among patients that completed treatment (17% of patients).

In order to improve the survival of this group there is a need to decrease the rate of progressive disease and non-responders (30% in 99.4) in the initial part of the treatment, by increasing the efficacy of induction chemotherapy. Rapid COJEC was the only European induction chemotherapy regimen that proved better results in a randomized study when compared to equivalent drugs and overall dose but given in a conventional every 3 to 4 week dosing scheme.

For the above reasons the study committees of the infants and high risk trials have agreed to integrate the high risk infant population into the HR-NBL-1.5/SIOPEN Study.

Major concerns about its use in this very young population are related toxicities as this approach has not been previously used in infants. Nevertheless, some hints on toxicity have been obtained: Data from the ENSG5 provided by Dr.A.Pearson and Dr. M.Gerrard in the population aged 12 to 18 months and treated with Rapid-COJEC (n=15) shows 13% of fever episodes, 13% of septicemia episodes (without G-CSF support), 20% of gastrointestinal toxicity as the most relevant ones. There were two toxic deaths (1 Acute Tumour Lysis Syndrome (ATLS) and 1 interstitial lung infection resulting in respiratory failure after megatherapy. Although there was more reported toxicity in young children treated with Rapid-COJEC compared to those infants treated with the standard regimen, this was not very different from that seen in older children treated with Rapid-COJEC. The preliminary toxicity data kindly provided by Dr. Ladenstein in the population aged 1-2 years actually included in the HR-NBL1/SIOPEN shows a high hematological toxicity (as expected, see figure 1), but 10.6 % of CTC grade 3-4 infective episodes, 5% CTC grade 3-4 gastrointestinal toxicity, 2% Brock grade 3-4 ototoxicity. Twenty seven out of the 35 SAE reported have been related to Rapid-COJEC, but only 3 of these have resulted in death (1 Multiorgan Failure and PD, 1 ATLS, 1 candidiasis). Seventeen out of these 27 rapid-COJEC related SAE have been infections (4 central venous line, 3 aspergillosis, 1 klebsiella, 1 candida, 1 pseudomonas, 1 pseudomembranous colitis and 1 neutropenic enterocolitis, 5 fever and pancytopenia).
In view of the dismal prognosis both study committees agreed that these high risk infants could benefit from this regimen if accompanied by adequate supportive care. Special awareness and monitorization during the Rapid COJEC induction phase is warranted. Experience with BuMel MAT has increased over the last years in the 99.4 and it has proved to be manageable in this population.

**Figure 9: Grade 3-4 toxicity in Rapid COJEC.**
Expressed in % (Y axis). A total of 394 courses are shown (107 A, 194 B and 93 C) in X axis. Other toxicities are also below 5% and are not shown. Brock grade 3/4 ototoxicity is currently underestimated as it is not adequately monitored in all countries in young children and will only be obtained definitively at follow-up.

4.10 Rationale for treatment reduction in children 12-18 months at diagnosis with favourable biology

Children diagnosed with INSS stage 4 neuroblastoma between 12 and 18 months of age form a particular group where a small cohort, defined by favourable biology, are thought to have a good prognosis and may not benefit from MAT and PBSCR [143-145]. In this amended version this group will be randomised in R3. However, children in this group without MYCN amplification and without segmental chromosomal alterations (SCA) will stop treatment after induction therapy and surgery to the primary tumour. All other patients in this group will continue in the trial.
5 Eligibility and Patient Entry Criteria

5.1 Eligibility Criteria for the Study

- Established diagnosis of neuroblastoma according to the International Neuroblastoma Staging System (INSS). See Section 18.3.
- Age below 21 years.
- High-risk neuroblastoma, defined as either:
  a) INSS stages 2, 3, 4 or 4s with MYCN amplification, or
  b) INSS stage 4 without MYCN amplification aged ≥ 12 months at diagnosis
- Patients who have received no previous chemotherapy except for 1 cycle of etoposide and carboplatin (VP/Carbo). In this situation patients will receive Rapid COJEC induction and the first COJEC cycle may be replaced by the first cycle of VP/Carbo.
- Written informed consent, including agreement of parents or legal guardian for minors, to enter a randomised study if the criteria for randomisation are met.
- Tumour cell material available for determination of biological prognostic factors.
- Females of childbearing potential must have a negative pregnancy test. Patients of childbearing potential must agree to use an effective birth control method. Female patients who are lactating must agree to stop breast-feeding.
- Registration of all eligibility criteria with the data centre within 6 weeks from diagnosis.
- Provisional follow up of 5 years.
- National and local ethical committee approval.

Any negative answer will render the patient ineligible.

The date of diagnosis is defined as the date when the pathological diagnosis is obtained. It is essential that this is prior to any treatment other than surgery.
The date of eligibility is defined as the date at which all criteria for entry into the study have been checked by the co-ordinating centre along with the referring physician. The date of diagnosis will be the starting point for subsequent follow up.

5.2 R0 Randomisation closed in November 2005

5.3 Eligibility Criteria for the R3 Randomisation

- Diagnosis of neuroblastoma confirmed.
- Stage 4 or Stage 4s with MYCN amplification.
- No prior chemotherapy.
- Written informed consent to participate in R3 randomisation, and for minors an agreement by parents or legal guardian.
- Females of childbearing potential must have a negative pregnancy test. Patients of childbearing potential must agree to use an effective birth control method. Female patients who are lactating must agree to stop breast-feeding.

NOTE: Patients with MYCN-amplified localised disease (INSS stages 2 and 3) of any age should be enrolled in the trial, but are not eligible for the R3 randomisation and will receive Rapid COJEC induction.
5.4 Eligibility Criteria for BuMel MAT (R1 Randomisation closed October 2010)

- Localised patients, without evidence of disease progression, must start BuMel no later than day 150 after the start of Rapid COJEC induction.
- Patients in metastatic CR following front-line induction (Rapid COJEC or modified N7) must start BuMel MAT no later than day 150 after the start of induction.
- Patients receiving two courses of TVD to achieve an adequate metastatic response (metastatic CR or PR) must start BuMel MAT no later than day 210 after the start of induction.

CR or PR at metastatic sites:
- at least 50% reduction in skeletal mIBG positivity and not more than 3 positive, but improved spots on mIBG
- cytomorphological CR in 2 BM aspirates and no positive BM biopsy

- Complete restaging of disease as close as possible before the start of BuMel MAT must take place following front-line induction (Rapid COJEC or modified N7) or two courses of TVD.

- Organ functions (liver, kidney, heart, lungs) fulfilling criteria prior to MAT:
  - ALT, bilirubin ≤ 2 x normal (see SmPC)
  - Creatinine clearance and/or GFR ≥ 60 ml/min/1.73m² and serum creatinine < 1.5mg/dl. Call study co-ordinator for MAT dose modifications if GFR < 60ml/min/1.73m² and serum creatinine ≥ 1.5mg/dl.
  - Shortening fraction ≥ 28%, or ejection fraction ≥ 55%, no clinical congestive heart failure.
  - Normal chest X-ray and oxygen saturation.

- Sufficient stem cells available. Minimum required: 3 x10⁶ CD34 cells /kg body weight, if a BM harvest was unavoidable at least 3 x 10⁸ MNC /kg body weight.
- Females of childbearing potential must have a negative pregnancy test. Patients of childbearing potential must agree to use an effective birth control method. Female patients who are lactating must agree to stop breast-feeding.

Any negative answer will render the patient ineligible for BuMel MAT on-study.

Note:
- The result of surgery will not influence eligibility for BuMel MAT.
- With a GFR rate ≥ CTC- Grade 2 the patient is ineligible for BuMel MAT on-study. Since recovery of GFR rate is observed in particular post surgery, at least two GFR rates need to be evaluated post surgery if the first postsurgical one shows a GFR grade 2 to 4 prior to day 150 (with at least a two weeks interval but ideally 4 weeks if feasible) or prior to day 210 respectively.

Thoracic primaries
The point as to whether children with thoracic primaries may be eligible for BuMel MAT has been discussed in various SIOPEN Study Committee meetings: Following these debates it was confirmed that these patients would be eligible for BuMel MAT. However, caution is recommended when considering radiation therapy in patients presenting at diagnosis with a large abdominal or large pulmonary primary. Patients fitting this criteria should be discussed with the current Radiotherapy Panel (see page 121 for a list of members).

- Patients aged 12-18 months at diagnosis, with stage 4 neuroblastoma, no MYCN amplification and without segmental chromosomal alterations (SCA) are not eligible
5.5 Eligibility Criteria for the R2 Randomisation (2nd Revision activated)

- All patients must be enrolled on the study and have completed therapy including intensive induction (Rapid COJEC or modified N7) with or without two additional cycles of TVD.
- BuMel MAT (minor modifications for toxicity concerns allowed) are permitted to render a patient eligible for R2 randomization, even after more than one line of induction treatment. However, patients must be no more than 9 months from the date of starting the first induction chemotherapy after diagnosis to the date of PBSCR.
- Complete re-staging, which shows no evidence of progression, following recovery from major transplant related toxicities.
- Stable WBC above $2 \times 10^9/L$ (or stable neutrophil count greater than $0.5 \times 10^9/L$) in 2 counts taken 48h apart after cessation of G-CSF.
- Written informed consent to participate in R2 randomisation, and for minors an agreement by parents or legal guardian.
- Females of childbearing potential must have a negative pregnancy test. Patients of childbearing potential must agree to use an effective birth control method. Female patients who are lactating must agree to stop breast-feeding.
- Radiotherapy must be scheduled to stop at least 7 days prior to the start of immunotherapy.
- Immunotherapy (starting with week 1 isotretinoin) must start no later than day 120 post PBSCR.

Any negative answer will render the patient ineligible.

Note.

- The patient may be randomised (via the SIOPEN-R-NET) once all eligibility criteria have been met from day 60 post PBSCR.
- Randomisation should take place at least two weeks prior to the start of immunotherapy, so that the antibody can be ordered and delivered in time.
- Patients aged 12-18 months at diagnosis, with stage 4 neuroblastoma, no MYCN amplification and without segmental chromosomal alterations (SCA) are not eligible for R2. These patients are thought to have a good prognosis and will stop treatment after induction therapy and surgery to the primary tumour.

6 Assessment of Extent of Disease, Response and Toxicity

Disease assessment will be according to the Revised International Neuroblastoma Criteria for Diagnosis, Staging and Response to Treatment published in 1993 [146] (see chapter 18).

6.1 Pre-treatment Investigations

It is essential that disease evaluation is carried out prior to any therapy. If treatment is required as an emergency, pre-treatment investigations must be completed within seven days of the start of therapy.
6.1.1 **FULL HISTORY**
- With attention to presence and duration of symptoms, such as pallor, sweating, weight loss, diarrhoea, irritability

6.1.2 **CLINICAL EXAMINATION**
- Measurements of weight, height and blood pressure
- Note signs of spinal cord compression

6.1.3 **HEMATOLOGY**
- Full blood count including haemoglobin, white blood cell, neutrophil, lymphocyte and platelet counts.
- Coagulation profile

6.1.4 **SERUM**
- Renal and liver function (Na, K, Ca, Mg, PO₄, urea, creatinine, glucose, total protein, bilirubin, transaminases)
- Serum lactate dehydrogenase (LDH)
- Serum ferritin, serum NSE
- Pre-transfusion serum tests

6.1.5 **URINE ANALYSIS**
- Catecholamine metabolites: Determination of vanillomandelic acid (VMA), homovanillic acid (HVA) and dopamine, expressed in relation to creatinine excretion
- Strip test for albumin, glucose, ketones, blood, pH prior to platinum derivatives to exclude underlying renal disease

6.1.6 **IMAGING**
- Isotope scintigraphy preferably I¹²³-mIBG: mIBG scan assessing the uptake on the primary tumour, the number and the location of bone metastases and any other metastatic sites.
- If negative mIBG, bone scintigraphy with ⁹⁹ᵐTc-hydroxy-methylene-diphosphonate scintigraphy (⁹⁹ᵐTc scan)
- AP chest x-ray
- CT or MRI scan of primary tumour (with 3D measurements) including search for dumbbell extension in relevant regions
- Radiological visualisation of any other evaluable disease

6.1.7 **BONE MARROW, APHAERETIC PRODUCT AND PERIPHERAL BLOOD SAMPLING AND EVALUATION**

Detailed information on handling of material is given in the Appendix on Guidelines for Bone Marrow Evaluation (section 19.4, page 169), in sections 14.11 (page 133) and 14.12 (page 137) and Appendix 24.1 (page 231).

Diagnostic Evaluation (DE) and Response Evaluation (RE) time points correspond to the timpanist in Table 1: Diagnostic and Response Evaluations (page 50).
Evaluation of the bone marrow (BM) is mandatory. Bone marrow aspirates and trephines should be obtained from right and left posterior iliac crests, i.e. a total of four samples, two aspirates and two trephines according to INSS guidelines.

- **BM will be centrally reviewed**
  - Cytospins at diagnosis and at RE time points to establish Europe-wide standardised immunocytology including controls based on tumour cell specific biological markers and development of standardised BM response criteria.
  - BM trephines (Europe-wide retrospectively)

Genetic verification of the infiltrated tumour cells will be done by the AIPF (automatic immunofluorescence plus FISH) technique at the CCRI, Vienna (Peter Ambros).

An overview of tumour, bone marrow, aphaeresis and peripheral blood sampling procedures is given in the following Figure.
Figure 10: Tumour, BM, aphaeresis and PB sampling procedures

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Local Tasks</th>
<th>Send to</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour Biopsy</td>
<td>Pathologist does the histological examination and delivers this information to the clinician and freezes sample</td>
<td>Send for MYCN FISH, pan-multigenomic analyses, expression profile, tissue bank to Biology Reference Centre¹</td>
</tr>
<tr>
<td>Tumour Surgery</td>
<td>Pathologist does the histological examination and delivers this information to the clinician and freezes sample</td>
<td>Send for MYCN FISH, pan-multigenomic analyses, expression profile, tissue bank to Biology Reference Centre¹</td>
</tr>
<tr>
<td>BM trephine</td>
<td>Pathologist does the histological examination and delivers this information to the clinician</td>
<td>Local Clinician/Data manager enters information in the SIOPEN-R-NET, Send to Oslo, Norway² or Genova, Italy³ for central review and entry of review results in the SIOPEN-R-NET</td>
</tr>
<tr>
<td>BM aspiration</td>
<td>Priority BM smears, e.g. Pappenheim staining delivers this information to the clinician 2 unstained BM smears per side BM cytospins, 6x antiGD2 staining (3x10⁶ MNC per side) or sends at room temp. for preparation at national reference laboratory 6x unstained BM cytospins (3x10⁶ MNC in total) &gt; AIPF the transfer in 2 PAXgene tubes (l.+r.), storage at -80°C</td>
<td>Local Clinician/Data manager enters information in the SIOPEN-R-NET, Send for AIPF (genet. verif.) to CCRI, Vienna⁴ National reference laboratory enters information in SIOPEN-R-NET and central review for patients with &lt;10 positive cells per million will be organised by Klaus Beiske Send for AIPF (genet. verif.) to CCRI, Vienna⁴ Send for QRT-PCR to national reference laboratory⁵ or to Leeds, UK⁶</td>
</tr>
<tr>
<td>Peripheral Blood</td>
<td>the transfer in 1 PAXgene tube, storage at -80°C</td>
<td>Send for QRT-PCR to national reference laboratory⁵ or to Leeds, UK⁶</td>
</tr>
<tr>
<td>Aphaeretic Product</td>
<td>Erythrocyte lysis, cytospins, at least 4x antiGD2 staining (3x10⁶ MNC) or sends at room temp. for preparation at national reference laboratory At least 4x unstained cytospins (minimum 3x10⁶ MNC) &gt; AIPF</td>
<td>Send for QRT-PCR to national reference laboratory⁵ or to Leeds, UK⁶ National reference laboratory enters information in the SIOPEN-R-NET and central review will be organised by Klaus Beiske Send for AIPF (genet. verif.) to CCRI, Vienna⁴</td>
</tr>
</tbody>
</table>

For more details refer to section 6.1.7 and for the sampling time points refer to the Investigation Tables (6.3, 6.4, 6.5 and 6.6)

Legend:
* All samples must be labelled with the patient's study number, date of birth, date sample taken, and the study time point (e.g. DE, RE1 etc)
**TTF is the “time to freeze” and is the duration in minutes from the time the sample was taken until the time it was frozen.
1 For contact information see section 31.16, page 316.
2⁳ Send to Oslo, Norway or Genova, Italy (contact details in section 6.1.7)
4 Send at room temperature to the CCRI in plastic boxes to prevent breakage (Peter Ambros, more information and contact details in sections 6.1.7 and 32.4.
5 Tubes provided by the reference laboratory in Leeds (Sue Burchill or Virginie Viprey, more information and contact details in section 6.1.7 and 32.5) unless specific national reference laboratory has been agreed within the Molecular Monitoring Group
6 Ship batches of samples on dry ice in an insulated container to Gareth Veal, University of Newcastle (Appendix 24).
7 Ship batches of samples on dry ice in an insulated container to Angelo Paci, Institut Gustave Roussy (Appendix 24).
8 Ship batches of samples on dry ice to Holger Lode (for details see 14.12 and 32.6).
Each National Study Coordinator is requested to indicate clearly in the Appendix 31.16, page 293 to national investigators the respective national contact details of reference sites to interact with the respective SIOPEN speciality committees.

- **TUMOUR BIOPSY AND TUMOUR SURGERY**

  The local pathologist performs the histological examination and delivers the diagnosis to the clinician. This information is entered into the SIOPEN-R-NET database by the clinician or the data manager.

  The frozen samples have to be sent to the **biology reference centre**, where *MYCN* FISH, segmental chromosomal alteration (SCA), expression profile assays etc. are performed and a tissue bank is established.

- **2 BONE MARROW TREPHINES (2 sides-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!

  10 unstained sections from each core biopsy and a copy of the local pathology report must be sent for central review to either: (a) Dr. Klaus Beiske, Dept. of Pathology, Oslo University Hospital Rikshospitalet, Sognsvannsveien 20, N-0027 Oslo, Norway or (b) Dr. Angela Sementa, Dept. of Pathology, Giannina Gaslini Children's Hospital, Largo Gaslini 5 1 - 16148 Genova, Italy.

- **BONE MARROW ASPIRATES (2 sides – right and left posterior iliac crests)**

  There is a general international consensus on the necessity to establish an international standardisation of both immunocytology (IC) and QRT-PCR in order to make results from different centres **comparable** and to agree upon **cut-off values** for the clinical management of MRD [147].

  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:

  - **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).

    - For comparison with the results obtained on cytopsins it is necessary to send 2 unstained smears per side within 3 days to the CCRI for genetic verification of the GD2 positive cells.

  **Aspiration for AIPF, immunocytology and QRT-PCR:** Draw 5-10 ml bilateral aspirate into Heparin (5000IE/ml). Of these:

    - Isolate mono nuclear cells (MNCs) by Ficoll gradient (see Appendix 19). Prepare at least 12 BM cytopsins (e.g. Hettrich centrifuge) with a minimum of $3 \times 10^6$ MNCs per side; this is done locally if possible or send at room temperature to national reference laboratory for preparation.
- Send six unfixed and unstained cytospins (in total at least 3x10^6 MNCs) directly to the CCRI (Peter Ambros email: peter.ambros@ccri.at) for genetic verification of the infiltrated tumour cells. Review of these results will be organised by Peter Ambros.
- Six antiGD2 stained cytospins (at least 3x10^6 MNCs per side) are sent to (or kept at) the national reference laboratory for immunocytological investigations. If <10 positive cells per million these slides will be centrally reviewed [147]; this review will be organised by Klaus Beiske, Oslo.

- Transfer immediately, 0.5ml BM from each side (unpooled) into two single PAXgene™ tubes for QRT-PCR studies. Send the filled PAXgene™ tubes to the national reference laboratory or to Prof. Sue Burchill, Leeds*.

* 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.

- **PERIPHERAL BLOOD**

  The **PATIENT’S PERIPHERAL BLOOD is needed for molecular-biologic studies as reference for QRT-PCR and pharmacokinetic and immunological studies.** Peripheral blood should be sent to the reference biology laboratory together with the tumour specimens or to the Molecular Monitoring Group Reference Laboratory.

  - Sample for QRT-PCR: peripheral blood; draw 2 ml of whole peripheral blood and transfer immediately into PAXgene™ tube. Send the filled PAXgene™ tube to the national reference laboratory or to Prof. Sue Burchill, Leeds.

During BuMel MAT sampling is require for:

- Pharmacokinetic studies of Busilvex and melphalan in heparinised tubes. For details of samples and time points see sections 24.1.4 and 24.1.5 (page 232), as well as the sampling sheets in Appendix 24.

During Immunotherapy sampling is require for:

- Pharmacokinetics and immunological monitoring of ch14.18/CHO. For details of samples and time points see sections 14.11 (page 133) and 14.12.1 (page 137).

- Immune effector cell activation. For details of samples and time points see section 14.12.2 (page 139).

- Pharmacokinetics and pharmacogenetics of isotretinoin (13-cis-RA). For details of samples and time points see section 24.2 (page 233).
• Aphaeretic Product

Samples for AIPF, immunocytoLOGY and QRT-PCR: Draw 2ml of aphaeretic product (PBSCH). Of these:

- Transfer immediately, 0.5 ml PBSCH into PAXgene™ tube. Send the filled PAXgene™ tube to the national reference laboratory or to Prof. Sue Burchill, Leeds.
- Lyse erythrocytes (when necessary) and prepare 8-15 cytospins (e.g. Hettrich centrifuge); this is done locally if possible or send at room temperature to national reference laboratory.
  - Send at least 4 unfixed and unstained cytospins (minimum 3x10^6 MNCs) directly to the CCRI (Peter Ambros email: peter.ambros@ccri.at) for genetic verification of the infiltrated tumour cells. Review of these results will be organised by Peter Ambros.
  - At least 4 antiGD2 stained cytospins (3x10^6 MNCs) are sent to (or kept at) the national reference laboratory for immunocytological investigations. Central review of the PBSCH immunological investigations will be organised by Klaus Beiske, Oslo.

Reference laboratory in Vienna supply transport boxes and performs genetic verification of the infiltrated tumour cells (Assoc. Prof. Peter F. Ambros, CCRI, Zimmermannplatz 10, 1090 Vienna, Austria, email: peter.ambros@ccri.at)

Reference laboratory in Leeds supply PAXgene™ tubes and LAM tubes (Prof. Sue Burchill/ Dr V Viprey/ Ms. D Roebuck; Cancer Research UK Clinical Centre, St James University Hospital, Leeds, LS9 7TF. Tel 00 44 113 2064917. s.a.burchill@leeds.ac.uk; v.f.viprey@leeds.ac.uk; d.roebuck@leeds.ac.uk)

For samples collected into PAXgene™ tube, mix contents by gently inverting tube and either i) transferred to -80°C locally or ii) mailed to arrive within 24h to country reference centre.

6.1.8 HistoLOGY OF PRIMARY TUMOUR

Detailed information on handling and storage of material is given in the Appendix on Pathology Guidelines (chapter 19).

It is recommended that in all children material from the primary tumour, even in the presence of metastatic disease (providing this can be obtained with minimal trauma to the child) is obtained. A biopsy of the primary tumour is desirable, allowing histopathological assessment.

In cases where gain of primary tumour material is considered too hazardous by virtue of the site of the tumour or the condition of the child, cytological diagnosis is accepted, provided adequate and sufficient material from heavily invaded bone marrows is obtained to enable identification of tumour cells and MYCN status and other biological and genetic markers.

The SIOPEN Biology Group stresses the importance of collecting both tissues, primary tumour and BM. Primary tumour material may be gained with true cut biopsies under imaging control (CT or sonography as appropriate).
6.1.9 Biological Studies
Rationale and handling of material is given in detail in the Appendix on Biological Guidelines (chapter 19).

The strong need to study primary tumour samples, samples from second look surgery and bone marrow aspirates and peripheral blood is stressed and advised by the SIOPEN Biology Group. Only the analysis of all these samples will enable the study of metastatic processes and the study of clonal evolution during cytotoxic treatment. Besides the direct work-up of the tissue samples and BM (see Appendix, chapter 19), it is strongly advised by the SIOPEN Biology Group that tumour material is snap frozen in the laboratory as soon as possible after the operation (primary tumour: -80° or liquid nitrogen) and that isolated BM cells are frozen also in liquid nitrogen with DMSO. In addition, laboratories will store material, extracted DNA (4°C), RNA (-80°) for genomic and expression studies.

The following studies have been agreed upon by the ENQUA group within the scope of the High-Risk NBL 1/SIOPEN Study. Material has to be adequately handled and prepared in local centres and is to be transferred to the national reference laboratory for specific studies.

Address of your national reference laboratory is given in Section 31.16
(If not supplied contact your National Coordinator for details)

6.1.9.1 Priority investigations within this protocol
- MYCN copy number
- Segmental chromosomal aberrations (SCA) by pan genomic or multi-locus techniques
- Ploidy
- Expression studies (by QRT-PCR or chip based technologies e.g. Affymetrix)
- Biology/genetics of disseminated tumour cells

6.2 Necessary Interactions to Secure Tumour and Biological Sample Flow

Check of National Coordinators and Committee Chairs needed on availability of secured materials as outlined here!

Co-ordinating clinician:
Major co-ordinating function by phone to secure the necessary work-up of tumour material by specialists, performs bone marrow aspirates and trephines, and sends out peripheral blood.

Pathologist:
Establishment of histological diagnosis (on tumour and BM trephines). Sectioning, securing and sending of tumour material (touch slides, frozen and native tumour material, freezing), to the national SIOPEN Biology Reference centre. Definition of tumour cell content on material further investigated for biological profile.

Biologist: (National Biology Reference Centre)
Securing, culturing, biological diagnosis (MYCN copy number, ploidy, cytogenetics, CGH, MLPA, expression studies, biology of disseminated tumour cells).

Cytologist:
Analysis of tumour cells in BM and aphaeresis products will be performed by ICH and AIPF analysis.
**Immunocytologist (National Immunocytology Reference Centre)**
Processing of 2 times $3 \times 10^6$ bone marrow cells on a total of 12 cytopsins and analysis of tumour cell number: 6 slides will be used for ICH and central review is guided by Klaus Beiske (Rikshospital, Oslo, Norway) and 6 further cytopsin slides should be sent unfixed and unstained to the CCRI.

**Molecular Monitoring Group (MMG) Reference Centre (MMG RC)**
Bone marrow, peripheral blood and PBSCH samples collected and analysed for circulating RNAs using QRT-PCR.
6.3 Investigations at Diagnosis, during Rapid COJEC Induction and TVD

- Early timing of investigations is an important issue!
- Register the patient immediately at diagnosis directly through the RDE System!
- At diagnosis adequate material from the primary tumour and BM, aspirates and peripheral blood for biology, molecular monitoring and immunocytology studies is mandatory!
- Informed consent signature!
- Departments performing imaging, PBSC harvest, surgery and MAT should be informed early!
- On study documentation within first 40 days!

### Flow Sheet 1

<table>
<thead>
<tr>
<th>Course</th>
<th>Diagnosis and RAPID COJEC induction phase</th>
<th>TVD phase to be given if metast. CR is not achieved following Rapid COJEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle Number</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Days (approx)</td>
<td>0</td>
<td>DE</td>
</tr>
<tr>
<td>Height</td>
<td>●</td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Physical examination</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Full blood counts</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Biochemistry renal/liver function, CRP, electrolytes</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Serum markers</td>
<td>●</td>
<td>LDH,NSE</td>
</tr>
<tr>
<td>Ferritin</td>
<td>●</td>
<td></td>
</tr>
<tr>
<td>Urine Catecholamines</td>
<td>●</td>
<td></td>
</tr>
<tr>
<td>Urine check + prox. tubular function</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Creatinine Clearance*</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Audiology</td>
<td>●</td>
<td></td>
</tr>
<tr>
<td>Echocardiogram/ECG</td>
<td>●</td>
<td></td>
</tr>
<tr>
<td>mIBG (± SPECT)</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Primary Tumour Scan: CT/ MRI</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Optional PET</td>
<td>●</td>
<td></td>
</tr>
<tr>
<td>Primary Tumour Ultrasound</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Tumour Biopsy</td>
<td>●</td>
<td></td>
</tr>
<tr>
<td>2 BM trephines</td>
<td>●</td>
<td></td>
</tr>
<tr>
<td>2 BM aspirates</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Peripheral Blood</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Apheretic product</td>
<td>●</td>
<td></td>
</tr>
</tbody>
</table>

The required tests/evaluations should be done PRIOR to the indicated cycle. Green shaded squares are mandatory for diagnostic and response evaluations.

* Patients in metastatic CR may move on to PBSC harvest

** GFR EDTA is suggested if concerns about renal function arise

For colour code guidance refer to Figure 10, page 80
### Investigations at Diagnosis, during modified N7 Induction and TVD

- Early timing of investigations is an important issue!
- Register the patient immediately at diagnosis directly through the RDE System!
- At diagnosis adequate material from the primary tumour and BM, aspirates and peripheral blood for biology, molecular monitoring and immunocytology studies is mandatory!
- Informed consent signature!
- Departments performing imaging, PBSC harvest, surgery and MAT should be informed early!
- On study documentation within first 40 days!

#### Flow Sheet 2

<table>
<thead>
<tr>
<th>Investigations</th>
<th>Diagnosis and modified N7 induction phase</th>
<th>TVD phase to be given if metast. CR is not achieved following modif. N7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Course</strong></td>
<td><strong>CAV</strong></td>
<td><strong>CAV</strong></td>
</tr>
<tr>
<td><strong>Cycle Number</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>Days (approx.)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DE</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Height</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Weight</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Physical examination</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Full blood counts</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Biochemistry renal/liver function, CRP, electrolytes</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Serum markers LDH, NSE</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Ferritin</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Urine Catecholamines</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Urine check + prox. tubular function</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Creatinine Clearance**</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Audiology</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Echocardiogram/ECG</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>mIBG (SPECT)</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Primary Tumour Scans: CT/ MRI</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Optional PET</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Primary Tumour Ultrasound</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Tumour Biopsy</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>2 BM trephines</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>2 BM aspirates</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Peripheral Blood</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Aphaeretic product</td>
<td>●</td>
<td>●</td>
</tr>
</tbody>
</table>

The required tests/evaluations should be done PRIOR to the indicated cycle. Green shaded squares are mandatory for diagnostic and response evaluations.

* Patients in metastatic CR may move on to PBSC harvest

** For colour code guidance refer to Figure 10, page 80

** GFR EDTA is suggested if concerns about renal function arise
### 6.5 Investigations at pre- and post-MAT Phase

- Careful timing is an important issue
- Contact the department of radiotherapy early to be able to start radiotherapy on time
- Plan R2 randomisation with results of post MAT investigations early

<table>
<thead>
<tr>
<th>INVESTIGATIONS FLOW SHEET 3</th>
<th>Post-TV D and PRE-MAT PHASE</th>
<th>Post Surgery</th>
<th>Prior to MAT</th>
<th>POST-MAT PHASE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time Point</td>
<td>RE after 1 TVD and prior to PBSC harvest</td>
<td>Post Surgery</td>
<td>Prior to MAT</td>
<td>Post MAT &amp; prior to radiation†</td>
</tr>
<tr>
<td>Days without TVD (approx.)</td>
<td>RE 2b</td>
<td>N/A for patients without TVD</td>
<td>RE 3</td>
<td>100-140</td>
</tr>
<tr>
<td>N7</td>
<td>105-130</td>
<td>115-140</td>
<td>175-210</td>
<td></td>
</tr>
<tr>
<td>Days with TVD (approx.)</td>
<td>from day 126</td>
<td>135-165</td>
<td>205-270</td>
<td></td>
</tr>
<tr>
<td>N7</td>
<td>from day 141</td>
<td>150-180</td>
<td>220-270</td>
<td></td>
</tr>
</tbody>
</table>

- Height
- Weight
- Physical examination
- Blood pressure
- Full blood counts
- Biochemistry, Renal/liver function, CRP, electrolytes
- Serum markers
- LDH, NSE
- Urine Catecholamines
- Urine check + prox. tubular function
- Creatinine Clearance
- GFR EDTA
- Audiology
- EEG
- Chest X-Ray
- Echocardiogram/ECG
- Pulmonary Function Test
- mIBG (± SPECT)
- Primary Tumour Scans: CT/ MRI
- Optional PET
- Ultrasound
- Attempt full tumour resection
- 2 BM trephines
- 2 BM aspirates
- Peripheral Blood
- MAT PK samples
- Apheretic product

Green shaded squares are mandatory for response evaluation.

* Response evaluation prior to MAT is only necessary if the time interval between the end of induction and/or TVD and the beginning of MAT is greater than 60 days and if surgery has been performed.
† Radiotherapy will be given after MAT/PBSCR and prior to the start of isotretinoin (13-cis-RA). After MAT the interval must be greater than 60 days after stem cell transplantation due to the risk of Busulfan-enhanced radiotoxicity.

For colour code guidance refer to Figure 10, page 80
6.6 Investigations during Immunotherapy Phase until the End of Treatment

The required tests/evaluations should be done PRIOR to the indicated week. Green shaded squares are mandatory for Response Evaluation.

* End of treatment or in case of early clinical progression
** Radiographic contrast materials have been associated with increased risk of anaphylactic reactions if used during aldesleukin (IL-2) infusion. Therefore, avoid contrast for at least 1 week after the completion of aldesleukin (IL-2).
† Only need for patients randomised to receive aldesleukin (IL-2).

Tests during immunotherapy are required as often as requested so a clear toxicity profile is gained. The toxicity profile is needed for the registration of ch14.18/CHO and the off-label use of aldesleukin (IL-2).

For colour code guidance refer to Figure 10, page 80

<table>
<thead>
<tr>
<th>INVESTIGATIONS</th>
<th>MRD PHASE</th>
<th>END*</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLOW SHEET 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13-cis RA Course Number</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Week 13-cis RA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ch14.18/CHO course</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Height</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Weight</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Physical examination</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Full blood counts</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Biochemistry</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Renal/liver function; CRP, electrolytes</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Serum markers</td>
<td>LDH, NSE</td>
<td>●</td>
</tr>
<tr>
<td>Urine Catecholamines</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Creatinine Clearance</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Audiology</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Ophthalmology</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>EEG</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Chest X-Ray</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Echocardiogram/ECG</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Pulmonary Function Tests</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>mIBG (+ SPECT)**</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Primary Tumour Scans: CT/ MRI**</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Optional PET</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Primary Tumour Ultrasound</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>2 BM trephines</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>2 BM aspirates</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Peripheral Blood</td>
<td>●</td>
<td>●</td>
</tr>
</tbody>
</table>

During immunotherapy samples are required at various time points for PK & immunological monitoring of ch14.18/CHO, NK cell activation, PK and pharmacogenetic analysis of isotretinoin (13-cis-RA). Refer to sections 14.11, 14.12.1, 14.12.2 and 24.2 for details of sampling time points.
6.7 Follow Up Investigations

6.7.1 TUMOUR ASSESSMENT/DETECTION
In an asymptomatic patient, the following are recommended:

6.7.1.1 Imaging of the Primary Site (ultrasound or CT scan as appropriate)
   a) every 3 months for the first year
   b) every 6 months in the second and third years
   c) yearly until 5 years

6.7.1.2 Metastatic Assessment:
   a) If residual skeletal mIBG positive, repeat mIBG scan every 3 months until negative or progression.
   b) If stable over 1 year, repeat it on a yearly basis for up to 5 years.
   c) If residual bone marrow (BM) disease at the end of differentiation therapy, repeat BM aspirates every three months until negative or progression.

6.7.1.3 Urine catecholamine assessment:
For patients with positive urine catecholamines at diagnosis, repeat assessment:
   a) every 3 months for the first year
   b) every 6 months in the second and third years
   c) yearly until 5 years

6.7.2 TOXICITY ASSESSMENT
The toxicity assessment needs to be related to the randomised treatment received by the patient. All children require renal, audiological and fertility follow up. Those who have had extensive abdominal or pelvic radiotherapy may have prolonged thrombocytopenia.

6.7.2.1 General Assessment
All patients should have a physical examination, height, weight, and full blood count (with differential and platelets) according to the following time points:
   a) every month for the first year
   b) every 6 months in the second and third years
   c) then yearly until 5 years

6.7.2.2 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 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.

6.7.2.3 Auditory Follow-Up
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. Otoxicity is usually permanent or irreversible. 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 repeated after 6 months.
6.7.2.4  **Cardiac Follow-Up**
Patients receiving anthracyclines (modified N7 or TVD) should have yearly echocardiograms and ECGs for the first 5 years and then as clinically indicated.

Patients receiving Rapid COJEC only do not receive anthracycline, thus no life-long late effects are anticipated. Cardiac follow-up is only indicated for individual patients experiencing cardiac toxicity on treatment.

6.7.2.5  **Pulmonary Follow-Up**
All patients receiving MAT should have yearly pulmonary function tests for the first 5 years and then as clinically indicated.

6.7.2.6  **Etoposide: second malignancy follow up**
It is essential that any second malignancy occurring amongst the children treated with chemotherapy is registered urgently with the data centre.

6.7.2.7  **I.V. Busulfan (Busilvex®): Fertility/ Ovarian Failure**
In patients who have received busulfan and boys who received melphalan reduced fertility is to be expected. The results of this follow-up will be part of a long-term follow-up study. In particular, the problem of busulfan in girls is the ovarian failure, since these girls need hormonal substitution to become pubescent.

7  **Surgery**

7.1  **Introduction**

Operation for high risk neuroblastoma is difficult and time consuming. 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 [20, 148-150]. 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 [104]. Treatment included induction chemotherapy and surgery with or without local irradiation. Patients then received total body irradiation including megatherapy (MGT) and autologous bone marrow transplantation. Since then, US groups have focused on local control and have been using multimodal techniques [103]. Not withstanding the above, there are well established biological principles to support complete removal of the primary tumour, prior to MGT. The goal of induction chemotherapy is to clear metastatic disease and achieve maximum response at the primary site. In this way the number of viable cells capable of developing drug resistance is minimised. Complete surgical resection of the primary is clearly consistent with this aim.

It is apparent that there is a large variation in the volume of tumour remaining after attempted excision. It is also apparent that, in some centres, almost all of the patients have almost their entire tumour excised, without unacceptable morbidity or mortality. In order to minimise the inherent variability of surgery there must be a commitment by those participating in the study to attempt complete excision. The principles of the vascular dissection required have been eloquently described [151]. If a centre does not feel able to give this commitment then the patient should be transferred for surgical treatment to one which does.
7.2 Aims

(i) 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.

(ii) 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).

7.3 Timing

Surgery should be undertaken after the end of induction chemotherapy and ideally after peripheral stem cell harvest.

This will occasionally mean excising the primary tumour when metastatic disease is still present. The justification for this is that the primary will need to be excised at some point and that further metastatic response may be anticipated with additional treatment. Patients with an overall partial response at metastatic sites may proceed to MAT. An overall partial response is defined as:

- at least 50% reduction in skeletal mIBG positivity and not more than 3 positive, but improved spots on mIBG
- cytomorphological CR in 2 BM aspirates and no positive BM biopsy

If the primary tumour shows a partial response (PR) to induction chemotherapy, but is still judged inoperable, surgery may be further postponed. If a patient needs additional surgery to the primary tumour site, this should be considered.

7.4 Definition of Procedures

7.4.1 Biopsy (Check also Pathology Guidelines)

Biopsy should be the first procedure on all tumours. Collection of tumour samples for review in the reference laboratories is extremely important for this trial!

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

- Open biopsies are most satisfactory but many may feel this an unacceptable additional procedure given that the diagnosis can be made by less invasive means. Multiple needle core biopsies can provide sufficient tissue for diagnostic studies. Optimum treatment is critically dependent on correct tissue handling. It is mandatory that the pathologist is prepared to receive the samples. Fresh tissue should be delivered to the pathologist immediately under sterile conditions. Formalin or other preservatives must NOT be used.

- Fine needle aspiration is acceptable if there is no other alternative and is an effective source for additional tumour cells for biological studies.

7.4.2 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.
7.4.3 **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.

7.4.4 **Incomplete Excision**
- More than 5% or 5ml of tumour remain after attempted excision.
- The amount should be estimated by the surgeon in mls and as a percentage and evaluated by post-operative imaging.

7.5 **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 National and International Data Centre through the RDE System

7.6 **Aspects of Surgical Procedures**

7.6.1 **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.

7.6.2 **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).

7.6.3 **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.

7.6.4 **Nephrectomy**
Nephrectomy is not associated with a survival advantage and should be avoided if possible. 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. 

A synopsis of the aims of the different stages in the protocol is as follows:

- The goal of induction chemotherapy is to eradicate metastatic disease and reduce tumour volume to increase the efficacy of operation.
- The goal of operation is complete tumour excision.
- The goal of myelo-ablative chemotherapy (MAT) is to eradicate minimal residual disease.
- The goal of radiotherapy is to eradicate minimal residual disease in the primary site.

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.

**7.6.5 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.

**7.6.6 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.

**7.7 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 hepatitis
- 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

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

8 **Pathology**

8.1 **General Remarks**
The Neuroblastoma Pathology Guidelines (Appendix, chapter 19) for resectable and unresectable neuroblastic tumours have been produced for SIOP/Europe Neuroblastoma Group and were accepted as such by the board and group members as common rules and should serve as reference.
The National reference pathologists will constitute a central review panel which will histologically review all tumour specimens derived from patients in this Study (responsible SIOPEN NBL contact person: Klaus Beiske).

8.2 **Recommendations for Handling of Tumour Material**
- Various biopsy techniques are applicable for collection of tissue material from unresectable tumours.
  - **TRU CUT BIOPSIES**: preferably four biopsies (at least two biopsies in case of small lesions) from different areas of the tumour should be obtained.
  - **FINE NEEDLE ASPIRATIONS**: at least two separate punctures/aspirations should be performed from each tumour. *(REMARK: two aspirations as only step for diagnosis will not achieve enough tumour material as needed !)*
  - **OPEN BIOPSY**: two different areas of the tumour should be biopsied by the surgeon. Specimen size should be at least 1 cm³.
• The tumour material should be transferred immediately from the operation theatre to the local pathology department under sterile conditions. The splitting of the tumour material must be done by the pathologist as soon as possible after the operation. The time of splitting up the tumour should be stated.

• The paediatric oncologist in charge and/or the surgeon has to inform the local Pathologist and Biologist (local and/or national) in a timely manner about the new patient and the material to be expected.

• Further close co-operation between pathologists and biologists is strongly encouraged. Pathologists should inform the biologists if morphologically unfavourable looking areas are present in the paraffin embedded material but most likely not in the specimens selected for molecular-genetic/biologic investigations. These areas should also be specifically analysed using the paraffin material (see below).

Caution: All the recommendations for open biopsies, tru cut biopsies and fine needle aspirations (e.g. number of different tumour areas) concern only those patients for whom the risk of taking tumour material is considered to be reasonable. In some instances this risk has to be weighed carefully (especially in case of patients with stage 4s neuroblastomas with coagulation disorders, but also in case of patients with large and fragile tumours). It is clear that if the procedure aiming at retrieving material carries an increased risk, it should not be performed! In this instance material should be obtained from secondaries e.g. cutaneous lesions, lymph nodes, or bone marrow if adequate, versus visceral specimen of tumour.

Address of your national reference laboratory see Chapter 31.16, page 293
(If not supplied contact your National Coordinator for details)

8.3 Role of Pathologist

• The handling of the tumour tissue should always be performed by the pathologist according to the Pathology Guidelines given in detail in the Appendix section (chapter 19, page 159). A copy of these guidelines should be available to all pathologists dealing with tumour material from neuroblastoma patients.

• Besides the important task of making morphological diagnoses and giving prognoses based on histopathological findings, another major task of the local pathologists is to choose the relevant tumour areas for molecular-genetic/biological analyses.

• The material selected for molecular-genetic/biological investigations should be frozen as soon as possible and sent as fast as possible to the National Reference Biology Laboratory. Please refer to the Appendix (chapter 19) for details specific to your National Group. In case of queries please contact the National group co-ordinator.

• In all instances, tumour material from different tumour areas (nodules are of special interest!) ought to be taken for histologic and molecular-genetic/biologic examination. The reason for this recommendation is based on the observation of tumour heterogeneities at the genetic level (e.g. for the MYCN copy number, the presence of SCAs and expression status) and/or at the histologic level (Ganglioneuroblastoma, nodular subtype according to the International Neuroblastoma Classification, INPC [152]), both of which have prognostic implications.

• To enable reliable interpretation of the molecular-genetic results, the exact tumour cell content of the specimen used for these investigations has to be determined. This is
possible only if the pathologist evaluates the specimens adjacent to those used for molecular-genetic/biological analyses (for details see below).

- In case the tumour pieces selected for molecular-genetic/biologic investigations were not appropriate for getting reliable results, MYCN, ploidy and chromosome 1p36.3 status can be determined on the paraffin embedded material. Laboratories which do not perform this kind of investigations have the possibility to send the paraffin blocks, ideally with H&E slides, to Drs. Peter and Inge Ambros, Vienna, Austria.

9 Biological Studies

Adequate material for biological studies is mandatory for all patients in this trial! It is particularly important for the 12-18 month age group and patients with locoregional disease. MYCN results have to reach the data centre within 10 days in patients with locoregional disease to be able to fulfil entry and eligibility criteria in case of MYCN-amplification and within 6 weeks for patients with primary metastatic disease prior to the first randomisation.

9.1 Type of Biological Studies

Material has to be adequately handled and prepared in local centres and has to be transferred to the national reference laboratory for specific studies.

Address of your national reference laboratory see chapter 31.16
(If not supplied contact your National Coordinator for details)

9.1.1 First Priority Investigations within This Protocol

- MYCN copy number
- Ploidy
- CGH or MLPA for study of other chromosomal gains and losses (i.e. SCA)
- Expression studies
- Biology of disseminated tumour cells

9.2 Material for Biological Studies of Neuroblastoma

Samples should be taken for all patients and sent to the National Biology Reference Laboratory (Contacts given in the Appendix section, chapter 32.4 and 32.16).

9.2.1 Primary Tumour

Pathology, compulsory
- Frozen tumour (biopsy, or surgical material) compulsory - MYCN, compulsory
- pan- or multi-genomic techniques (aCGH, MLPA)
- Ploidy by FACS (on native cells)

9.2.2 Blood Samples
- Constitutional DNA
- Serum

9.2.3 Frozen Tissue
- Primary tumour (- 80°, liquid nitrogen)
- Molecular biology
  DNA (+ 4°)
  RNA (- 80°, sterile)
9.3 Important Remarks

- Analyses of MYCN and tumour cell DNA content are obligatory investigations which have to be carried out in the National Biology Reference Laboratories i.e. laboratories participating in the European Neuroblastoma Quality Control Assessment (ENQUA) study. Results from other laboratories will not be used!
- It is of crucial importance that frozen tumour material (biopsy or surgical material) is sent in time to the Reference Laboratory!
- The MYCN copy number can be analysed by fluorescence based in situ hybridisation (FISH). Further details and the terminology used for description of MYCN results are given in the Appendix on Biological Guidelines (chapter 19).
- To be eligible for the entry in the Study it is essential that the results for the MYCN studies are available within 10 days in patients with locoregional disease of the tumour resection or the diagnostic biopsy (six weeks for patients with primary metastatic disease).
- The DNA content can be determined by Flow Cytometry or Image Cytometry.
- The evaluation of SCA (segmental chromosomal aberrations) can be done by a CGH or MLPA. For these studies the tumour cell content has to be over 60 per cent. Further details are given in the Appendix on Biological Guidelines (chapter 19).
- For expression studies frozen tumour material is essential.

10 Peripheral Stem Cell Harvest – Aphaeretic Product

10.1 Introduction
The aim of the PSC Harvest is to collect a sufficient number of blood progenitors in order to allow safe and prompt haematological recovery following the high dose chemotherapy (HDC) regimen and autograft.

The target dose of collected CD34+ cells is ≥ 3 x 10^6 CD34 positive cells/kg for one BuMel MAT procedure. In case of a circulating level still higher than 20 CD34 positive cells per μL the leukapheresis procedure should be started, and a second procedure on the next day is recommended, if the amount of first apheresis is near by or less than 3 x 10^6 CD34 positive cells/kg. The total harvest should always be divided into minimum two bags. The amount of dimethyl sulphoxide (DMSO) should not be more than 1g/kg body weight (bw) for one reinfusion per day to avoid toxicity. If less than 3 x 10^6 CD34 /kg is obtained from the first mobilization attempt discuss with study co-ordinator. There is no ideal timing for PBSC harvest in patients with metastatic neuroblastoma, except that early collection increases the risk of tumour cell contamination and later ones of poor progenitor cell collection. The monitoring of both tumour cell contamination and progenitors in the graft using standard procedures will enable us to better define the impact of possible tumour cell contamination on EFS.

It is recommended that all procedures should be carried out in institutions with particular expertise in stem cell harvest in small children.

10.2 Timing of Peripheral Stem Cell Harvest
- The disease response status should be evaluated before performing the PBSC harvest procedure. The bone marrow staging on two aspirates and on two bone marrow biopsies should be cytomorphologically and histologically negative and skeletal response meet recommended criteria (partial remission according to INSS criteria). In case of bone marrow MRD positivity it is strictly recommended to analyse the harvest for MRD, at minimum 1x10E06 MNC should be examined.
- If the patient fulfils the response eligibility criteria for MAT at least 3 x 10^6 CD34 + cells/kg BW should be harvested.
There are various time points for stem cell collection, the decision about which ones to use is left to the discretion of centre, since this is closely related to individual local organisation. It is recommended to start early with harvest efforts. The first harvest option is following recovery from aplasia after the end of induction (after day 70 of COJEC chemotherapy or after the last course of modified N7 chemotherapy).

**SUMMARY OF ALTERNATIVE HARVEST OPTIONS**

- Mobilisation with (5-) 10 µg/kg BW g-CSF s.c. after day 70 of COJEC or after the last course of modified N7
- Steady state mobilisation after COJEC or modified N7, but prior to surgery with 10 µg/kg BW g-CSF s.c.
- Steady state mobilisation after surgery with 10 µg/kg BW g-CSF s.c.
- Consider BM-Harvest
- No stem cells by day 150 for patients after front-line induction (rapid COJEC or modified N7) or 210 for patients after TVD: Ineligible for BuMel MAT on-study.

If the patient has to go off study because of an unsatisfactory response at this particular time point a different harvest procedure will be followed according to the type of phase II study he/she enters. Therefore both the mIBG and bone marrow response should be known before starting the first leukapheresis.

**10.3 Recommended Procedure for PBSC Mobilisation and Day of Collection**

There are at least two options for stem cell mobilisation:

a) The use of G-CSF (5-) 10 µg/kg/day following the last cycle of chemotherapy, i.e. day 72 of rapid COJEC or after recovery from last modified N7 course (approximately day 100), with monitoring of the presence of peripheral blood CD34+ cells until a sufficient number is reached (minimum >20 CD34+ cells/µL are required to start the first leukapheresis).

b) Steady state mobilisation after haematological recovery following the last course of front-line induction chemotherapy (Steady state defined as >1 neutrophils and >100 platelets x 10^9/l) giving G-CSF 10 µg/kg/day for 5 days by subcutaneous injection. Collection should be started ideally on the 4th or 5th day.

The symptoms of fever and bone pain that may occur with G-CSF administration can be reduced by regular paracetamol, but be aware of the possibility of masking fever due to infection.

- The number of collections depends on the quantity of peripheral blood stem cells harvested, evaluated by the number of CD34 positive cells.
- If the sample is insufficient, it can be repeated following surgery using the steady state method with G-CSF at a dose of 10 µg/kg/day.

**10.4 Vascular Access**

PBSC may be collected using a central Broviac or Hickman line together with a large peripheral vein. Most patients will have a Broviac or Hickman catheter as central venous line for treatment and blood samples during the induction therapy. This line could be used as the inlet or return line in the leukapheresis procedure. As opposite line, i.e. a 22 – 20 G peripheral venous access (PVA) could be established for the procedure. Otherwise also two PVA can be used as inlet line in combination with a second PVA (22 – 20 G).

When necessary a central venous line should be established before leukapheresis according to the in- house standard techniques, i.e. a double lumen central venous catheter specifically designed for apheresis may be placed for the procedure. It is anticipated that most neuroblastoma patients will require placement of a temporary or tunnelled apheresis catheter.
10.5 Standard Procedure
Leukapheresis will be performed according to current recommended practice. However, the procedure should be performed by an operator who is experienced in paediatric procedures. In small children, i.e. less than 20 or 15kg, the extracorporeal volume of the machine used has to be considered. The priming procedure can be performed with human albumin 5% instead of saline solution in cases where a hypoproteinemia and consecutively edema can be expected, and a further priming step with packed red blood cells, irradiated and leukocyte depleted, should be carried out if the loss of RBC volume calculated for the machine priming exceeds 30% or in case of hemodynamically instable patients.
In children weighing less than 10 kg, it is recommended that the cell separator is primed with irradiated, white cell depleted, packed red cells resuspended in 5% albumin. A dilution with saline to match the patient’s haematocrit depends on the apheresis system used. It is strictly recommended to have internal SOP’s on this issue in each centre.
Once an adequate harvest is obtained the cells should be frozen in at least two separate bags, pending use after BUMEL. After leukapheresis a sample of each buffy coat should be analysed for CD34+ cell content and tumour cell contamination.

10.6 Freezing Procedure and Storage
The buffy coat should be frozen after mixing with DMSO (maximum concentration after mixing 10%) in a rate controlled freezer. The amount of DMSO should not exceed 1g/kg bw for one reinfusion per day. Samples should be stored in the liquid phase of nitrogen. Two small samples (i.e. 1 ml) should also be frozen and stored under the same conditions for backup examinations and quality controls.

10.7 Purging of PBSC Harvests
No in vitro purging of stem cells is envisaged. It is strongly recommended that stem cell harvest and MAT/PSCR are carried out in institutions with particular expertise in this procedure. Consideration for referral to such an institution is advised and should be planned early.

11 Chemotherapy Regimen Details
Any organ dysfunction which could lead to an important alteration of drug elimination should be discussed with the national trial co-ordinator.

The administration regimens that appear below are guidelines only. In cases where national and/or local institutional guidelines differ to these, the local/national guidelines may be used; this is at the discretion of the local/national investigators.

11.1 Rapid COJEC Induction
This induction treatment will be applied over ten weeks and proceed regardless of neutrophil or platelet counts and controlled infection. Three different courses are given every 10 days. Consult study co-ordinators if glomerular filtration rate < 80 ml/min/1.73m². COURSE A starts on days 0 and 40, COURSE B on days 10, 30, 50 and 70 and COURSE C on days 20 and 60.

- **COURSE A** consists of vincristine 1.5 mg/m² (maximum dose 2 mg), carboplatin 750 mg/m² and etoposide 2 x 175mg/m².
- **COURSE B** uses vincristine 1.5 mg/m² (maximum dose 2 mg) and cisplatin 80 mg/m²/ctn over 24 hours.
- **COURSE C** consists of vincristine 1.5 mg/m² (maximum dose 2 mg), etoposide 2 x 175mg/m² and cyclophosphamide 2 x 1050mg/m².
In case of a body weight below 12kg it is recommended to calculate the dose according to kg using the formular that one m² equals 30 kg (see Section 3.1.2, page 43). For infants with a weight < or equal to 5 kg, a further 1/3 reduction is advised.

<table>
<thead>
<tr>
<th>Dose /day</th>
<th>days</th>
<th>days</th>
<th>days</th>
<th>days</th>
<th>days</th>
<th>days</th>
<th>days</th>
<th>days</th>
<th>days</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-CSF</td>
<td>5µg/kg</td>
<td>3-8</td>
<td>12-18</td>
<td>23-28</td>
<td>32-38</td>
<td>43-48</td>
<td>52-58</td>
<td>63-68</td>
<td>72 till harvest</td>
</tr>
<tr>
<td>CBDA</td>
<td>750mg/m²</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Vp16</td>
<td>175mg/m²</td>
<td>↓↓</td>
<td>↓↓</td>
<td>↓↓</td>
<td>↓↓</td>
<td>↓↓</td>
<td>↓↓</td>
<td>↓↓</td>
<td>↓↓</td>
</tr>
<tr>
<td>VCR</td>
<td>1.5mg/m²</td>
<td>◇</td>
<td>◇</td>
<td>◇</td>
<td>◇</td>
<td>◇</td>
<td>◇</td>
<td>◇</td>
<td>◇</td>
</tr>
<tr>
<td>CDDP</td>
<td>80mg/m²</td>
<td>◇ctn</td>
<td>◇ctn</td>
<td>◇ctn</td>
<td>◇ctn</td>
<td>◇ctn</td>
<td>◇ctn</td>
<td>◇ctn</td>
<td>◇ctn</td>
</tr>
<tr>
<td>CYC</td>
<td>1050mg/m²</td>
<td>↓↓</td>
<td>↓↓</td>
<td>↓↓</td>
<td>↓↓</td>
<td>↓↓</td>
<td>↓↓</td>
<td>↓↓</td>
<td>↓↓</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DAY</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
<th>110</th>
</tr>
</thead>
<tbody>
<tr>
<td>COURSE</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>B</td>
</tr>
<tr>
<td>HARVEST</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>SURGERY</td>
<td>Sx</td>
<td>Sx</td>
<td>Sx</td>
<td>Sx</td>
<td>Sx</td>
<td>Sx</td>
<td>Sx</td>
<td>Sx</td>
<td>Sx</td>
<td>Sx</td>
<td>Sx</td>
<td>Sx</td>
</tr>
<tr>
<td>STAGING</td>
<td>◆</td>
<td>◆</td>
<td>◆</td>
<td>◆</td>
<td>◆</td>
<td>◆</td>
<td>◆</td>
<td>◆</td>
<td>◆</td>
<td>◆</td>
<td>◆</td>
<td>◆</td>
</tr>
</tbody>
</table>

◆ Primary site MRI or CT, ultrasound, mIBG, Bone Marrow: 2 aspirates/2 biopsies
◆ Bone Marrow: 2 aspirates, primary site ultrasound
▪ Primary site: control of surgical success (method optional ultrasound, MRI, CT: 2 weeks after surgery; if positive repeat it but not prior to day 60 post MAT)
- Disease assessment according to the International Neuroblastoma Staging and Response Criteria.

The following guidelines are aimed at enabling the prescription of the chemotherapy to be easier. Infusion rates are slightly higher than required to infuse the chemotherapy over the stated time due to the fact that there will invariably be interruptions in the infusion.

### COURSE A

**START ON DAY 0 AND 40**

<table>
<thead>
<tr>
<th>DAYS of course A</th>
<th>1&lt;sup&gt;st&lt;/sup&gt;</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>VINCRISTINE</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>CARBOPLATIN</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>ETOPOSIDE</td>
<td>4h ©</td>
<td>4h ©</td>
</tr>
</tbody>
</table>

**DRUG** | **Time** | **Dose**(mg/m²) | **Administration**
---|---|---|---
VINCRISTINE | 0 hrs | 1.5 mg/m² | (maximum dose 2 mg) as a single iv bolus
CARBOPLATIN | 0 hrs | 750 mg/m² | standard size bags infused over 1 hour
1 - 250mg of carboplatin in 50 ml of 5% dextrose
>250 - 500mg of carboplatin in 100 ml of 5% dextrose
>500 -1000mg of carboplatin in 250 mls of 5% dextrose
ETOPOSIDE  1 hrs  175 mg/m²  standard size bags infused over 4 hours
0 - 40mg of etoposide in 100 mls of 0.9% saline
>40 - 50mg of etoposide in 150 mls of 0.9% saline
>50 - 100mg of etoposide in 250 mls of 0.9% saline
>100 - 200mg of etoposide in 500 mls of 0.9% saline
>200 - 300mg of etoposide in 750 mls of 0.9% saline
>300 - 600mg of etoposide in 1000 mls of 0.9% saline

In case of body weight below 12kg the following dose calculation is recommended:
VINCRISTINE  0.05 mg/kg, CARBOPLATIN 25 mg/kg, ETOPOSIDE (VP16)  5.833 mg/kg

The use of G-CSF 5µg/kg/day s.c. during Rapid COJEC induction is recommended starting 24h after last chemotherapy, and to be stopped the day prior to commencing the next course with an interval of at least 24 hours between the last G-CSF injection and the start of chemotherapy, i.e. days 3 to 8, and days 43 to 48.

**COURSE B**

START ON DAYS  10 - 30 - 50 - 70

<table>
<thead>
<tr>
<th>DRUG</th>
<th>Time</th>
<th>Dose(mg/m²)</th>
<th>Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>VINCRISTINE</td>
<td>0 hrs</td>
<td>1.5 mg/m²</td>
<td>(maximum dose 2 mg) iv bolus pre-hydration for cisplatin infused at 200 ml/m²/hr for 3 hours 0.9% sodium chloride with 10 mmol/l potassium chloride</td>
</tr>
<tr>
<td></td>
<td>0 hrs</td>
<td></td>
<td>Mannitol 20% 0 hrs short infusion in a dose of 40ml/m²</td>
</tr>
<tr>
<td></td>
<td>2.5 hrs</td>
<td></td>
<td>Mannitol 20% 2.5 hrs short infusion in a dose of 40ml/m²</td>
</tr>
<tr>
<td>CISPLATIN</td>
<td>3 hrs</td>
<td>80 mg/m²/24 hours</td>
<td>in a mini-bag alongside the hydration for 24h 1- 50mg cisplatin in 100ml of 0.9% sodium chloride &gt;50-100mg cisplatin in 150ml of 0.9% sodium chloride &gt;100-200mg cisplatin in 200mls of 0.9% sodium chloride Commence hydration in parallel with cisplatin 1.5 l/m²/24 hrs of 0.9% sodium chloride 1.5 l/m²/24 hrs of 5% glucose 30 mmol/m²/24 hrs of potassium chloride 2.5 mmol/m²/24 hrs of calcium gluconate 10 mmol/m²/24hrs of magnesium sulphate</td>
</tr>
<tr>
<td></td>
<td>9 hrs</td>
<td></td>
<td>Mannitol 20% 9 hrs if diuresis falls below 400 ml/m²/6 hours, short infusion in a dose of 40ml/m² repeat thereafter whenever indicated</td>
</tr>
</tbody>
</table>
27 hrs

Commence **post cisplatin hydration**

- 1.5 l/m²/24 hours of 0.9% sodium chloride
- 1.5 l/m²/24 hours of 5% glucose
- 60 mmol/m²/24 hours of potassium chloride
- 2.5 mmol/m² calcium gluconate
- 10 mmol/m²/24hrs of magnesium sulphate

51 hrs

Complete therapy

In case of body weight below 12kg the following dose calculation is recommended

**VINCRISTINE 0.05 mg/kg, CISPLATIN 2.666 mg/kg. For Infants with a weight < or equal to 5 kg, a further 1/3 reduction is advised.**

During prehydration, the cisplatin infusion together with its parallel hydration and post-cisplatin hydration, a careful record of fluid input and output should be kept to prevent hydration overload and ensure diuresis. Cisplatin is given as a continuous infusion over 24h combined with forced mannitol diuresis: Mannitol 20% in a dose of 40ml/m² should be administered 3 hours and 30 minutes prior to starting cisplatin and thereafter if diuresis falls below 400 ml/m²/6 hours (during the cisplatin infusion furosemide should be avoided because of its enhancing effect on ototoxicity). The addition of magnesium during cisplatin treatment is recommended at a daily dose of 180mg/m²/day during the induction period but may need to be adjusted following strict monitoring of Mg levels. Mannitol and magnesium are not to be given con-currently as mannitol and magnesium are not compatible. The addition of calcium, potassium and phosphate may also be modified according to serum levels.

The use of G-CSF 5µg/kg/day s.c. during Rapid COJEC induction is recommended in between Courses B to C or A respectively starting 24h after last chemotherapy, and to be stopped the day prior to commencing the next Course with an interval of at least 24 hours between the last G-CSF injection and the start of chemotherapy, i.e. days 12-18, days 32 to 38 and 52 to 58 and after day 72 till PB recovery (either directly to be used for mobilisation and PBSC harvest or with 10µg/kg out of steady state after recovery).

### COURSE C

**START ON DAYS 20 - 60**

<table>
<thead>
<tr>
<th>DAY</th>
<th>1st</th>
<th>2nd</th>
</tr>
</thead>
<tbody>
<tr>
<td>VINCRISTINE</td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>CYCLOPHOSPHAMIDE</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>ETOPOSIDE</td>
<td>4h ☄</td>
<td>4h ☄</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DRUG</th>
<th>Time</th>
<th>Dose(mg/m²)</th>
<th>Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vincristine</td>
<td>0 hrs</td>
<td>1.5 mg/m²</td>
<td>(maximum dose 2 mg) iv bolus</td>
</tr>
</tbody>
</table>
| Etoposide  | 0 hrs| 175 mg/m²   | **standard size bags infused over 4 hours**
|            |      |             | 0-40mg of etoposide in 100 ml of 0.9% saline
|            |      |             | >40-50mg of etoposide in 150 ml of 0.9% saline
|            |      |             | >50-100mg of etoposide in 250 ml of 0.9% saline
|            |      |             | >100-200mg of etoposide in 500 ml of 0.9% saline
|            |      |             | >200-300mg of etoposide in 750 ml of 0.9% saline
|            |      |             | >300-600mg of etoposide in 1000 ml of 0.9% saline |
Mesna Bolus 4hrs 200mg/m² i.v. bolus

Cyclophosphamide 4 hrs 1.05 gm/m² as iv bolus followed by post-cyclophosphamide infusion for 24 hours with:
1.2 g / m² / 24 hrs mesna
1.5 l /m² / 24 hrs of 0.9% sodium chloride
1.5 l /m² / 24 hrs 5% glucose
60 mmol/m²/24 hours of potassium chloride

Etoposide 28 hrs 175 mg/m² standard size bags infused over 4 hours as above

Cyclophosphamide 32 hrs 1.05 gm/m² as iv bolus followed by post-cyclophosphamide infusion for 24 hours with:
1.2 g / m² / 24 hrs mesna
1.5 l /m² / 24 hrs of 0.9% sodium chloride
1.5 l /m² / 24 hrs 5% glucose
60 mmol/m²/24 hours of potassium chloride

56 hrs Complete therapy

During post-cyclophosphamide infusion a careful record of fluid input and output should be kept to prevent fluid overload and ensure an adequate diuresis. If diuresis falls less than 400 ml/m²/6 hours, furosemide 0.5 – 1.0 mg/kg should be given.

In case of body weight below 12kg the following dose calculation/per dose is recommended. Vincristine 0.05 mg/kg, Cyclophosphamide 35 mg/kg, Etoposide (VP16) 5.8333 mg/kg. For Infants with a weight < or equal to 5 kg, a further 1/3 reduction is advised.

11.2 Modified N7 Induction

The modified N7 induction regime is the comparator arm. By current Clinical Trial Definition/International Guidelines of Harmonisation (CTD/ICH) all drugs of modified N7 are investigational medicinal products (IMPs). Use of this regimen has been well documented [2, 3, 93, 94]. Therefore, the drugs will not be supplied by the Sponsor. All the drugs used in this regimen are commercially available.

The modified N7 induction is a dose intensive induction chemotherapy regimen including two putatively non cross-resistant drug combinations: high-dose cyclophosphamide plus doxorubicin/vincristine (CAV) and high-dose cisplatin/etoposide (P/E).

1. The original regimen with 7 cycles was modified reducing the number of cycles to 5, with a lower dosage of VCR and using G-CSF [3].
2. Cycles 1, 2, and 4 consist of CAV: Cyclophosphamide (70 mg/kg) is infused intravenously (i.v.) for 6 hours on days 0 and 1 (140 mg/kg/course). Beginning on day 0, doxorubicin (75 mg/m² over 3 days, i.e. 25 mg/m²/day) and vincristine (0.067 mg/kg over 3 days, i.e. 0.022 mg/kg/day, up to a maximum of 2.0mg/course) are given by 72-hour i.v. infusion.
3. Cycles 3 and 5 consist of P/E, with 1-hour i.v. infusions of 50 mg/m² of cisplatin on days 0 to 3 (200 mg/m²/course), and 2-hour i.v. infusions of 200 mg/m² of etoposide on days 0 to 2 (600 mg/m²/course).
4. G-CSF 5 μg/kg is administered for every cycle. Administration is started 72 hours after the end of chemotherapy and given until the absolute neutrophil count (ANC) is > 1.0 International Units.

5. Cycles of chemotherapy are started on count recovery (ANC ≥ 500/uL and platelets ≥ 75,000/uL). The patient should have been off G-CSF for at least 48 hours. It is expected that courses will be delivered approximately every three weeks. If there are concerns regarding delays to chemotherapy, because of protracted haematological recovery contact your National Coordinator to discuss the possibility of a dose reduction.

6. The cardioprotectant dexrazoxane should NOT be administered [4].

<table>
<thead>
<tr>
<th>G-CSF</th>
<th>Dose/day</th>
<th>INTRAVENOUS</th>
<th>6h</th>
<th>6h</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYC</td>
<td>70 mg/kg</td>
<td>6h</td>
<td>6h</td>
<td>6h</td>
</tr>
<tr>
<td>DOXO</td>
<td>25 mg/m²</td>
<td>72 h ctn</td>
<td>72 h ctn</td>
<td>72 h ctn</td>
</tr>
<tr>
<td>VCR</td>
<td>0.022 mg/kg</td>
<td>72 h ctn</td>
<td>72 h ctn</td>
<td>72 h ctn</td>
</tr>
<tr>
<td>CDDP</td>
<td>50 mg/m²</td>
<td></td>
<td>72 h ctn</td>
<td>72 h ctn</td>
</tr>
<tr>
<td>Vp16</td>
<td>200 mg/m²</td>
<td></td>
<td>72 h ctn</td>
<td>72 h ctn</td>
</tr>
</tbody>
</table>

IN CASE OF A BODY WEIGHT BELOW 12KG IT IS RECOMMENDED TO CALCULATE THE DOSE ACCORDING TO KG USING THE FORMULAR THAT ONE M² EQUALS 30 KG; DRUGS ALREADY PRESCRIBED USING MG/KG (CYC AND VCR) WILL BE PRESCRIBED AT 2/3 OF THE ORIGINAL DOSE (SEE SECTION 3.1.4, PAGE 45). FOR INFANTS WITH A WEIGHT < OR EQUAL TO 5 KG, A FURTHER 1/3 REDUCTION IS ADVISED.

The following guidelines are aimed at enabling the prescription of the chemotherapy to be easier. Infusion rates are slightly higher than required to infuse the chemotherapy over the stated time due to the fact that there will invariably be interruptions in the infusion.

**Course CAV**

**Start on approximately* days 0, 21 and 63**

<table>
<thead>
<tr>
<th>DAY</th>
<th>0</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYCLOPHOSPHAMIDE</td>
<td>6h ©</td>
<td>6h ©</td>
<td></td>
</tr>
<tr>
<td>DOXORUBICIN</td>
<td>72h ©</td>
<td>©©</td>
<td>©©</td>
</tr>
<tr>
<td>VINCristine</td>
<td>72h ©</td>
<td>©©</td>
<td>©©</td>
</tr>
</tbody>
</table>

* Cycles of chemotherapy are started on count recovery (ANC ≥ 500/uL and platelets ≥ 75,000/uL). It is expected that courses will be delivered approximately every three weeks.
DRUG      Time      Dose      Administration

Hydration  0hrs      300 mL/m²/hr  i.v. infusion over 2 hours (day 0 only) using D5/1/2 normal saline with:
           (day 0 only)            potassium chloride 30 mEq/L
           2 hours                magnesium sulphate 500 mg/L
           normal saline          calcium gluconate 250 mg/L

Mesna Bolus  0hrs      20 mg/kg    i.v. bolus (day 0 only)

Cyclo-       1 hrs      70 mg/kg    i.v. infusion over 6 hours in 250mL of phosphamide
phosphamide  (days 0-1) normal saline (days 0-1)

Hydration  1hrs      2,500 mL/m²/d  i.v. infusion over 24 hours (days 0-1)
           using D5 2/3 normal saline (1/3) with:
           potassium chloride 2 g/L
           magnesium sulphate 2 g/L
           calcium gluconate 2 g/L

Mesna       1 hrs      70 mg/kg    i.v. infusion over 24 hours (days 0-1)

Doxorubicin 1hrs      25 mg/m²    i.v. infusion over 24 hours in 100 mL of normal saline (days 0-2)

Vincristine 1 hrs      0.022 mg/kg  i.v. infusion over 24 hours in 100 mL of
(maximum dose 2.0 mg/course) normal saline (days 0-2)

In infants and children with a body weight below 12kg the following dose calculation is
recommended: CYCLOPHOSPHAMIDE 46.7 mg/kg/d, DOXORUBICIN 0.83 mg/kg/d, VINCRISTINE
0.015 mg/kg/d. For infants with a weight ≤ 5 kg, a further 1/3 reduction is advised.

NOTE:

- During hydration a careful record of fluid input and output should be kept to prevent fluid
  overload and ensure an adequate diuresis. If diuresis falls to < 400 ml/m²/6 hours,
  furosemide 0.5 – 1.0 mg/kg should be given.
- To avoid fluid overload the total intake of fluid should be no more than 4.5 L/m²/d.

The use of G-CSF 5µg/kg/day s.c. during modified N7 induction is recommended to start 72 hours
after the end of chemotherapy and until the ANC is > 1.0 International Units. There should be an
interval of at least 48 hours between the last G-CSF injection and the start of the next course of
chemotherapy.

**COURSE P/E**

**START ON APPROXIMATELY* DAYS 42 AND 84**

<table>
<thead>
<tr>
<th>DAY</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td>1h ©</td>
<td>1h ©</td>
<td>1h ©</td>
<td>1h ©</td>
</tr>
<tr>
<td>Etoposide</td>
<td>2h ©</td>
<td>2h ©</td>
<td>2h ©</td>
<td></td>
</tr>
</tbody>
</table>

* Cycles of chemotherapy are started on count recovery (ANC ≥ 500/uL and platelets ≥ 75,000/uL). It is expected that courses will be delivered approximately every three weeks.
<table>
<thead>
<tr>
<th>DRUG</th>
<th>Time</th>
<th>Dose</th>
<th>Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydration</td>
<td>0hrs</td>
<td><strong>300 mL/m²/hr</strong></td>
<td>i.v. infusion over 2 hours (day 0 only) using D5/1/2 normal saline with: potassium chloride 30 mEq/L magnesium sulphate 500 mg/L calcium gluconate 250 mg/L</td>
</tr>
<tr>
<td><strong>Mannitol 20%</strong></td>
<td>0hrs</td>
<td></td>
<td>short infusion in a dose of 40ml/m² (days 0-3)</td>
</tr>
<tr>
<td><strong>Cisplatin</strong></td>
<td>2 hrs</td>
<td><strong>50 mg/m²</strong></td>
<td>i.v. infusion over 1 hour (days 0-3) 1-50mg cisplatin in 100mL of 0.9% sodium chloride 50-100mg cisplatin in 100mL of 0.9% sodium chloride</td>
</tr>
<tr>
<td><strong>Hydration</strong></td>
<td>2hrs</td>
<td><strong>125 mL/m²/hr</strong></td>
<td>i.v. infusion over 24 hours (days 0-3) using D5/1/2 normal saline with: potassium chloride 30 mEq/L magnesium sulphate 500 mg/L calcium gluconate 250 mg/L</td>
</tr>
<tr>
<td><strong>Mannitol 20%</strong></td>
<td>2.5hrs</td>
<td></td>
<td>short infusion in a dose of 40ml/m² (days 0-3)</td>
</tr>
<tr>
<td><strong>Etoposide</strong></td>
<td>4hrs</td>
<td><strong>200 mg/m²</strong></td>
<td>i.v. infusion over 2 hours (days 0-2)</td>
</tr>
</tbody>
</table>

In infants and children with a body weight below 12kg the following dose calculation is recommended: **Cisplatin 1.7 mg/kg/d, Etoposide 6.7 mg/kg/d.** For infants with a weight ≤ 5 kg, a further 1/3 reduction is advised.

**NOTE:**
- During hydration a careful record of fluid input and output should be kept to prevent fluid overload and ensure an adequate diuresis.
- If diuresis falls below 400 ml/m²/6 hours give a short infusion of Mannitol 20% in a dose of 40ml/m². Repeat whenever indicated. Furosemide should be avoided, because of the increased risk of ototoxicity.
- To avoid fluid overload the total intake of fluid should be no more than 4.5 L/m²/d.

The use of G-CSF 5µg/kg/day s.c. during modified N7 induction is recommended to start 72 hours after the end of chemotherapy and until the ANC is > 1.0 International Units. There should be an interval of at least 48 hours between the last G-CSF injection and the start of the next course of chemotherapy.
11.3 Topotecan, Vincristine and Doxorubicin (TVD) Strategy

All drugs given during TVD are non-investigational medicinal products (IMPs).

Patients not achieving a complete metastatic response following induction (rapid COJEC or modified N7) should receive two cycles of TVD. This treatment option also includes the infant population with insufficient response or early progression during induction. Patients receiving two cycles of TVD will still be eligible for MAT and PBSCR. Full tumour re-evaluation must be performed after two cycles of TVD to evaluate whether or not the patient has achieved a sufficient metastatic response (negative bone marrow evaluation, \( \leq 3 \) MIBG bone spots) to re-enter the HR-NBL-1.5/SIOPEN study. Randomisation in R2 is allowed providing that PBSCR is within 9 months of the first induction chemotherapy following diagnosis (see section 5.5, page 78). Patients with an inadequate response after two cycles of TVD may be eligible for an open phase I or II study. If in doubt, check with your National Coordinator for treatment recommendations.

11.3.1 CHEMOTHERAPY REGIMEN DETAILS OF TVD

**Topotecan**
To be administered i.v. in the morning, as a 30 minute infusion in saline 100 ml/m² at a dose of 1.5 mg/m²/day for 5 consecutive days (days 1 to 5).

**Vincristine**
To be administered as a 48-hour continuous infusion at a dose of 1 mg/m²/day in 50 ml/m²/day 0.9% saline (maximum dose 1mg/day), starting one hour after the final topotecan infusion (days 5 and 6).

**Doxorubicin**
To be administered simultaneously with vincristine as a 48-hour continuous infusion at a dose of 22.5 mg/m²/day in 50 ml/m²/day of 0.9% saline solution (days 5 and 6).

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

G-CSF, 5 \( \mu \)g/kg/day s.c, should be started 72 hours after conclusion of vincristine and doxorubicin infusion and continued until neutrophil recovery (ANC > 1.0 International Units). A second cycle will be administered at the same dose 21-28 days from the start of the first cycle, provided that:

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

Also, the patient should have been off G-CSF for at least 48 hours. A limited disease evaluation after the first cycle of TVD should include ultrasound of primary tumour, 24hr urinary catecholamine estimation, and plasma LDH. Full tumour re-evaluation must be performed after two cycles of TVD to evaluate if the patient has achieved sufficient response to be eligible for MAT.

NOTE: Infants and children with a body weight below 12kg should be dosed according to their weight in kg instead of their body surface area (m²) according to the known formula of 30 kg = 1m².

- **Topotecan** 0.05 mg/kg/day
- **Vincristine** 0.033 mg/kg/day
- **Doxorubicin** 0.75 mg/kg/day

In infants weighing \( \leq 5 \) kg, a further 1/3 dose reduction is advised.
11.4 Check List prior to MAT

Investigations necessary prior to MAT are outlined in Section 6.3.

- Localised patients, without evidence of disease progression, must start BuMel no later than day 150 after the start of Rapid COJEC induction.
- Patients in metastatic CR following front-line induction (Rapid COJEC or modified N7) must start BuMel MAT no later than day 150 after the start of induction.
- Patients receiving two courses of TVD to achieve an adequate metastatic response (metastatic CR or PR) must start BuMel MAT no later than day 210 after the start of induction.

CR or PR at metastatic sites:
- at least 50% reduction in skeletal mIBG positivity and not more than 3 positive, but improved spots on mIBG
- cytomorphological CR in 2 BM aspirates and no positive BM biopsy

- Complete restaging of disease as close as possible before the start of BuMel MAT must take place following front-line induction (Rapid COJEC or modified N7) or two courses of TVD.

- Organ functions (liver, kidney, heart, lungs) fulfilling criteria prior to MAT:
  - ALT, bilirubin $\leq 2 \times$ normal (see SmPC)
  - Creatinine clearance and/or GFR $\geq 60$ ml/min/1.73m² and serum creatinine $< 1.5$mg/dl. Call study co-ordinator for MAT dose modifications if GFR $< 60$ml/min/1.73m² and serum creatinine $\geq 1.5$mg/dl.
  - Shortening fraction $\geq 28\%$, or ejection fraction $\geq 55\%$, no clinical congestive heart failure.
  - Normal chest X-ray and oxygen saturation.

- Sufficient stem cells available. Minimum required: $3 \times 10^6$ CD34 cells /kg body weight, if a BM harvest was unavoidable at least $3 \times 10^8$ MNC /kg body weight.

- Females of childbearing potential must have a negative pregnancy test. Patients of childbearing potential must agree to use an effective birth control method. Female patients who are lactating must agree to stop breast-feeding.

Any negative answer will render the patient ineligible for BuMel MAT on-study.

Note:
- The result of surgery will not influence eligibility for BuMel MAT.
- With a GFR rate $\geq$ CTC- Grade 2 the patient is ineligible for BuMel MAT on-study. Since recovery of GFR rate is observed in particular post surgery, at least two GFR rates need to be evaluated post surgery if the first postsurgical one shows a GFR grade 2 to 4 prior to day 150 (with at least a two weeks interval but ideally 4 weeks if feasible) or prior to day 210 respectively.

Thoracic primaries

The point as to whether children with thoracic primaries may be eligible for BuMel MAT has been discussed in various SIOPEN Study Committee meetings: Following these debates it was confirmed that these patients would be eligible for BuMel MAT. However, caution is recommended when considering radiation therapy in patients presenting at diagnosis with a large abdominal or large pulmonary primary. Patients fitting this criteria should be discussed with the current Radiotherapy Panel (see page 121 for a list of members).
INELIGIBILITY FOR MAT
Patients with neoplastic or non-neoplastic disease of any major organ system that would compromise their ability to withstand the pre-transplant conditioning regimen. This will include:
- Patients with a response less than metastatic PR following induction and TVD therapy. These patients may enter on to available Phase II studies.
- Patients who are pregnant or lactating.
- HIV seropositive patients.
- Patients aged 12-18 months at diagnosis, with stage 4 neuroblastoma, no MYCN amplification and without segmental chromosomal alterations (SCA) are not eligible for MAT. These patients are thought to have a good prognosis and will stop treatment after induction therapy and surgery to the primary tumour.

11.5 Monitoring for Ototoxicity
In children over the age of 3.5 years pure-tone audiometry should be carried out as near to the beginning of treatment as possible, preferably before starting cisplatin. This should be classified according to the Brock criteria [153].
In children under the age of three an experienced audiologist should be able to get good results using distraction techniques if the child is not in pain. The results from distraction techniques may be graded according to the Brock criteria.
Table: Brock Grading System for Cisplatin-Induced Bilateral High-Frequency Hearing Loss[153]

<table>
<thead>
<tr>
<th>BILATERAL HEARING LOSS ¹</th>
<th>GRADE</th>
<th>DESIGNATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 40 dB at all frequencies²</td>
<td>0</td>
<td>Minimal</td>
</tr>
<tr>
<td>&gt; 40 dB at 8,000 Hz only²</td>
<td>1</td>
<td>Mild</td>
</tr>
<tr>
<td>&gt; 40 dB at 4,000 Hz and above²</td>
<td>2</td>
<td>Moderate</td>
</tr>
<tr>
<td>&gt; 40 dB at 2,000 Hz and above²</td>
<td>3</td>
<td>Marked</td>
</tr>
<tr>
<td>&gt; 40 dB at 1,000 Hz and above²</td>
<td>4</td>
<td>Severe</td>
</tr>
</tbody>
</table>

Notes
1 The results are obtained by pure-tone audiometry, from the "better" ear.
2 < 40 dB at lower frequencies

11.5.1 Audiometry

11.5.1.1 General notes
It is accepted that this trial, presents a challenge to oncology departments to liaise with their respective audiology departments in different countries. Some units will be better served than others. It might mean that the child needs to move to another centre to be tested and this should be made clear to the family at the start of treatment.

Baseline hearing evaluations should ideally be completed before the first dose of cisplatin whenever possible. Definitive audiology evaluation should be completed for all children from the age of 3.5 years onwards (or as soon as a reliable hearing test can be obtained).

ASHA criteria and Brock grading are available in Appendix 21.
11.5.1.2 Audiologic evaluations will include:

- measurement of bilateral pure tone air conduction thresholds at 8, 6, 4, 2, 1 and 0.5 kHz starting with the high frequencies
- otoscopy
- immittance evaluation or tympanometry
- measurement of transient evoked otoacoustic emissions (TEOAE’s) and distortion product otoacoustic emissions (DPOAE’s), at facilities where this equipment is available.

If a child is unco-operative or too unwell to give reliable responses on behavioural assessment and has middle ear effusion and hence TEOAE and DPOAE cannot be done then we strongly recommend obtaining thresholds using bone conduction ABR. Ideally using tone burst but click evoked ABR would also be helpful. See Appendix 21.

Procedures for obtaining pure tone thresholds should include standard behavioural measurement techniques as appropriate for the age and development of the child, including conditioned play audiometry and visual reinforcement audiometry. For the purposes of this study, a descending testing procedure, starting at 8000 Hz, is recommended for all children and is especially important to be used in children with limited cooperation, as any decrease in auditory sensitivity will most likely occur at the higher frequencies. Obtaining 0.5 kHz is recommended as tester can reassess reliability of responses. Insert earphones or headphones should be used to obtain ear-specific thresholds whenever possible. Bone conduction thresholds should be measured if baseline testing indicates pre-existing hearing loss, if a child has a significant decrease in hearing at the definitive evaluation, and/or if immittance results indicate conductive middle ear pathology. When behavioural test results cannot be reliably obtained, auditory thresholds should be estimated using an electrophysiologic test procedure, specifically click or tone burst evoked auditory brainstem response (ABR).

Immittance evaluation should include the measurement of middle ear pressure and compliance and acoustic reflex thresholds. Probe tones equal to or greater than 660 Hz instead of 226 Hz in babies. Tympanograms should be classified as normal if the static admittance is 0.2 mmho or greater, the peak pressure is between –150 to +200 daPa, and the tympanometric width is less than 160 daPa. Acoustic reflex thresholds should be measured at 500, 1000, 2000 and 4000 Hz ipsilaterally in both ears.

TEOAEs and DPOAEs should be collected at facilities where this equipment is available. If a child exhibits evidence of active middle ear disease, as evidenced by abnormal tympanometry and/or conductive hearing loss, OAE measurement should be deferred until the middle ear pathology resolves. For TEOAEs click stimuli should be presented at 80 dB peak equivalent SPL. The two TEOAE parameters that should be used to compare results will be total emissions level (mean response) and the reproducibility of the waveforms in the frequency region 1000–4000 Hz. For DPOAEs to be added.

11.5.1.3 The following test protocol is recommended:

Evaluation for children younger than 12 months of age will include:

- measurement of minimal thresholds by visual reinforcement audiometry (VRA) preferably with insert ear phone, if the child does not accept that, then on soundfield setting with dBA weighting which should then be converted to equivalent of hearing level (HL) by the central audiology committee through the RDE web-site to equate to Pure Tone's (Appendix 21).
- tympanometry
- measurement of TEOAE’s
- measurement of DPOAE’s

Click or tone burst evoked ABR’s should be used to estimate auditory thresholds if possible. If ABR is not available, acoustic reflex thresholds should be measured.

**Children aged 12-42 months will have:**

- Evaluation by VRA or conditioned play audiometry, if thresholds are estimated on soundfield setting in dBA weighting then must be converted to HL.
- tympanometry
- TEOAE’s
- DPOAE’s

If reliable results cannot be obtained by behavioural testing, then ABR or acoustic reflex threshold measurement is recommended.

**Children 3.5 years and older will have**

- evaluation using play audiometry as described above or standard pure tone threshold
- tympanometry
- TEOAE’s
- DPOAE’s

11.5.1.4 Guidelines for visual reinforcement audiometry (VRA):

Visual reinforcement audiometry is the standard accepted method for obtaining frequency and ear specific hearing thresholds in children between the ages of 6 months through 30 months. Animated and/or lighted toys are used to condition and reinforce a head-turning response to sound. Insert earphones should be used whenever possible.

If a child will not tolerate wearing earphones, testing may be completed using warbled pure tone presented through calibrated soundfield speakers. These thresholds measured as dBA should be converted to dB HL for purpose of this study, table in Appendix 21.

11.5.1.5 Guidelines for auditory brainstem response (ABR):

Testing for air conduction (AC) threshold estimation is difficult in very young children who will not cooperate for behavioural assessment or for OAE’s. Measurement of auditory evoked brainstem potentials is an electrophysiologic procedure, which allows for evaluation of peripheral auditory function and threshold determination in subjects who are not able to participate in behavioral testing due to cooperation or state of health. Although ABR does not measure hearing sensitivity, ABR thresholds are strongly correlated with thresholds of hearing sensitivity and allow for an estimation of auditory thresholds.

Frequency specific tone burst stimuli should be used to measure ABR thresholds whenever possible for 500, 1000, 2000, and 4000 Hz stimuli. Thresholds for click stimuli should be obtained if evaluation with frequency specific stimuli is not available. ABR thresholds should be determined as the lowest level at which detectable, repeatable wave V responses are obtained. Responses should be labeled only when replicable and compared with normative pediatric data.

Frequency specific stimulus, i.e. tone burst low, mid and high frequency or click evoked ABR, is delivered via earphones. If the AC thresholds are raised, i.e greater than 20 dB nHL or where due to time constraints it is not possible to do full audiological evaluation, then bone conduction (BC)
testing should be carried out to determine the thresholds of the better hearing ear. Thresholds should be determined using 10 dB steps down to 20 dB nHL. All threshold obtained in dB nHL should be converted to HL using the conversion table.

11.5.1.6 Guidelines for otoacoustic emissions (OAE)

Otoacoustic emissions were first reported by Kemp in 1978 [154]. These are sounds that are produced by healthy ears in response to acoustic stimulation. They are products of the activity of the outer hair cells in the cochlea.

OAEs are measured by presenting a series of very brief acoustic stimuli, clicks, to the ear through a probe that is inserted in the outer third of the ear canal. The probe contains a loudspeaker that generates clicks and a microphone that measures the resulting OAEs that are produced in the cochlea and are then reflected back through the middle ear into the outer ear canal. The resulting sound that is picked up by the microphone is digitised and processed by specially designed hardware and software. The very low-level OAEs are separated by the software from both the background noise and from the contamination of the evoking clicks.

Three types of OAE measurements have received concentrated attention; spontaneous, transiently evoked, and distortion product. OAE assessment is specifically sensitive to the status of outer hair cells in the cochlea and is a relatively efficient objective test. It has been used to assess cochlear function in patients receiving Cisplatin with promising results [155, 156]. Although OAE testing presents a new and exciting tool for cochleotoxicity monitoring, its application has not been evaluated sufficiently to enable formulation of specific guidelines.

We suggest the following as a minimum:

TEOAE: One level (e.g. 80 dB pSPL) click stimulus should be completed. Normal distributions for this condition for normal hearing are documented in the literature [157, 158].

DPOAE: One level of L1 and L2 65/55 dB SPL at least at four frequencies. Normal distributions for this condition for normal hearing are documented in the literature [157].

More detailed testing would include:

TEOAE: Two levels (e.g. 80 dB pSPL and a lower level) may be completed and/or one level using click and multiple frequencies for stimuli, or

DPOAE: Use of three levels (e.g. 65/55 and lower levels, as shown by Kummer, Janssen & Arnold 1998; Kummer, Janssen, Hulin & Arnold 2000) should be completed to obtain DPOAE input-output functions, or at one level for multiple (more than four) frequencies, or

Comparison of TEOAE (e.g. single level, single stimulus) and DPOAE (e.g. single level): The TEOAE is a better predictor of low frequency and DPOAE of high frequency sensitivity [159, 160].

11.5.1.7 Monitoring during treatment

After the initial baseline evaluation, interim audiometry is recommended after every second course of cisplatin. In children younger than 3.5 years of age interim audiometry is strongly recommended.
11.5.1.8 Definitive evaluation at end of treatment and age 3.5 years
All children should have a definitive evaluation when they have completed treatment and are aged 3.5 years or older. If the child is old enough the evaluation should be done within 6-12 weeks after the last cisplatin dose. Ototoxicity should be assessed using both Brock’s grading and ASHA guidelines wherever pre-chemotherapy hearing thresholds are available. If the children have hearing loss equal to or greater than Brock’s grade 1 on the definitive audiologic evaluation that should be noted as Ototoxicity.
Ototoxicity will be defined (according to ASHA) as hearing loss
- 20 dB HL decrease at any one test frequency or
- 10 dB decrease at any two adjacent frequencies or
- loss of response at three consecutive test frequencies where responses were previously obtained (this refers to high frequencies) as described by ASHA criteria.

In cases of asymmetric hearing loss, results should be reported for the ear with better hearing, as this is the primary determinant of overall auditory performance.

11.6 Monitoring of Renal Function

11.6.1 EVALUATION OF RENAL FUNCTION
Renal function should be evaluated at the time of diagnosis and prior to MAT. It should be assessed by the glomerular filtration rate and serum magnesium. The GFR should be measured by a radio-isotope method preferably with Cr51 EDTA otherwise DPTA clearance. The GFR needs to be adjusted for surface area giving a result in ml/min/1.73m². The same method should be used in the same child throughout the treatment.

11.7 BUMEL MAT Regimen
The current protocol amendment highly recommends the use of intravenous busulfan. Since the use of oral busulfan is related to a very large therapeutic window, with an important percentage of patients outside the target AUC of 900 – 1400 units (personal communication G. Vassal, April 2011) and a significantly higher VOD rate of up to 30% [5], its use is discouraged. The prophylactic use of defibrotide is not encouraged, because the rate of VOD grade 3 (severe) is documented at 3% within this trial while the complete rate of VOD (grade 1 and 2 included) is 18%. However, its therapeutic use is recommended (see section 16.9.3, page 147).

The BUMEL MAT regimen consists of intravenous administration of busulfan (Busilvex®) as a two-hour infusion every 6 hours over 4 (or 5) consecutive days through a central venous catheter only (dosage according to BW as outlined in the table below) and the short I.V. infusion of melphalan (140mg/m²). The SmPC recommends at least a day of rest (24h) between the last dose of Busilvex® and Melphalan administration.
In case i.v. busulfan (Busilvex®) is not available, the use of oral busulfan is permitted, although not recommended. Oral busulfan is given at 37.5 mg/m²/dose every 6 hours for a total of 16 doses over 4 consecutive days (total 600 mg/m²).

Even patients presenting at diagnosis with a large abdominal or large pulmonary primary should receive BuMel MAT. However, the radiation field and dose should be discussed with the current Radiotherapy Panel (see page 121 for a list of members).

In case of body weight below 12kg the melphalan dose calculation is recommended to be dosed by kg/BW, i.e. 4mg/kg. For Infants with a weight ≤ 5 kg, a further 1/3 reduction is advised.
Oral Busulfan - Dose adjustment if patient less than 12kg to 480mg/m² instead of 600mg/m² total dose, i.e. 4 x 120 mg/m²/day (30 mg/m²/dose).

<table>
<thead>
<tr>
<th>DRUG</th>
<th>DOSE</th>
<th>DAY</th>
<th>-7</th>
<th>-6</th>
<th>-5</th>
<th>-4</th>
<th>-3</th>
<th>-2</th>
<th>-1</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUSILVEX®</td>
<td>&lt; 9 kg: 1.0mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9 kg to &lt; 16 kg: 1.2 mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16 kg to 23 kg: 1.1 mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;23 kg to 34 kg: 0.95 mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;34 kg: 0.8 mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MELPHALAN</td>
<td>140 mg/m² I.V. short infusion (15’) not before 24h after last Busilvex® dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydration</td>
<td>3/l/m²/day = 125 ml/m²/hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clonazepam</td>
<td>0.025 – 0.1 mg/kg/day total dose i.v. as ctn infusion or divided in 3 doses p.o./day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral Stem Cells</td>
<td>Minimum: 3x 10⁶ /kg/CD34 + i.v.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

11.7.1 I.V. BUSULFAN (BUSILVEX®)

Concentrate (6 mg/ml) for solution for infusion; after dilution 1 ml of solution contains 0.5 mg of busulfan. For details of Busilvex® Appendix “Drug Information”, 23.9, page 201).

Give every 6h starting for a total of 16 doses. The dosage follows the guidelines below:

<table>
<thead>
<tr>
<th>Actual body weight (kg)</th>
<th>Busilvex® dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;9</td>
<td>1.0</td>
</tr>
<tr>
<td>9 to &lt; 16</td>
<td>1.2</td>
</tr>
<tr>
<td>16 to 23</td>
<td>1.1</td>
</tr>
<tr>
<td>&gt; 23 to 34</td>
<td>0.95</td>
</tr>
<tr>
<td>&gt; 34</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Preparation

(Busilvex® for intravenous administration, ampoules of 10 ml, 60mg per ampoule, Pierre Fabre Médicament) Busilvex® must be diluted prior to administration (see Appendix “Drug Information”, 23.9, page 201). A final concentration of approximately 0.5 mg/ml busulfan should be achieved. Busilvex® should be administered by intravenous infusion via central venous catheter. Busilvex® should not be given by rapid intravenous, bolus or peripheral injection. 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. However, caution is recommended (see Appendix “Drug Information”, 23.9, page 201).

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 “Drug Information”, 23.9, page 201)

Drug delivery
Busilvex® is commercially available.

Oral Busulfan
Tablets at 2mg
The child should fast 2 hours before and 30 minutes after administration.

11.7.2 MELPHALAN

TOTAL DOSE = 140 mg/m²/day and at least 24 h after last busulfan dose. There will be no melphalan dose adjustment based on GFR in BUMEL-MAT.

Preparation
(ALKERAN® for intravenous administration, 50 mg vials, Welcome)
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 5mg in 1 ml anhydrous Melphalan.

Administration
Either give undiluted or further dilute 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
Start hydration at a rate of 125ml/m²/hr three hours prior to melphalan administration. Continue until at least 12 hours 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/hour pre-melphalan and for two hours post-melphalan administration. Give increased fluids and/or furosemide to achieve this urine output.

STEM CELLS: should not be re-infused until at least 12 hours after the end of the melphalan infusion.

Remember:
The prospective PK/PD evaluation of both busulfan and melphalan should be continued within the HRNBL protocol, in order to better understand pharmacological determinant of HVOD occurring after BUMEL regimen. (for sampling guidelines see Appendix- Pharmacology.)
Ancillary Treatments

- During busulfan treatment no anti-emetics are indicated according to the French experience when the individual dose is administered in capsule form (has to be provided if possible by the local pharmacy). 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. G-CSF will continue until a stable increase of WBC > 5 x 10⁹/l or ANC >0.5 x 10⁹/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 day 0 until at least day +10 or until WBC ≥ 1.0 x 10⁹/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.

11.7.3 Drug Sampling

Since the targeted AUC has never been correlated to outcome data in a large, retrospective trial, sampling for Busilvex® PK data is still highly relevant and encouraged. The sampling details are outlined in chapter 25.1. Equally, the sampling of melphalan PK data is of interest as drug interactions may trigger VOD.

The sampling sheets can be found in Appendix 25 (page 231).

11.8 Stem Cell Reinfusion

11.8.1 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, premedicate with acetaminophen (10mg/kg p.o.) and diphenhydramine (1 mg/kg i.v.).

- Ambubag, diphenhydramine and epinephrine 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.

11.8.2 Dosage/Timing

Peripheral Stem Cells

A minimum of 3 x 10⁶ CD34 cells/kg (optimum 5 x 10⁶ /kg) must be available. A maximum of 10 x 10⁶ CD 34 cells/kg should be used.
Or

**Bone Marrow**

A minimum of 3 x 10^8 mononuclear bone marrow cells/kg or > 8 x 10^4 CFU-GM/kg should be used.

**Reinfusion of Stem Cells**

Stem cells will be infused intravenously on Day 0 with specified rest, according to the MAT regimen, following completion of chemotherapy, within 1½ hours of thawing.

## 12 Radiotherapy

### 12.1 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 MAT. 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 (see page 121 for a list of members). 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.

### 12.2 Timing of Radiotherapy

Radiotherapy will be given after MAT/PSCR and prior to the start of isotretinoin (13-cis-RA) treatment. After BUMEL MAT the interval must be greater than 60 days after stem cell transplantation, due to the risk of busulfan-enhanced radiotoxicity. A negative interaction between radiotherapy and isotretinoin (13-cis-RA) has been described (Philadelphia 1996), so these should not be used together.

### 12.3 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 preoperative 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 vertebrae. 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 beam 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.

### 12.4 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 $V_{12}$ of 50 % of total lung volume and a $V_{15}$ 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.
12.5 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 in Vienna 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
Prof. Karin Dieckmann  E-mail: karin.dieckmann@meduniwien.ac.at
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 in Vienna 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. The Sponsor’s address for sending the above information is:

Department Studies and Statistics (S²IRP)
HR-NBL-1.5/SIOPEN Radiotherapy Review
St. Anna Kinderkrebsforschung
Zimmermannplatz 10
A-1090 Wien
Austria

13 Isotretinoin (13-cis-RA) Therapy for patients not randomised in R2

13.1 Treatment Schedule for isotretinoin (13-cis-RA)

Isotretinoin (13-cis-RA) is a non-investigational medicinal product (IMP).

Aim to begin at day 90, but not later than day 120 after MAT/PBSCR and local radiation, if the ANC > 0.5 International Units, liver function < CTC grade 2 toxicity, and renal function, calcium,
uric acid and triglycerides ≤ 2 x normal values. Common toxicity criteria (CTC) can be found in the Appendix on toxicity (chapter 28).

Patients will receive **isotretinoin (13-cis-RA)** 160 mg/m²/day divided into two equal doses given orally twice a day for 14 days, followed by a 14 day rest for a total of six cycles (six **months**). Patients ≤ 12 kg will not be given a dose reduction; the rationale for this is outlined in section 4.2.6.4 (page 64). Doses will need to be rounded to the nearest 10 mg. Capsules come as 10, 20, and 40 mg sizes, and can be emptied into a high fat food such as ice cream or chocolate mousse to administer. It is acknowledged in the product information sheet for Roaccutane® that peanut protein is used in the production of this medication. To date, there has been no significant reaction to Roaccutane® in children treated for high risk neuroblastoma. It is, however, advisable to watch carefully any child with known peanut allergy whilst on this treatment, and to be certain that an epipen is available close to the child for immediate use should it be required.

<table>
<thead>
<tr>
<th>Treatment Schedule for isotretinoin (13-cis-RA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1</td>
</tr>
<tr>
<td>rest</td>
</tr>
</tbody>
</table>

| W13 | W14 | W15 | W16 | W17 | W18 | W19 | W20 | W21 | W22 |
| RA | RA | RA | RA | RA | RA |

W: weeks related to start of isotretinoin (13-cis-RA) treatment

**13.2 Suggested Supportive Care for isotretinoin (13-cis-RA)**

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

**13.3 Criteria prior to each Cycle of isotretinoin (13-cis-RA)**

- Total bilirubin ≤ 1.5 x normal, and (SGPT) ALT ≤ 5 x normal. Veno-occlusive disease if present, should be stable or improving.
- Skin toxicity no greater than grade 1
- Serum triglycerides < 500 mg/dL
- No haematuria and/or proteinuria on urinalysis
- Serum calcium < 11.6 mg/dL
- Serum creatinine based on age/gender as follows:

<table>
<thead>
<tr>
<th>Age</th>
<th>Maximum Creatinine (mg/dL)</th>
<th>Serum Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>1 month to &lt; 6 months</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>6 months to &lt; 1 year</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>1 to &lt; 2 years</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>2 to &lt; 6 years</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>6 to &lt; 10 years</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>10 to &lt; 13 years</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>13 to &lt; 16 years</td>
<td>1.5</td>
<td>1.4</td>
</tr>
<tr>
<td>≥ 16 years</td>
<td>1.7</td>
<td>1.4</td>
</tr>
</tbody>
</table>
• Patients with seizure disorder must be well controlled and taking anticonvulsants. CNS toxicity < grade 2.

13.4 Dose Modifications for isotretinoin (13-cis-RA)

a. A dose reduction of 25% (to 120 mg/m²/day) for subsequent cycles should be made for the occurrence of any grade 3 or 4 CTC toxicity. EXCLUDING: grade 3 or 4 haematologic, grade 3 hepatic, grade 3 nausea, grade 3 vomiting, or grade 3 fever. If the same grade 3 or 4 toxicity recurs after a 25% dose reduction, then decrease the dose by another 20% (to 100 mg/m²/day). If the same grade 3 or 4 toxicity recurs after two dose reductions, then discuss with study co-ordinator before continuing further therapy.

b. It has been reported (rarely) that some patients treated with isotretinoin (13-cis-RA) develop new areas of abnormal uptake on bone scan. This is likely to be due to increased bone resorption. If such changes occur during the isotretinoin (13-cis-RA) phase in the absence of any other evidence of tumour recurrence, discuss with study co-ordinator before reporting as disease progression.

c. If the criteria to begin the next cycle are not met by the date the cycle is due to begin, delay the cycle for one week. If the criteria are still not met, treat at 25% dose reduction (120 mg/m²/day). An additional dose reduction to 100 mg/m²/day should occur if criteria are not met within one week after due date for subsequent cycles.

d. If serum creatinine increases by > 50% in any cycle, measured GFR should be carried out prior to commencing the next cycle. If GFR is < 50 ml/min/1.73 m², then call the study co-ordinator for dose adjustment.

e. If patient develops haematuria, proteinuria, and/or hypertension during any cycle of therapy, withhold medication and contact study co-ordinator.

f. For localised cheilitis, apply topical vitamin E to lips for subsequent cycles. If this does not control symptoms sufficiently to allow sufficient oral intake, then decrease dose by 25% to 120 mg/m²/day.

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

Limited PK sampling study of patients under isotretinoin (13-cis-RA) treatment on the high-risk study:
This would also help to clarify future recommendation about the dose to be given depending if capsules are swallowed with milk or if capsules are snipped / mixed with ice-cream. First observation suggests that children receiving snipped capsules may be underdosed. Currently there is no adopted dosing recommendation given, but centres are highly encouraged to participate in the PK studies to help to clarify this issue soon (details outlined in the Appendix section 25.3 – Pharmacology, page 233 ).
14 Immunotherapy with Ch14.18/CHO with or without aldesleukin (IL-2) for patients randomised in R2

14.1 Indication

Both ch14.18/CHO and aldesleukin (IL-2) are investigational medicinal products (IMPs). Aldesleukin (IL-2) is commercially available and will not be supplied by the Sponsor. Ch14.18/CHO is supplied by the Sponsor and in order to achieve timely delivery randomisation must take place at least two weeks prior to the start of immunotherapy.

The ch14.18/CHO will be given to all patients fulfilling R2 criteria and being randomised to immunotherapy. Prior to the start of ch14.18/CHO treatment, patients have to be in good health without clinical or laboratory signs of infection. In case of infection postpone cycle.

Patients randomised to receive additional immunotherapy will be given aldesleukin (IL-2) in addition to isotretinoin (13-cis-RA) and ch14.18/CHO.

14.2 Treatment Schedule for patients randomised to ch14.18/CHO alone

Patients randomised to ch14.18/CHO alone will receive **ch14.18/CHO at a dose of 20mg/m²/day over 5 days every 4 weeks for 5 courses***. The first course will start three weeks after initiation of isotretinoin (13-cis-RA) (week 4).

**Treatment schedule for isotretinoin (13-cis-RA) and ch14.18/CHO alone**

<table>
<thead>
<tr>
<th>W1</th>
<th>W2</th>
<th>W3</th>
<th>W4</th>
<th>W5</th>
<th>W6</th>
<th>W7</th>
<th>W8</th>
<th>W9</th>
<th>W10</th>
<th>W11</th>
<th>W12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>rest</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>rest</td>
</tr>
</tbody>
</table>

**RA** | **GD2** | **RA** | **RA** | **GD2** | **RA** | **GD2**

**RA** | **RA**

<table>
<thead>
<tr>
<th>W13</th>
<th>W14</th>
<th>W15</th>
<th>W16</th>
<th>W17</th>
<th>W18</th>
<th>W19</th>
<th>W20</th>
<th>W21</th>
<th>W22</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>rest</td>
<td></td>
</tr>
</tbody>
</table>

| RA   | RA   | GD2  | RA   | RA   | GD2  | RA   | RA   |

W: weeks related to start of isotretinoin (13-cis-RA) treatment

GD2: ch14.18./CHO

*Infants and children with a body weight below 12kg should be dosed according to their weight in kg instead of their body surface area (m²) according to the known formula of 30 kg = 1 m². In infants weighing ≤ to 5 kg, a further 1/3 dose reduction is advised. Ch14.18/CHO will be dosed at 0.67mg/kg/day.

As mentioned in section 3.7, patients should not receive a dose reduction for isotretinoin (13-cis-RA).

The guidelines for pain must be strictly followed (see section 14.6, page 126).

14.3 Mode of Action of ch14.18/CHO

After binding to neuroblastoma cells, ch14.18/CHO induces killing of tumour cells by complement dependent (CDC) and antibody dependent cytotoxic lysis (ADCC). Background is given in detail in Section 4.2.7.
14.4 Mode of Administration of ch14.18/CHO

Ch14.18/CHO is given as an 8 hour intravenous infusion. Intensive visceral pain and pain in the extremities is expected and all patients must follow the supportive care during ch14.18/CHO treatment (see section 14.6, page 126).

- Admit to hospital on the day/or the evening before starting ch14.18/CHO infusion
- Preferably the central venous line will still be in place
- G-CSF should not be given prior to any ch14.18/CHO cycle

Premedication with paracetamol and anti-histamine medication (preferably parental) prior to each ch14.18/CHO anti-GD2 mAb infusion on day 1 through day 5 should be administered as an analgesic and allergic prophylaxis (if not clinically indicated avoid steroids). Regular antiemetics should be prescribed during the infusion.

<table>
<thead>
<tr>
<th>ch14.18/CHO</th>
<th>DOSE/CYCLE</th>
<th>DOSE/DAY</th>
<th>ADMINISTRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100mg/m²/cycle</td>
<td>20mg/m²/day*</td>
<td>as 8 hour infusion in 100ml NaCl 0.9% + 5 ml human albumin 20%</td>
</tr>
</tbody>
</table>

Hydration

With additional NaCl and KCl according to institutional guidelines

*Infants and children with a body weight below 12kg will be dosed at 0.67mg/kg/day. In infants weighing ≤ to 5 kg, a further 1/3 dose reduction is advised.

For information regarding the drug preparation see the Drug Information Appendix, section 23.13, page 221.

14.5 Toxicity of ch14.18/CHO

Close monitoring of the patient will be required during the administration of ch14.18/CHO. Each cycle requires adequate nursing support during the first 2 hours of each infusion.

14.5.1 Pain

During the infusion intensive visceral pain and pain in the extremities has to be anticipated. Therefore, the supportive care during ch14.18/CHO treatment must be carefully followed (see section 14.6, page 126). Pain symptoms are usually quickly reversible once the ch14.18/CHO infusion is stopped.

14.5.2 Anaphylactic Reactions

Allergic reactions are seen quite frequently, with associated symptoms such as tachycardia, coughing attacks, fever and elevation of CRP (reactive protein C) levels.

14.5.3 Reversible Pupil Palsy

Pupil palsy was previously observed in early dose escalation studies, but was reported to resolve within a few weeks [81].
14.6 Special Supportive Care during ch14.18/CHO Treatment

14.6.1 Mandatory Medication for Pain during ch14.18/CHO infusion

Severe visceral and neuropathic pain is an anticipated side effect of ch14.18/CHO treatment. Therefore, one of the following pain schedules must be strictly followed.

**Pain schedule A**
Precipitation with gabapentin (Neurontin®) 3 days prior to the start of ch14.18/CHO and intravenous morphine as previously established will be given.

**Premedication**
Prior to each cycle of ch14.18/CHO the patient should be primed with oral gabapentin at a dose of 10 mg/kg/dose once daily three days prior to the start of ch14.18/CHO treatment. The first dose will be given on day 19 of cycle 1 once a day, twice daily on day 20 and three times daily on day 21 and all subsequent days during the antibody administration (days 22–26). Gabapentin is available as oral solution containing 250 mg/5 mL of gabapentin or in capsules (100 mg, 300 mg, and 400 mg).

Recommended dosages:
**Gabapentin** PO (10 mg/kg/dose)

**Morphine hydrochloride**

<table>
<thead>
<tr>
<th>Bolus Rate</th>
<th>Infusion Rate during ch14.18/CHO infusion</th>
<th>Interval Infusion Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05 mg/kg/h</td>
<td>0.03 mg/kg/h</td>
<td>0.01 mg/kg/h</td>
</tr>
</tbody>
</table>

(2 hours prior to starting the ch14.18/CHO infusion)

**Morphine Infusion Schedule**

<table>
<thead>
<tr>
<th>Duration</th>
<th>mg/kg/h</th>
<th>ml/kg/h (0.25mg=1ml)</th>
<th>mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-infusion rate 2 h</td>
<td>0.05 mg/kg/h</td>
<td>0.2 ml/kg/h</td>
<td>0.1 mg/kg</td>
</tr>
<tr>
<td>Infusion rate during ch14.18/CHO infusion 8 h</td>
<td>0.03 mg/kg/h</td>
<td>0.12 ml/kg/h</td>
<td>0.24 mg/kg</td>
</tr>
<tr>
<td>Interval infusion rate 14 h (4 h)</td>
<td>0.01 mg/kg/h</td>
<td>0.04 ml/kg/h</td>
<td>0.14 (0.04) mg/kg</td>
</tr>
<tr>
<td>Total dose mg/kg/24 h</td>
<td></td>
<td></td>
<td>0.48 (0.38) mg/kg</td>
</tr>
</tbody>
</table>

The aim is to achieve absence of pain. Dosing may need to be adapted to obtain complete analgesia. The individual dose may vary widely. According to WHO recommendations the concomitant use of at least one non-opioid analgesic is encouraged.

**Further analgesics (optional)**

<table>
<thead>
<tr>
<th>Analgesic</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracetamol</td>
<td>10 – 15mg/kg x dose orally every 4 hrs or</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>5 mg/kg x dose orally every 6 – 12 hrs or</td>
</tr>
<tr>
<td>Metamizol</td>
<td>10 – 15 mg/kg x dose orally every 4 hrs</td>
</tr>
</tbody>
</table>
If a patient still experiences pain, stop the ch14.18/CHO infusion for 30 minutes, add a non-opioid analgesic and/or give a morphine bolus or increase the permanent morphine infusion rate. In the German experience morphine infusions of up to 1.2mg/kg/h over 24 hours were required, however such high doses were rarely needed in the Bridging Study (data in the IMPD) even with the use of a higher ch14.18/CHO dose.

**Pain schedule B**
Nurse controlled (NSA or patient controlled analgesia (PCA) require special “infusion pump settings” and may be followed when institutions are accordingly equipped. A number of patients on the trial have previously stopped ch14.18 treatment due to the experienced pain breakthroughs. Therefore, close surveillance of tolerance of children is needed when working with a pain triggered, titrated approach.

**Gabapentin** may be effective in alleviating pain associated with the infusion of ch14.18/CHO and have an opioid sparing effect. The maximal analgesic benefit may take several weeks to develop. Gabapentin should therefore be started in week 1. The initial dose is 10mg/kg (max 300mg) orally once a day, preferably given at night. This should be increased to twice a day on week 2 and then to three times a day on week 3. This will ensure that the patient is established on gabapentin before the first ch14.18/CHO infusion in week 4. Gradually increasing the dose over three weeks will hopefully reduce the severity of common side effects, such as somnolence and dizziness. If tolerated, a maintenance dose of 10mg/kg gabapentin (max 300mg) three times a day should be continued until the completion of the last cycle of ch14.18/CHO. Gabapentin should not be stopped in between cycles of ch14.18/CHO and must not be abruptly withdrawn on completion of the treatment period.

**Paracetamol** should be given regularly throughout each treatment cycle of ch14.18/CHO, even if there is no apparent pain. The pre-emptive use of paracetamol will have an opioid sparing effect. Intravenous paracetamol may be more efficacious than the oral preparation, so this is the preferred route of administration. Paracetamol 15mg/kg should be given intravenously or orally four times a day. The dose of intravenous paracetamol should be reduced from 15mg/kg to 7.5mg/kg in children who weigh less than 10kg.

Non-steroidal anti-inflammatory drugs are not routinely recommended.

**Morphine** is the first line opioid used to provide analgesia during the administration of ch14.18/CHO. The morphine is administered intravenously as a continuous infusion or alternatively either as nurse controlled analgesia (NCA) or patient controlled analgesia (PCA). To allow local hospitals to use their usual strengths of morphine preparation, only the recommended doses are given below.

Recommended initial programming, with typical ranges shown in brackets:

<table>
<thead>
<tr>
<th></th>
<th>NCA</th>
<th>PCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bolus</td>
<td>20mcg/kg (10-20mcg/kg)</td>
<td>20mcg/kg (10-20mcg/kg)</td>
</tr>
<tr>
<td>Background</td>
<td>20mcg/kg/h (0-20mcg/kg/h)</td>
<td>4mcg/kg/h (0-20mcg/kg/h)</td>
</tr>
<tr>
<td>Lockout</td>
<td>10 minutes (10-30 minutes)</td>
<td>5 minutes (3-10 minutes)</td>
</tr>
</tbody>
</table>

The NCA/PCA settings may require adjustment so that the dose of morphine administered is titrated to the pain experienced.
The PCA/NCA should be started 2 hours before the commencement of the first ch14.18/CHO infusion of each cycle. Typically a patient will require 50mcg/kg/h over the first 2 hours to load with sufficient morphine to provide analgesia when the infusion is started. This is achieved by a combination of the background rate and presses of the NCA/PCA.

The PCA/NCA is used to control pain during the infusions of ch14.18/CHO. Nurse or patient controlled boluses of morphine allow careful titration of the morphine to the pain experienced. Following the first infusion of each cycle the background rate is maintained until the start of the second infusion on the subsequent day.

Between subsequent infusions an attempt should be made to reduce the background rate or temporarily turn it off. Following the completion of an infusion the pain usually subsides within a few hours. Reducing or stopping the background 2 hours after completing an infusion will reduce the total amount of morphine given. The bolus facility should always remain available. If this is poorly tolerated, the previous background rate should be reinstated. The background rate should be restarted 2 hours before the start of the next ch14.18/CHO infusion.

Pain scores should be recorded hourly using a pain assessment tool which is appropriate to the age and development of the child. The NCA/PCA settings should be reviewed if a satisfactory level of analgesia is not being achieved. If pain remains inadequately controlled on morphine alone then the addition of intravenous low dose ketamine should be considered to provide additional analgesia. An example of a suitable regimen may be found at http://www.ich.ucl.ac.uk/website/gosh/clinicalservices/Pain_control_service/Custom Menu 03 (UCL Institute of Child Health).

Following completion of the fifth ch14.18/CHO infusion of each cycle the morphine PCA/NCA is discontinued. Pain usually resolves quickly following cessation of the ch14.18/CHO. After 5 days the patient may have received a significant total amount of morphine. Therefore patients sometimes have withdrawal symptoms. Oramorph may be helpful in alleviating these symptoms. Typically 200-300mcg/kg oramorph are required. The duration and frequency are specific to the individual, but oramorph is rarely required for more than a few days.

### 14.6.2 Prohibited Treatments

The following treatments are not permitted while patients are receiving R2 treatment:

- Chemotherapy, hormonal anticaner therapy, or experimental anticancer medications other than those that are study-related.
- Glucocorticoids, or other drugs with known immunosuppressive activity, may not be used during and for two weeks prior to entry onto this trial except for life threatening symptoms.
- Radiotherapy.
- The use of IVIG post-PBSCR is discouraged. If necessary, its use should be limited to the first 100 days post-PBSCR, because IVIG may interfere with the antibody (ch14.18/CHO) dependent cellular toxicity. For patients randomised in R2, IVIG should not be given within 2 weeks of starting ch14.18/CHO and 1 week after completing ch14.18/CHO; i.e. if necessary, it may be given during the first week and at any time from week 22.

### 14.6.3 Mandatory Surveillance and Monitoring During Antibody Infusion

Monitor regularly:

- Respiration and oxygen saturation (equipped with pulse oximetry)
- Blood pressure: every 10 min for the first 30 minutes, then every 30 minutes for the next 2 hours and then hourly, if stable.
Fluid balance should be monitored throughout the infusion, diuretics such as furosemide may be required if symptomatic with a positive fluid balance.

Urine output should be monitored very closely. Morphine may induce urinary retention requiring urinary catheterisation.

Daily weight should be documented.

Monitor daily:
Blood counts, electrolytes, bilirubin and creatinine.
Aim to keep haemoglobin > 8g/dL and platelets > 30g/L with appropriate transfusions. Correct electrolyte imbalances.

Refer to section 14.11 (page 133) and 14.12 (page 137) for details of pharmacokinetic and immunological monitoring.

14.6.4 STANDARDISED TREATMENT OF ANAPHYLACTIC REACTIONS

This part serves only as a suggestion and may be adapted to each centres guidelines. It is the treating physician’s responsibility to judge the severity of the reaction, but it is recommended to introduce medication stepwise, i.e. allergic skin reaction may be well controlled with antihistamines alone.

- Stop ch14.18/CHO infusion for at least 30 minutes
- Volume substitution, i.e. 5% albumin 10ml/kg x doses
- Antihistamine as per institutional practice (e.g. Clemastin: 0.6 mg/ 10 kg per dose i.v. or Chlorphenamine maleate i.v.)
  If not successful, next step:
  - Methylprednisolone: 8-10mg/kg per dose i.v. (i.e.: Urbanson®, 5ml= 250mg)
  If not successful, next step:
  - Norepinephrine (i.e. Arterenol® 1:1000, 1ml=1000µg) 1ml Arterenol 1: 1000 + 9ml NaCl 0.9%; 0,2-1ml i.v.

If the reaction resolves easily, continue the ch14.18/CHO infusion. If the reaction is severe, stop the infusion for the day, but try again the next day with appropriate premedication and reduce the antibody dose to 50% for one day. Try to increase to 75% the day thereafter if the 50% regimen was well tolerated. Any dose reduction has to be documented and reported on the case report forms sent to the study centre.

14.6.5 SCIENTIFIC ADDITIONAL EXAMINATIONS

- A 1 ml serum sample needs to be frozen and stored before the first cycle and 4 weeks after the last cycle of immunotherapy, for the determination of human anti-chimeric antibodies (HACHA) and anti-idiotypic antibodies.

14.7 Treatment schedule for patients randomised to ch14.18/CHO and subcutaneous aldesleukin (IL-2)

Patients randomised to receive ch14.18/CHO and subcutaneous aldesleukin (IL-2) will start their immunotherapy with aldesleukin (IL-2) at week 3. Aldesleukin (IL-2) will be given according to the following administration schedule:
During weeks 3, 7, 11, 15 and 19 aldesleukin (IL-2) will be given at a dose of 6 MIU/m²/day over five days subcutaneously (Monday-Friday).

During weeks 4, 8, 12, 16 and 20 aldesleukin (IL-2) will be given two hours after the stop of the anti-body infusion at a dose of 6 MIU/m²/day over five days subcutaneously (Monday-Friday).

Aldesleukin (IL-2) must be given at least 2 hours after the stop of the ch14.18/CHO infusion with prophylactic paracetamol. The use of local anaesthetic cream (EMLA) (applied 30 minutes prior) or cold spray prior to injection is highly recommended. Rotation of the injection sites should be practiced. In case of persistent Grade III or higher fever, a dose reduction of 50% is recommended.

NOTE: A temperature spike 2-4 hours after the administration of aldesleukin (IL-2) is often seen. For this reason the administration of aldesleukin (IL-2) is:

a) given at least 2 hours after the stop of the ch14.18/CHO infusion to decrease the chance of allergic reaction, and

b) recommended after 6 p.m., so that the patient will sleep through.

Ch14.18/CHO will be given at a dose of 20mg/m²/day over 5 days every 4 weeks for 5 courses, starting in week 4. Guidelines for pain prophylaxis must be considered (Chapter 14.6.1).

Infants and children with a body weight below 12kg should be dosed according to their weight in kg instead of their body surface area (m²) according to the known formula of 30 kg = 1 m². In infants weighing ≤ to 5 kg, a further 1/3 dose reduction is advised.

Ch14.18/CHO will be dosed at 0.67mg/kg/day and aldesleukin (IL-2) will be dosed at 0.2 MIU/kg/day.

As mentioned in section 3.7, patients should not receive a dose reduction for isotretinoin (13-cis-RA).

### Treatment schedule for isotretinoin (13-cis-RA), ch14.18/CHO and aldesleukin (IL-2)

<table>
<thead>
<tr>
<th></th>
<th>W1</th>
<th>W2</th>
<th>W3</th>
<th>W4</th>
<th>W5</th>
<th>W6</th>
<th>W7</th>
<th>W8</th>
<th>W9</th>
<th>W10</th>
<th>W11</th>
<th>W12</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>RA</td>
<td></td>
<td></td>
<td>GD2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>IL-2</td>
<td>RA</td>
<td>RA</td>
<td>IL-2</td>
<td>RA</td>
<td>IL-2</td>
<td>RA</td>
<td>GD2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GD2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>W13</th>
<th>W14</th>
<th>W15</th>
<th>W16</th>
<th>W17</th>
<th>W18</th>
<th>W19</th>
<th>W20</th>
<th>W21</th>
<th>W22</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>RA</td>
<td>IL-2</td>
<td>RA</td>
<td>IL-2</td>
<td>RA</td>
<td>IL-2</td>
<td>IL-2</td>
<td>RA</td>
<td>RA</td>
<td></td>
</tr>
</tbody>
</table>

GD2: ch14.18/CHO

### 14.8 Criteria prior to immunotherapy

For **patients randomised to ch14.18/CHO alone** the following tests/examinations should be done as close as possible prior to each week of ch14.18/CHO (i.e. beginning of weeks 4, 8, 12, 16 and 20).

For **patients randomised to ch14.18/CHO plus aldesleukin (IL-2)** the following tests/examinations should be done as close as possible prior to each week of aldesleukin (IL-2) alone (i.e. beginning of weeks 3, 7, 11, 15 and 19) as well as prior to each week of ch14.18/CHO + aldesleukin (IL-2) (i.e. beginning of weeks 4, 8, 12, 16 and 20).
Required tests/examinations (see section 6.6, page 90, for an overview):

- Normal physical examination
- At least 0.5 abs./G/L lymphocytes, 30 G/L platelets, and 8g/dL haemoglobin without transfusion support for at least one week.
- More than 0.5 abs./G/L neutrophils after a minimum of 3 days discontinuation from GCSF treatment.
- Serum bilirubin, creatinine and blood coagulation within less than 25% more than age suggested upper institutional limit.
- Normal chest x-ray
- Normal ECG. Fractional shortening of ≥ 30% by echocardiogram, or if shortening fraction abnormal, ejection fraction of ≥ 55% by gated radionuclide study.
- Patients with seizure disorder must be well controlled and taking anticonvulsants. CNS toxicity < grade 2.
- FEV₁ and FVC > 60% of predicted by pulmonary function test (PFT). For children who are unable to do PFTs, no evidence of dyspnea at rest, no exercise intolerance, and a pulse oximetry > 94% on room air.

The tests are repeated so often, because a clear toxicity profile is needed for:

- Registration of ch14.18/CHO
- Off-label use of aldesleukin (IL-2)

14.9 Dose Modifications for patients randomised to ch14.18/CHO alone

14.9.1 Expected toxicities that do not require CH14.18/CHO dose modification

The following expected toxicities will NOT need dose modification when observed, provided that these toxicities are judged to be tolerable by the responsible clinician, as well as the patient and family.

a. Grade 4 pain (requires intravenous narcotics).
b. Grade 3 nausea and vomiting and diarrhoea.
c. Grade 3 fever.
d. Grade 3 skin toxicity that remains stable and tolerable, or improves with treatment (e.g., IV diphenhydramine or chlorpheniramine maleate) within 24 hrs.
e. Grade 3 electrolytes (especially hyponatremia <124 mEq/l in the absence of CNS symptoms and sequelae) that improve with treatment within 24 hrs.
f. Grade 3 hepatic toxicity that returns to Grade 1 prior to the time for next ch14.18/CHO treatment course.
g. Grade 3 neurotoxicity with subjective findings (e.g., tingling, hot or cold hands, etc.)
h. Grade 4 hematologic toxicity, which improves to at least Grade 2 or baseline pretherapy values within one week of completing aldesleukin (IL-2).
i. Grade 3 performance (30 - <50%, see chapter 26, Appendix: Performance Scales)
j. Impaired visual accommodation, correctable with eye glasses.
k. Altered taste.

14.9.2 Dose modification of CH14.18/CHO for hypersensitivity reactions

- Mild symptoms (eg. localized cutaneous reactions or rigor): Decrease the rate of ch14.18/CHO infusion to 50% until recovery from symptoms, remain at bedside and monitor subject; complete infusion at the initial planned rate. Diphenhydramine may be administered q 4-6hr at the discretion of the treating physician.
- For moderate symptoms (eg. hypotension): Interrupt ch14.18/CHO infusion, administer supportive care, remain at bedside and monitor subject until resolution of symptoms. At

HRNBL1.5/SIOPEN valid per 01.06.2011
the discretion of the treating physician, infusion may be resumed at 50% of the initial infusion rate.

- For severe symptoms (any reaction such as bronchospasm, angioedema or anaphylactic shock): Immediately discontinue infusion. Give epinephrine, diphenhydramine and corticosteroids, bronchodilator or other medical measures as needed. Patients should be monitored as in-patient for at least 24 hours AND until the symptoms have resolved. Refer to section Fehler! Verweisquelle konnte nicht gefunden werden. for stopping ch14.18/CHO. If ch14.18/CHO is stopped, resume next course with isotretinoin (13-cis-RA).

14.9.3 CRITERIA FOR STOPPING CH14.18/CHO
Patients should be taken off ch14.18/CHO if the following toxicities occur. Please note that patients should continue to receive isotretinoin (13-cis-RA). For any of these toxicities, please notify the International Coordinator and your National Coordinator immediately.

- Grade 3 (bronchospasm) and 4 (anaphylaxis) allergic reaction.
- Grade 3 serum sickness.
- Grade 4 severe, unrelenting neuropathic pain unresponsive to continuous infusion of narcotics and other adjuvant measures including lidocaine infusions.
- Neurotoxicity: 1) Grade 3 sensory changes interfering with daily activities >2 weeks after completing ch14.18/CHO therapy; 2) Objective motor weakness; 3) Grade 3 vision toxicity (i.e., subtotal vision loss per toxicity scale).
- Grade 4 hyponatremia (<120 mEq/L) despite appropriate fluid management.
- Grade 4 capillary leak syndrome (Grade 4 includes ventilator support).
- Grade 4 Skin Toxicity

14.10 Dose Modifications for patients randomised to ch14.18/CHO plus aldesleukin (IL-2)

For toxicities related to ch14.18/CHO that do not require dose modification refer to section 14.9.1. The details of dose modifications for ch14.18/CHO hypersensitivity reactions can be found in section 14.9.2.

14.10.1 CRITERIA FOR DOSE MODIFICATION OF ALDESLEUKIN (IL-2) WHEN GIVEN ALONE

Treatment with aldesleukin (IL-2) should be continued unless Grade III toxicity occurs, at which time aldesleukin (IL-2) should be discontinued until a return to baseline pre-therapy values or Grade I toxicity.

In the case of Grade IV toxicity from aldesleukin (IL-2), aldesleukin (IL-2) should be discontinued. If Grade III or Grade IV toxicity occurs after resumption at 50% of the initial dose, aldesleukin (IL-2) will be discontinued and the subject will be given ch14.18/CHO alone for subsequent courses of immunotherapy.

14.10.2 CRITERIA FOR DOSE MODIFICATIONS OF CH14.18/CHO AND ALDESLEUKIN (IL-2) WHEN GIVEN TOGETHER

For any of the following toxicities, please notify the International Coordinator and your National Coordinator immediately.
a. Grade 3 hypotension:

Dose modification instructions during the course of immunotherapy:

- Discontinue aldesleukin (IL-2) and ch14.18/CHO infusion.
- If hypotension resolves or improves to Grade 1 with fluid boluses, may resume ch14.18/CHO at 50% rate 1 hr later to complete the prescribed dose. If ch14.18/CHO infusion is tolerated, give 50% of the aldesleukin (IL-2) dose as scheduled for the remainder of the course.
- If hypotension (grade 3) recurs with ch14.18/CHO, then discontinue ch14.18/CHO.

Dose modification instructions for subsequent courses:

- If patient tolerated 50% infusion rate of ch14.18/CHO and 50% aldesleukin (IL-2) dose, subsequent courses of ch14.18/CHO with aldesleukin (IL-2) should start at 50% dose aldesleukin (IL-2). If tolerated, may increase aldesleukin (IL-2) to full dose.
- If aldesleukin (IL-2) is not tolerated during immunotherapy and has to be discontinued, ch14.18/CHO should be given as the sole immunotherapy.
- If hypotension unresponsive to supportive measures or requires ventilator support, patient will be off immunotherapy.

b. Grade 3 capillary leak syndrome:

Dose modification instructions during the course of immunotherapy:

- Discontinue aldesleukin (IL-2) and ch14.18/CHO infusion.
- If capillary leak syndrome resolves or improves to Grade 1 with supportive measures, may resume ch14.18/CHO at 50% rate 1 hr later to complete the prescribed dose. If ch14.18/CHO infusion is tolerated, give 50% of the aldesleukin (IL-2) dose as scheduled for the remainder of the course.
- If capillary leak syndrome (grade 3) recurs, or if vasopressor support is needed for control of hypotension, permanently discontinue aldesleukin (IL-2) and give ch14.18/CHO alone.
- If capillary leak syndrome (grade 3) recurs with ch14.18/CHO alone, then discontinue immunotherapy.

Dose modification instructions for subsequent courses:

- If patient tolerated 50% aldesleukin (IL-2) dose and 50% infusion rate of ch14.18/CHO, subsequent course of ch14.18/CHO with aldesleukin (IL-2) should start at 50% dose aldesleukin (IL-2). If tolerated, may consider advancing ch14.18/CHO infusion to full rate as tolerated but keep aldesleukin (IL-2) at 50% dose.
- If aldesleukin (IL-2) is not tolerated during immunotherapy and has to be discontinued, ch14.18/CHO should be given as the sole immunotherapy.
- If capillary leak syndrome unresponsive to supportive measures or requires ventilator support, patient will be off immunotherapy.

c. Grade 3 pulmonary toxicity in the setting of capillary leak syndrome: follow the same dose modification guideline as for grade 3 capillary leak syndrome.

d. Grade 3 infection during infusion of ch14.18/CHO with aldesleukin (IL-2): abort the immunotherapy course. Missed doses will not be replaced. May proceed to the subsequent planned immunotherapy course only when infection resolves or under control (asymptomatic and negative blood culture).

14.11 Pharmacokinetics for ch14.18/CHO

Rationale:
The pharmacokinetics of ch14.18/CHO needs further analysis in view of the European Medicines Agency (EMA) requirements for marketing authorisation in order to make ch14.18 available to
children with neuroblastoma. This is particularly important since the number of investigated patients in the SIOPEN Bridging Study is too small to get the antibody approved by the EMA. Therefore it is crucial to continue pharmacokinetic sampling in patients randomised to either immunotherapy arm. This also allows for an assessment of the impact of s.c aldesleukin (IL-2) on ch14.18/CHO pharmacokinetics and pharmacodynamics.

**Patients assigned for PK sampling:**
120 patients randomised to immunotherapy (60 patients s.c. aldesleukin (IL-2)/ch14.18/CHO and 60 ch14.18/CHO alone) will have blood samples taken for pharmacokinetic studies.

Only the first treatment cycle for each patient will be used for PK analysis. Ideally no further pharmacokinetic studies will be necessary but the ultimate decision will depend on the results from the first 60 patients in each arm. Hence, each patient on trial needs to be considered for PK sampling.

**Time Points of PK sampling:**
To determine the pharmacokinetics of ch14.18/CHO given as repeated infusions, the first serum samples (Day 0-4) will be obtained before starting the ch14.18/CHO 8hr infusion.

**Important: ON DAY 4 take one additional sample AT THE END OF THE LAST INFUSION OF CH14.18! (PEAK SERUM CONCENTRATION).**
Take samples of days 5, 7, 14, 21 and 28 in the morning of each visit and indicate the time. All samples will be assessed centrally by ELISA for ch14.18/CHO.

All samples should be marked with patient study number, centre, date and time of sampling, cycle number and day of cycle. Please use the PK Sampling Sheet provided below.

**Instructions for serum sampling:**
- Draw a minimum of 2ml of blood into a serum sample tube.
- Send sample to laboratory for serum preparation.
- Remove serum from pellet and place in Eppendorf vial.
- Ensure clear labelling of the sample as above.
- Freeze serum at -80°C.

Ship batches of samples together with the sampling sheet (PK Analysis Sampling Sheet) on dry ice to:
Prof. Dr. med. Holger Lode
University of Greifswald
Children’s Hospital
Sauerbruchstrasse 1
17475 Greifswald
Germany

Notify Prof. Lode prior to sending off a sampling batch to make sure that samples will be appropriately received and looked after upon arrival.
Phone: +49 3834 86 6310/6300
Fax: +49 3834 86 6325
Email: lode@uni-greifswald.de
Figure 11: Pharmacokinetics of ch14.18/CHO (scIL2/ch14.18 and ch14.18)

PK time points, days 0-28

Weeks

<table>
<thead>
<tr>
<th>Days</th>
<th>W3</th>
<th>W4</th>
<th>W5</th>
<th>W6</th>
<th>W7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>± IL2</td>
<td>GD2 ± IL2</td>
<td>RA</td>
<td>RA</td>
<td>± IL2</td>
</tr>
</tbody>
</table>

ch14.18/CHO, 8hr infusion
# PK Analysis Sampling Sheet

**Patient ID:**

| Weight     | ____________ kg |
| Size       | ____________ cm |
| Body Surface Area | ____________ m² |

**Treatment arm:**
- ☑ ch14.18/CHO
- ☑ s.c. IL-2 + ch14.18/CHO

**Absolute amount ch14.18/CHO per infusion:** ____________ mg
**Absolute Amount of IL-2 per injection:** ____________ IU

<table>
<thead>
<tr>
<th>Day</th>
<th>Date of collection</th>
<th>Time of collection</th>
<th>Label</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>_ _ _ _ _ _ _ _ _ _</td>
<td>_ _ : _ _ _ _ _ _ _</td>
<td>1 + Pat. ID</td>
</tr>
<tr>
<td>(pre treatment sample)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>_ _ _ _ _ _ _ _ _ _</td>
<td>_ _ : _ _ _ _ _ _ _</td>
<td>2 + Pat. ID</td>
</tr>
<tr>
<td>Day 2</td>
<td>_ _ _ _ _ _ _ _ _ _</td>
<td>_ _ : _ _ _ _ _ _ _</td>
<td>3 + Pat. ID</td>
</tr>
<tr>
<td>Day 3</td>
<td>_ _ _ _ _ _ _ _ _ _</td>
<td>_ _ : _ _ _ _ _ _ _</td>
<td>4 + Pat. ID</td>
</tr>
<tr>
<td>Day 4</td>
<td>_ _ _ _ _ _ _ _ _ _</td>
<td>_ _ : _ _ _ _ _ _ _</td>
<td>5 + Pat. ID</td>
</tr>
<tr>
<td>Day 4</td>
<td>_ _ _ _ _ _ _ _ _ _</td>
<td>_ _ : _ _ _ _ _ _ _</td>
<td>6 + Pat. ID</td>
</tr>
<tr>
<td>(&lt; 10min end of infusion)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 5</td>
<td>_ _ _ _ _ _ _ _ _ _</td>
<td>_ _ : _ _ _ _ _ _ _</td>
<td>7 + Pat. ID</td>
</tr>
<tr>
<td>Day 7</td>
<td>_ _ _ _ _ _ _ _ _ _</td>
<td>_ _ : _ _ _ _ _ _ _</td>
<td>8 + Pat. ID</td>
</tr>
<tr>
<td>Day 14</td>
<td>_ _ _ _ _ _ _ _ _ _</td>
<td>_ _ : _ _ _ _ _ _ _</td>
<td>9 + Pat. ID</td>
</tr>
<tr>
<td>Day 21</td>
<td>_ _ _ _ _ _ _ _ _ _</td>
<td>_ _ : _ _ _ _ _ _ _</td>
<td>10 + Pat. ID</td>
</tr>
<tr>
<td>Day 28</td>
<td>_ _ _ _ _ _ _ _ _ _</td>
<td>_ _ : _ _ _ _ _ _ _</td>
<td>11 + Pat. ID</td>
</tr>
</tbody>
</table>
14.12 Immunological Monitoring for ch14.18/CHO

14.12.1 Determination of human anti-chimeric antibodies (HACHA) and anti-idiotypic antibodies.

All Patients will have additional blood samples taken for immunological monitoring. The serum samples for these studies should be obtained at 4 time points during the MRD treatment.

1st time point: W1
2nd time point: W11
3rd time point: W19
4th time point: Last visit.

<table>
<thead>
<tr>
<th>W1</th>
<th>W2</th>
<th>W3</th>
<th>W4</th>
<th>W5</th>
<th>W6</th>
<th>W7</th>
<th>W8</th>
<th>W9</th>
<th>W10</th>
<th>W11</th>
<th>W12</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>RA</td>
<td>GD2</td>
<td>RA</td>
<td>GD2</td>
<td>RA</td>
<td>IL-2</td>
<td>RA</td>
<td>IL-2</td>
<td>RA</td>
<td>IL-2</td>
<td>GD2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>W13</th>
<th>W14</th>
<th>W15</th>
<th>W16</th>
<th>W17</th>
<th>W18</th>
<th>W19</th>
<th>W20</th>
<th>W21</th>
<th>W22</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>RA</td>
<td>GD2</td>
<td>GD2</td>
<td>RA</td>
<td>IL-2</td>
<td>RA</td>
<td>IL-2</td>
<td>RA</td>
<td>RA</td>
</tr>
</tbody>
</table>

A 1ml serum sample needs to be frozen and stored.

Instructions for serum sampling:
- Draw a minimum of 2ml of Blood into a serum sample tube.
- Send sample to laboratory for serum preparation.
- Remove serum from pellet and place in Eppendorf vial.
- Ensure clear labelling of the sample as above.
- Freeze serum at -80°C.

Ship batches together with the sample sheet provided below (HaChA Analysis Sampling Sheet) on dry ice to:
Prof. Dr. med. Holger Lode
University of Greifswald
Children’s Hospital
Sauerbruchstrasse 1
17475 Greifswald
Germany

Notify Prof. Lode prior sending off the samples.
Phone: +49 3834 86 6310/6300
Fax: +49 3834 86 6325
Email: lode@uni-greifswald.de
**HaChA Analysis Sampling Sheet**

Patient ID: 

<table>
<thead>
<tr>
<th>Weight</th>
<th>____________ kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>____________ cm</td>
</tr>
<tr>
<td>Body Surface Area</td>
<td>____________ m²</td>
</tr>
</tbody>
</table>

Treatment arm:  
- [ ] ch14.18/CHO  
- [ ] s.c. IL-2 + ch14.18/CHO

Absolute amount ch14.18/CHO per infusion: ____________ mg  
Absolute Amount of IL-2 per injection: ____________ IU

1\(^{st}\) time point: W1  
2\(^{nd}\) time point: W11  
3\(^{rd}\) time point: W19  
4\(^{th}\) time point: Last visit.

<table>
<thead>
<tr>
<th>Day</th>
<th>Date of collection</th>
<th>Time of collection</th>
<th>Label</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>_ _ _ _ _ _ _ _ _ _ _ _</td>
<td>_ _ _ : _ _ _</td>
<td>1 + Pat. ID</td>
</tr>
<tr>
<td>(pre treatment sample)</td>
<td>d  m  y</td>
<td>h  min</td>
<td></td>
</tr>
<tr>
<td>Week 11</td>
<td>_ _ _ _ _ _ _ _ _ _ _ _</td>
<td>_ _ _ _ _ _</td>
<td>2 + Pat. ID</td>
</tr>
<tr>
<td></td>
<td>d  m  y</td>
<td>h  min</td>
<td></td>
</tr>
<tr>
<td>Week 19</td>
<td>_ _ _ _ _ _ _ _ _ _ _ _</td>
<td>_ _ _ _ _ _</td>
<td>3 + Pat. ID</td>
</tr>
<tr>
<td></td>
<td>d  m  y</td>
<td>h  min</td>
<td></td>
</tr>
<tr>
<td>End of treatment</td>
<td>_ _ _ _ _ _ _ _ _ _ _ _</td>
<td>_ _ _ _ _ _</td>
<td>4 + Pat. ID</td>
</tr>
<tr>
<td></td>
<td>d  m  y</td>
<td>h  min</td>
<td></td>
</tr>
</tbody>
</table>
14.12.2 IMMUNE EFFECTOR CELL ACTIVATION

Immune effector cell activation will be determined by flow cytometry locally and the results faxed to Holger Lode (using the NK cell activation report form on the following page). The analysis should involve CD16+ / CD56+ NK cells (cytotoxic NK cells).

For the analysis collect 2-5ml of blood in an EDTA tube on day 1 of weeks 3, 4 and 5 for every cycle and send to the local laboratory for immediate analysis.

Immune effector cell activation should be determined for all patients randomised in R2.

Time points for Analysis of Effector Cell activation

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Days</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>W3</td>
<td>+/-</td>
<td>IL2</td>
</tr>
<tr>
<td>W4</td>
<td>+/-</td>
<td>IL2</td>
</tr>
<tr>
<td>W5</td>
<td></td>
<td>RA</td>
</tr>
<tr>
<td>W6</td>
<td></td>
<td>RA</td>
</tr>
<tr>
<td>W7</td>
<td></td>
<td>+/- IL2</td>
</tr>
</tbody>
</table>

ch14.18/CHO, 8hr infusion
NK cell activation report form

Patient ID: 

Weight
________________kg
Size
________________cm
Body Surface Area
________________m²

Treatment arm:
☐ ch14.18/CHO
☐ s.c. IL-2 + ch14.18/CHO

Absolute amount ch14.18/CHO per infusion: ____________ mg
Absolute Amount of IL-2 per injection: ____________ IU

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Day 1, Week 3</th>
<th>Date</th>
<th>Day 1, Week 4</th>
<th>Date</th>
<th>Day 1, Week 5</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st sample</td>
<td>Pre IL-2 sample</td>
<td>d m y</td>
<td>Pre IL-2 + ch14.18/CHO sample</td>
<td>d m y</td>
<td>Post IL-2 + ch14.18/CHO sample</td>
<td>d m y</td>
</tr>
<tr>
<td>2nd sample</td>
<td>d m y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Absolute number of NK cells / µl
________________/ µl

Absolute number of CD16+ NK cells / µl
________________/ µl

Day 1 Week 4
____________
(Pre IL-2+ch14.18/CHO sample) d m y

Absolute number of NK cells / µl
________________/ µl

Absolute number of CD16+ NK cells / µl
________________/ µl

Day 1 Week 5
____________
(Post IL-2+ch14.18/CHO sample) d m y

Absolute number of NK cells / µl
________________/ µl

Absolute number of CD16+ NK cells / µl
________________/ µl

FAX Form to:
Prof. Dr. med. Holger Lode, University of Greifswald, Children’s Hospital, Sauerbruchstrasse 1, 17475 Greifswald, Germany
Fax: +49 3834 86 6325
15 Use of G-CSF

15.1 Use of G-CSF during Induction treatment

The use of G-CSF as primary prophylaxis throughout the induction period is now recommended on the bases of results of the R0 randomisation.

15.2 Summary of results of the closed randomisation within this trial on the use of G-CSF during Rapid COJEC Induction Treatment (Supportive Care Randomisation R0)

Randomisation 0: G-CSF question – information updated May 2009

R0 accrual was completed in November 2005, with a total of 239 patients accrued and 232 (97%) evaluable for the primary endpoint (mean number of febrile episodes).

The comparison of the primary endpoint showed a significant reduction of the mean number of febrile episodes (p=0.002). Patients randomised not to receive G-CSF (the control arm) had a median number of 0.75 febrile episodes per cycle compared to 0.50 for patients randomised to the G-CSF arm (difference 0.25, p=0.004). During cycle 4 this difference between both arms was most pronounced. There is a significant difference in number of days with fever and number of days with antibiotics in favour of the G-CSF arm on the basis of completely reported cycles.

Overall results are in favour of G-CSF. Cost estimates may be done, but we will need the cost of a hospital day and of 1 ampoule G-CSF in each participating country as there again appears to be quite a variation Europe-wide (and even within the same country) and therefore the economic aspect may reach different conclusions in different countries. In addition, this was not a study endpoint. However, a previous report on the use of prophylactic G-CSF in children with non-Hodgkin’s lymphoma treated with COPAD(M) intensive induction, was able to show that the costs of 2 more days of hospitalisation in the control arm would have to be weighted against the price of 17 days of G-CSF [161].

Fungal Infections
A total of 17 fungal infections were reported in the control arm of which 8 (7%) are reported as severe. A total of 13 fungal infections were reported in the G-CSF arm of which 5 (4%) are reported as severe. This difference was not significant.

Significant differences in favour of the G-CSF arm were also seen for general condition, WBC and granulocytes and for gut toxicities. No further difference in reported toxicities was observed.

No deaths occurred in the control arm; 4 deaths occurred in the G-CSF arm: 3 early tumour related deaths (on days 2, 10, 19) and one related to septic shock in an outpatient returning to hospital in neutropenia, febrile and miserable condition.

No major differences between the arms were seen in the response rates and the PBSC harvest success rate.

The results of the R0 randomisation have been published in JCO [1].

HRNBL1.5/SIOPEN valid per 01.06.2011
15.3 G-CSF for PBSC Mobilisation

Routine G-CSF for the mobilisation of PBSCs is recommended for children needing autologous PBSC transplantation.

- G-CSF can be administered following myelosuppressive therapy at a dose of 5µg/kg per day until a value of 0.02 x 10^9/l circulating CD34+ cells or higher is reached for the start of the PBSC collection.
- Alternatively, G-CSF can be administered alone at a dose of 10µg/kg per day for 4 days with collection on day 5, or for 5 days with collection on days 5 and 6.

15.4 G-CSF after MAT

15.4.1 BACKGROUND

Adult guidelines strongly recommend the use of G-CSF to shorten the period of neutropenia after autologous BMT. Data on G-CSF use in children as an adjunct to autologous BMT are limited and no randomised trial has been carried out. However, two open studies, with historical controls, have indicated the usefulness of G-CSF in this setting [162, 163]. There is no evidence that beneficial effects can be observed when G-CSF is given during the first days after autologous transplantation. Beginning the treatment after the first week seems adequate.

Recent data on G-CSF in the setting of PBSC transplantation is given in the table below summarizing numerous studies depicting an advantage for the use of G- and /or GM-CSF. There is however much less evidence in children to suggest that routine G-CSF administration to recipients of autologous PBSCs is effective in accelerating neutrophil recovery. In a non-randomised study of 98 children G-CSF had no effect on neutrophil recovery following autologous PBSC transplantation [164].

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Type</th>
<th>Pts No.</th>
<th>Dose of G-CSF / GM-CSF</th>
<th>Treatment duration</th>
<th>ANC&gt; 500/µl Days +/-G-CSF</th>
<th>Days in Hospital</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klumpp [165]</td>
<td>1995</td>
<td>+</td>
<td>41</td>
<td>G-CSF 5 µg/kg</td>
<td>D1- 0.5x10^9/l for 3 days</td>
<td>10.5 vs 16</td>
<td>18 vs 24</td>
<td>S</td>
</tr>
<tr>
<td>Tarella [166]</td>
<td>1998</td>
<td>-</td>
<td>40</td>
<td>G-CSF 5 µg/kg</td>
<td>D1 - 0.5x10^9/l for 3 days</td>
<td>10 vs 14</td>
<td>10 vs 16</td>
<td>S</td>
</tr>
<tr>
<td>Benedetti [167]</td>
<td>1997</td>
<td>-</td>
<td>40</td>
<td>G-CSF 5 µg/kg + Epo</td>
<td>D1-D12</td>
<td>9 (10) vs 11</td>
<td>18 (16) vs 20</td>
<td>S</td>
</tr>
<tr>
<td>Spitzer [168]</td>
<td>1994</td>
<td>+</td>
<td>37</td>
<td>G-CSF 7.5 µg/kg and GM-CSF 2.5 µg/kg</td>
<td>D1-&gt;1.5x10^9/l for 2 days</td>
<td>10 vs 16</td>
<td>19 vs 21</td>
<td>S</td>
</tr>
<tr>
<td>Bensinger [169]</td>
<td>1994</td>
<td>-</td>
<td>54</td>
<td>G-CSF 5 µg/kg or GM-CSF 250µg/m²</td>
<td>D1- 1.0x10^9/l for 2 days</td>
<td>12 vs 14</td>
<td>?</td>
<td>S</td>
</tr>
<tr>
<td>McQuaker [170]</td>
<td>1997</td>
<td>+</td>
<td>33</td>
<td>G-CSF 50µg/m²</td>
<td>D1- 0.5x10^9/l</td>
<td>10 vs 14</td>
<td>13 vs 16</td>
<td>S</td>
</tr>
<tr>
<td>Lee [171]</td>
<td>1998</td>
<td>+</td>
<td>31</td>
<td>G-CSF 300µg</td>
<td>D5- 1.0x10^9/l for 2 days</td>
<td>9.7 vs 13.5</td>
<td>12 vs 15</td>
<td>S</td>
</tr>
<tr>
<td>Shimazaki [172]</td>
<td>1994</td>
<td>-</td>
<td>20</td>
<td>G-CSF 50µg/m²</td>
<td>D1-0.5x10^9/l</td>
<td>10 vs 14</td>
<td>?</td>
<td>S</td>
</tr>
</tbody>
</table>

R: Randomised
Pts No.: Number of Patients

A recent prospective, randomized phase III AEIOP study of G-CSF following allogeneic, autologous bone marrow and peripheral blood progenitor cell transplantation in children was reported by S. Dallorso et al for the Italian group (Dallorso, S. et al. 2000. Acta Haematologica 103, S1, p.92.). Among the PBSC transplant group the majority of 76 children had solid tumours. The time to reach ANC 0.5x10^9/l was 11 days in the treatment group versus 14 days in the control
group (p=0.0001). No difference was depicted for platelet recovery, transfusional support, duration of fever, antibiotic therapy and length of hospitalisation. However, it is difficult to draw definite conclusions from this study on the possible impact of G-CSF in view of the number of patients and their treatment heterogeneity associated with various protocols.

A very recent French randomized trial compared 2 schedules of G-CSF (lenogastrim) after autologous peripheral stem cell transplantation (APSCCT) to a third group without Lenogastrim (D. Valteau-Couanet, et al for the Société Française de Greffe de Moelle (SFGM) (Valteau-Couanet, D. et al. 2001. Bone Marrow Transplant 27, S1, abstr. OS107). Both children and adults either with haematological malignancies or solid tumours underwent consolidation with high-dose chemotherapy (HDC) ± TBI and APBSCT and received after randomisation 150 µg/m²/d of intravenous G-CSF starting on day 1 post SCT (G1), day 5 post SCT (G5) or no Lenograstim (G0). Randomisation was stratified on the conditioning regimen (Busulfan vs TBI vs no Busulfan and no TBI) and the CD34+ cells count (> 3 x10⁶/kg vs < 3 x 10⁶/kg). Between September 1998 and December 1999, 240 patients entered this trial, 239 are evaluable, 80, 80 and 79 in the G5, G1 and G0 groups respectively. Patients in the 3 groups were comparable in terms of age, underlying malignancy, previous treatment, conditioning regimen and number of CD34+ cells. Median duration of neutropenia (<0.5 x10⁹/l) was significantly shorter in the 2 groups receiving G-CSF: 10 days (5-15) and 9 days (4-20) in G5 and G1 groups respectively compared to the G0 group: 13 days (7-36) (p<0.0001). Neutropenia duration was significantly longer (p < 0.01) after TBI containing regimens, but as the other regimens was significantly reduced when G-CSF was administered after APBSCT. Median duration of G-CSF administration was significantly shorter in the G5 group 8 days (4-15) compared to the G1 group 11 days (6-34) (p<0.0005). The median duration of thrombocytopenia did not differ in the 3 groups. It was 5 (1-65), 7 (1-21) and 6 (1-34) in the G5, G1 and G0 groups, respectively. The incidence of infectious complications, visceral toxicity and duration of hospitalisation were similar in the 3 groups.

The study concluded that

- Administration of G-CSF, post autologous PBSCT, was associated with a significantly more rapid recovery from neutropenia without impairing platelet recovery.
- No significant difference was observed between the groups where G-CSF was administered on day 1 versus day 5. No difference in non-haematological toxicities was observed between the 3 groups.
- A cost-efficacy study is in progress.

Therefore the administration of G-CSF starting at day 5 post SCT is recommended in this study.

15.4.2 MODE OF ADMINISTRATION

Based on the results of the above French trial, G-CSF administration following stem cell transplant will be given to all patients according to the established standard:

5µG/KG G-CSF FROM DAY 5 POST PBSCT UNTIL A STABLE INCREASE OF WBC > 5 x 10⁹/L OR ANC > 0.5 x 10⁹/L ON TWO CONSECUTIVE BLOOD CELL COUNTS WITH A 48H INTERVAL.

16 Supportive Care Guidelines

All the treatment described in this protocol, in particular the induction and MAT, is intensive and aggressive and will be followed by severe bone marrow depression. Hence, treatment according to this protocol should be restricted to institutions who are familiar with the
administration of intensive aggressive combination chemotherapy and where the full range of supportive care is available.

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

16.1 Anti-Emetics
Antiemetic therapy should be administered according to institutional guidelines, e.g. Ondansetron 5 mg/m² (maximum single dose 8 mg) p.o./i.v. every 12 hours for 5 days.

16.2 Hydration
Sufficient hydration (2 to 3 l/m²) with appropriate electrolyte supplementation must be provided during chemotherapy. Monitoring of blood pressure, cardiac and respiratory rates, body weight, and diuresis is mandatory. The application of diuretics may become necessary in the case of oedema or hypertension.

16.3 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 (except fresh frozen plasma) should be irradiated with at least 15 Gy prior to transfusion, according to national policies. The use of leukocyte filters for leukocyte depletion (CMV negativity) is advised.

Red blood cells
Keep the haemoglobin level above 8g/dl.

Platelets
Platelet substitution is advised when the platelets are < 10 x 10⁹/l, and/or there is clinical evidence of bleeding.

16.4 Central Lines
Since Busilvex® must be given through a central venous catheter, the use of central lines is mandatory. Especially in HDT patients, multi-lumen central lines are essential for PSC sampling and supportive care.

16.5 Treatment Guidelines for febrile Neutropenia
The HR-NBL 1/SIOPEN study is a very intensive protocol which is likely to result in prolonged episodes of neutropenia and infection.

The Rapid COJEC regimen has a similar myelosuppressive effect to that which occurs during the therapy of acute myeloid leukaemia resulting in a significant risk of fungal infection; therefore it is very important that investigators adhere to and follow this approach for therapy for febrile neutropenia.

All participating institutions must be familiar with managing such problems according to the accepted general principles of supportive care.
During episodes of fever and neutropenia patients need to be admitted to hospital for adequate diagnostic measures and appropriate treatment.
a. If there is fever (>38°C) and the neutrophil count is less than 1.0 X 10^9/L, then the centre’s usual combination of broad-spectrum antibiotics should be commenced.

b. If fever persists (>38°C) for 48 hours despite broad-spectrum antibiotics, then 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. In addition a chest X-ray (CXR) should be carried out.

Other antifungal therapy e.g. fluconazole is not permitted in view of the substantial risk that the underlying fungal infection is aspergillosis and fluconazole will not be active.

c. If fever persists for a further 48 hours (i.e. a total of 96 hours), without another identified cause then:-
   - The dose of ambisome should be escalated to 3mg/kg/day;
   - If the patient is receiving amphotericin B, then very careful consideration should be given to substituting ambisome for amphotericin B;
   - Careful consideration should be given to carrying out a CT scan of the chest,

If there are any abnormalities on the CT scan then:
   - G-CSF should be commenced at 5ug/kg/day;
   - Consideration should be given to the introduction of caspofungin or another antifungal agent. Itraconazole and voriconazole should not be considered since it should not be combined with vincristine;
   - Consideration should be given to other specific, appropriate investigations e.g. imaging, biopsies and broncho-alveolar lavage.
   - Granulocyte infusions may also be considered.

d. If the fever persists for a further 48 hours (i.e. a total of 144 hours) without CT scan changes) then:
   - GSCF should be commenced at 5ug/kg/day;
   - Consideration should be given to the introduction of caspofungin or another antifungal agent. Itraconazole and voriconazole should not be considered since it should not be combined with vincristine
   - Further dosage escalation of ambisome to 6mg/kg/day could be considered

The early (after 72 hours of fever) introduction of ambisome or amphotericin is the most important measure and investigators MUST adhere to this. **Investigators must have a very high index of suspicion of invasive fungal infections, in these myelosuppressed patients and vigorous, empirical antifungal therapy must be given early in an episode of febrile neutropenia**

16.6 Pneumocystis Pneumonitis Prophylaxis

Pneumocystis carinii pneumonitis prophylaxis is mandatory, but may be given according to the recommendations of each national group. Patients should start at the time of diagnosis up to 6 months after MAT.

**During COJEC**

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

**During MAT**
Stop prophylactic treatment with SMZ/TMP from day 0 until at least day +10 or until WBC $\geq 1.0 \times 10^9/l$ after stem cell reinfusion.

16.7 Nutrition
According to the ENSG5 experience patients on the COJEC schedule require close monitoring of patient weight, food intake and tolerance. The early start of supplementary nutrition is highly recommended. Once a 10% weight loss occurs, the institution of naso-gastric alimentation with a high caloric nutritional formula and/or parenteral nutrition via the central venous line according to institutional standards is recommended.

16.8 Additional Supportive Care during the Acute Phase of MAT
- Protective isolation per local institutional guidelines.
- Prophylactic antifungal treatment with ketokonazole, itraconazole or fluconazole should be avoided, because of the increased risk of VOD with these drugs in particular in association with busulfan.
- Prophylaxis against HSV, VZV and/or CMV will be given in seropositive patients as per institutional guidelines.
- Infections: Documented or suspected infections during the cytopenic phase will be treated with appropriate antibiotics, anti-fungals, and/or antivirals as determined by the treating physician. Prophylactic use of the latter, in view of side effects and drug interactions, is strongly advised against.

16.9 Prophylaxis, Diagnosis and Management of Hepatic Veno Occlusive Disease
In spite of the published results of low dose heparin prophylaxis against hepatic veno-occlusive disease (HVOD) [173], the administration of heparin did not, in the French experience, improve the incidence or the severity of HVOD after administration of a busulfan containing regimen.

Recently V. Lapierre et al. reported on further IGR studies, suggesting that G-CSF mobilized PBSCR results in an increased incidence of anti-HLA-immunization and further confirms that the use of such a graft alters allo-immune Ab responses. It was recommended to alter the transfusion policy and not use ABO-incompatible platelet transfusions. This may help to further reduce HVO-incidence [174].

16.9.1 PROPHYLAXIS OF HVOD
No prophylaxis for HVOD is recommended. However, careful observation of patients during BuMel phase is required and management of HVOD is outlined in 16.9.3.
16.9.2 DIAGNOSIS OF HVOD

Clinical features
According to McDonald [175] 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.

Biological features
Hepatic function is often disturbed, with hyperbilirubinemia in more than 90% of cases. Elevated transaminases occur in 60-70%. Coagulation abnormalities are less frequent, reduction in Factor VII & X are most frequent, and may occur before any clinical signs.
A decrease in urinary sodium output is an early finding and constant (< 10 mmol/l), 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 [176].

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 paraumbilical vein. Doppler ultrasound can be useful to confirm the diagnosis. The results can be predictive and of prognostic relevance [177].

Histological features
Hepatic biopsy should not be performed on these children who are often in a perilous clinical state, except in the rare situation where there is doubt about the diagnosis. Histologic features consist of concentric subendothelial thickening and luminal narrowing of terminal hepatic venules or small sublobular veins by either oedematous reticulum fibres or collagen [178].

16.9.3 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, note that platelet transfusions contain considerable amounts of sodium.
- Defibrotide at the earliest sign of HVOD is strongly encouraged (see below for dosing guidelines).
- 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). This diuretic treatment is not always effective.
- Furosemide is ineffective, and may exacerbate renal failure. It may sometimes be useful with blood product transfusions.
- Platelet transfusions should be given, to maintain the platelet count in excess of 20 x 10^9/l. This level may be difficult to maintain even with twice daily transfusions.
- Opiate analgesia may be required for abdominal pain.
Abdominal paracentesis to drain ascites should only be carried out where the volume of ascites is causing serious respiratory distress, or when pain cannot be controlled by analgesia. However pain may be related to the enlarged liver rather than the ascites.

Albumin administration is not recommended, but might be considered when the albumin level is profoundly low (< 15g/l).

**Defibrotide Dosing Guidelines**

<table>
<thead>
<tr>
<th>Early signs of HVOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start Defibrotide for the treatment of HVOD at: 25 mg/kg/d i.v. divided into 4 doses</td>
</tr>
<tr>
<td>Taper Defibrotide by 10 mg/kg/week in case of:</td>
</tr>
<tr>
<td>Complete resolution of ascites and Return of hepatopedal flow and Normalisation of total and direct bilirubin*</td>
</tr>
</tbody>
</table>

* Omitted if other causes for hepatic injury occurred, i.e. GvHD of the liver

Return to the therapeutic dose if signs of VOD re-occur (bimodal presentation)

---

**16.10 Renal Function Monitoring**

**16.10.1 GLOMERULAR FUNCTION – GFR**

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

According to Schwartz's formula [179], creatinine clearance (Ccrea) can be calculated from single serum samples:

\[
C_{\text{crea}} = \frac{F \times \text{Height [cm]}}{\text{Crea serum [mg/dl]} \times \text{Height [cm]}} \times \left[ \frac{\text{ml/min}/1.73\text{m}^2}{\text{cm}^2} \right]
\]

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):

- \( \geq 1 \) year: 120
17 Statistics and Biometrical Methodology

The main scientific aims of this trial address therapeutic options to improve control of disease in two sequential randomisations (R2 and R3). As of October 2010 R1 is closed.

17.1 End Points

17.1.1 PRIMARY ENDPOINTS

Randomisation 1: MAT-question (BUMEL vs CEM) (closed October 2010)
The primary endpoint was the event free survival (EFS) calculated from the date of the first randomisation. The following was considered as events:
- disease progression or relapse
- death from any cause
- second neoplasm.
Patients lost to follow-up without event were censored at the date of their last follow up evaluation.

Randomisation 2: Immunotherapy-Question (Ch14.18/CHO ± aldesleukin (IL-2))
The primary endpoint is 3-year event free survival calculated from the date of the second randomisation. The following will be considered as events:
- disease progression or relapse
- death from any cause
- second neoplasm.
Patients lost to follow-up without event will be censored at the date of their last follow-up evaluation.

Randomisation 3: Induction-Question (Rapid COJEC vs modified N7)

Two co-primary endpoints will be investigated:

1) **Complete Metastatic response after induction**
   Complete Metastatic response is defined as
   - No skeletal uptake on mIBG
   - Negative bone marrow aspirates (by cytomorphology) and trephines
   - Absence of other metastatic sites

Metastatic response is chosen as primary endpoint as
- it is an established measure of therapeutic activity
- it is not affected by subsequent therapies
- it is highly correlated with outcome (i.e. EFS and Overall survival)
- can be assessed immediately after study termination, thus shorter follow up is needed.

Disadvantages of response as primary endpoint:
- it is a measure of activity and not a direct measure of clinical benefit for the patient
- even if metastatic response is a true surrogate for survival (i.e. the effect of the intervention on response completely predicts its effect on survival), a big effect on metastatic response may translate only into a modest effect on survival/EFS.
- differences in response rates may not translate to outcome at all (i.e. EFS and Overall survival) and
- differences in EFS may be observed with similar metastatic response, for example due to interaction with subsequent therapies.

2) Event Free Survival
The second primary endpoint is event free survival calculated from the date of the R3-randomisation. The following will be considered as events:
- disease progression or relapse,
- death from any cause
- second neoplasm.
Patients lost to follow-up without event will be censored at the date of their last follow-up evaluation.

EFS is chosen as primary endpoint as
- it is a well established and universally accepted endpoint in pediatric oncology
- it is closely related to clinical efficacy
- it reflects the ultimate aim of the study to improve patients ultimate outcome

The disadvantage of EFS as primary endpoint.
- it is confounded by the crossover effect of subsequent therapies. If subsequent treatments for patients without metastatic CR are efficacious, differences between the induction regimens in EFS can be diluted. Thus the therapeutic impact on EFS can be underestimated.
- long-term EFS can be evaluated only after prolonged follow up. However, short-term differences in EFS may be more likely to translate in differences in long-term outcome than differences in response rates.

The trial will be positive and conclude superiority for one arm, if at least one of both endpoints reaches statistical significance. If significant differences in EFS will be observed, the arm with superior EFS will be considered for future use. If no significant differences in EFS are seen, the arm that achieves significant better response rates is considered superior. This is reasonable, as non-responders switch to more intense and experimental treatments. Patients will take advantages from better response rates as this prevents them to switch to more intense, experimental treatments.

17.1.2 SECONDARY ENDPOINTS

Secondary endpoints of the main trial
- Overall survival
  Calculated from date of randomisation to death from any cause. Patients lost to follow-up without an event will be censored at the date of their last follow-up evaluation.
- Cumulative incidence of relapse/progression and deaths without relapse/progression
- The cumulative incidence of treatment related mortality and of disease related mortality
- Response
  Overall response (incl. primary tumour after induction), skeletal response on MIBG, BM-response,
- Toxicity
  in particular comparison of the frequency of episodes of febrile neutropenia and grade 3-4 infections during induction (modified N7 vs. Rapid COJEC)
- Response rates, survival, EFS and the cumulative incidence of relapse/progressions will be related to potential prognostic factors including:
  - Biological factors (MYCN amplification, SCAs, expression signature) of neuroblastoma cells in the bone marrow and/or the primary tumour.
- Serological factors (serum concentrations at diagnosis of LDH, ferritin, neuron specific enolase).
- Urinary catecholamines at diagnosis (VMA, HVA, Dopamine)

17.2 Randomisation
Randomisation will be done using the web-based SIOPEN-HRNBL study site of the SIOPEN-R-NET (https://www.siopen-r-net.org/)

17.2.1 RANDOMISATION 1: MAT-QUESTION (CLOSED IN OCTOBER 2010)

17.2.2 RANDOMISATION 2: IMMUNOTHERAPY-QUESTION

17.2.2.1 Randomisation requirements
- eligibility criteria met for R2
- signed informed consent for R2

17.2.2.2 Timing of randomisation
All patients eligible for the second randomisation will be randomised
- after completion of disease restaging following MAT

17.2.2.3 Mode of randomisation
Randomisation will be stratified by the following factors:
- National group
- Allocated by previous treatment (R1: BuMel, R1: CEM, Non R1 patients)

17.2.3 RANDOMISATION 3: INDUCTION-QUESTION (RAPID COJEC VS MODIFIED N7)

17.2.3.1 Randomisation requirements
- eligibility criteria met for R3
- signed informed consent for R3

17.2.3.2 Mode of randomisation
Randomisation will be stratified by the following factors:
- National group
- Metastatic sites (BM only, Skeleton only, other only, combined)

17.3 Patient Number Estimates
Based on the annual accrual rates of previous national studies the estimated accruals per year:
Austria 5, Belgium 6, Czech Republic 5, France 40, Greece 5, Hungary 2, Israel 15, Italy 40, Nordic Countries (Denmark, Sweden, Norway) 10, Poland 12, Portugal 6, Spain 14, Switzerland 3, United Kingdom 40)
Thus a maximum accrual of approximately 200 per year is anticipated.
17.4 Power Considerations

17.4.1 RANDOMISATION R1: MAT QUESTION (SINCE AMENDMENT 2006 – CLOSED OCTOBER 2010)
The 3-year EFS in the CEM-group (without immunotherapy) was estimated to be 45%. This trial aimed to show an improvement of 10% for the BUMEL-group. With a recruitment period of 6 years (630 patients randomised) and a minimum follow up of 1.5 years the power to show a difference of 10% is 80% (two-sided logrank test with \( \alpha = 5\% \)) [180].

Original Version of the protocol until Amendment 2006
About 75% of the expected annual recruitment of 175 patients will enter the first randomisation, i.e. about 130 randomisations/year. The 3-year EFS in the CEM-group (without immunotherapy) is estimated to be 45%. This study aims to show an improvement of 10% for the BUMEL-group (3-year EFS of 55%). With a recruitment period of 6 years (1050 pts entered and 780 randomised) and a minimum follow up of 1.5 years the power to show a 10% difference is 89% (bilateral formulation of the logrank test and \( \alpha = 5\% \)) irrespective of whether there is a benefit from immunotherapy.

17.4.2 RANDOMISATION R2: IMMUNOTHERAPY QUESTION
The 3-year EFS in the group without aldesleukin (IL-2) (i.e. isotretinoin (13-cis-RA) and ch14.18/CHO) is anticipated to be 55%. This trial aims to demonstrate an improvement of 12.5% by the addition of aldesleukin (IL-2). With a sample size of 400 patients, a recruitment period of 4 years, a minimum follow up of 2 years, and two-sided \( \alpha = 5\% \) the study has a power of 80% [180].

17.4.3 RANDOMISATION R3: INDUCTION-QUESTION (RAPID COJEC VS MODIFIED N7)
The R3-randomisation investigates two primary endpoints. The Bonferroni inequality is used to control the overall type one error rate at 5% (i.e. the chance to erroneously conclude that there is a benefit in at least one endpoint, when there is none, is 5%). Thus, for each endpoint an alpha of 2.5% is used.

According to previous data, the 2-year EFS in the group with Rapid COJEC is 40%. This trial aims to demonstrate an improvement of 12.5% with modified N7 (2-years EFS is 52.5%). With a sample size of 630 patients, a recruitment period of 3.5 years, a minimum follow up of 0.5 years, and two-sided \( \alpha = 2.5\% \), the study has a power of 84% [180].

According to our experience the metastatic response rate after Rapid COJEC is 33%. This trial aims to improve metastatic response to 45%. With a sample size of 630 patients and two-sided \( \alpha = 2.5\% \) the study has a power of 80%.

The overall power of the study (i.e. probability of correctly rejecting at least one null hypothesis) depends on the dependence of both hypotheses regarding EFS and metastatic response. This dependence is anticipated to be high, as both endpoints are correlated and treatment effects are anticipated to be related to each other. In the unlikely event of low dependences between the hypotheses, the power to detect a difference for at least one endpoint is higher as the 80 and 84% given in the calculations of above.
17.5 Analysis

17.5.1 Final Analyses
The final analysis will be performed 18 months for R1 and R2 and 6 months for R3 after the inclusion of the last patient.

To evaluate the difference of EFS between the randomised arms all randomised patients will be analysed and the comparison of treatment regimens will be performed according to intention to treat.

The 3-year EFS will be estimated by the Kaplan-Meier method. The standard errors of these estimates will be calculated according to the method described by Peto et al [181].

17.5.1.1 Randomisation 1: MAT Question (BUMEL vs CEM) (closed in October 2010)
The difference in EFS will be analysed using the log rank-test adjusted by the prognostic factors of age, stage and the addition of TVD (see [182]).

17.5.1.2 Randomisation 2: Immunotherapy Question (Ch14.18/CHO with or without aldesleukin (IL-2))
To evaluate the effect of two immunotherapy schedules the log rank-test adjusted by the previous treatment group (R1: BuMel, R1: CEM, Non R1 patients) will be used (see [182]).

17.5.1.3 Randomisation 3: Induction-Question (Rapid COJEC vs modified N7)
The R3-randomisation investigates two primary endpoints. The Bonferroni inequality is used to control the overall type one error at 5%. Thus, for each endpoint an alpha of 2.5% is used and one arm is considered superior, if the p-value is below 0.025 for at least one hypothesis.

To compare metastatic response rates in patients with Rapid COJEC to patients with modified N7, logistic regression will be used. The analysis will be adjusted for metastatic involvement at diagnoses, i.e. BM-involvement (trephine and/or aspirates), skeletal uptake on MIBG, other metastatic sites.

To compare the induction regimens, Cox-regression adjusted for metastatic involvement at diagnoses, i.e. BM-involvement (trephine and/or aspirates), skeletal uptake on MIBG, other metastatic sites, will be used. (see [182])

If significant differences in EFS are observed, the arm with superior EFS will be considered for future use. If no significant differences in EFS are seen, the arm that achieves better response rates is considered superior. Non-responders switch to more intense and experimental treatments. If these subsequent therapies are efficacious, this may dilute the effect of induction regimens on EFS. Patients will take advantages from better response rates as this prevents them to switch to more intense, experimental treatments. In an exploratory Cox-regression analysis, the impact on EFS of the induction regimen and their interaction with subsequent rescue therapies will be investigated.

17.5.2 Interim Analyses
The study will be monitored by an annual report to an independent Data Monitoring and Safety Committee. It is anticipated, that the Peto stopping rule may be used as a guide by the DMSC but this alone will not provide the only basis for their recommendations. Thus the recommendation to close or continue a trial is essentially a medical one.

It is anticipated, even if the R1 randomisation were discontinued, that the trial will continue for the R2 randomisation on immunotherapy but with the recommended MAT arm only.
17.6 Comparison of oral Busulfan vs. I.V. Busulfan (Busilvex®) within the HRNBL1/SIOPEN trial

For the 3rd amendment of this protocol (July 2007) the study committee suggested the change from oral Busulfan to I.V. Busulfan (Busilvex®). Due to this the following additional hypothesis arises:

17.6.1 AIM

For patients above the age of one the primary aim of the study is the comparison of the VOD-rate of I.V. Busulfan (Busilvex®) and oral Busulfan. It is anticipated that I.V. Busulfan (Busilvex®) will produce lower VOD rates with equivalent efficacy in terms of Event Free Survival and Overall Survival.

In infants the aim is to estimate the VOD-rate. Infants were eligible to the trial since April 2006. Infants are not eligible to R1 randomisation and recommended to receive BUMEL-MAT regimen.

17.6.2 END POINTS

Primary Endpoints

The primary endpoint is the incidence of VOD (Bearman Score 1-3) after MAT.

Secondary Endpoints

- Overall Survival
- Event Free Survival
- CTC-Toxicities after MAT

17.6.3 PATIENT NUMBER ESTIMATES

Until the anticipated start of the use of I.V. Busulfan (Busilvex®), 160 patients above the age of one will be randomised to the BUMEL regimen. By that time additionally 320 patients will be necessary for the final evaluation of R1, thus 160 patients above the age of one will be randomised to receive BUMEL with I.V. Busulfan (Busilvex®).

Note: I.V. Busulfan (Busilvex®) is free of charge for 150 R1 randomised patients (total number for all participating countries except Israel) plus 10 R1 randomised patients from Israel.

We anticipate that until the closure of the study additionally 50 infants will be included in the trial and will receive the BUMEL regimen. However, I.V. Busulfan (Busilvex®) is not free of charge for infants.

17.6.4 POWER CONSIDERATIONS

In patients above the age of one the VOD rate with oral Busulfan is 30%. This trial aims to show an improvement for the I.V. Busulfan (Busilvex®) group with an anticipated VOD rate of about 15%. This improvement can be shown with a total sample size of 320 pts. above the age of one with a power of 80% (two-sided logrank test with $\alpha = 5\%$)
In infants, a sample size of 50 patients produces a 95% confidence interval equal to the sample proportion ± 9% (when the estimated proportion is 15%).

Note: I.V. Busulfan (Busilvex®) is not free of charge for infants.

17.6.5 Analysis

17.6.5.1 Final Analyses

In patients above the age of one, Chi Square test will be used to evaluate the differences of VOD with oral Busulfan compared to I.V. Busulfan (Busilvex®).

In infants, an exact 95% confidence interval of the sample proportion of VOD will be calculated.

17.6.5.2 Interim Analyses

The study will be monitored by an annual report to an independent Data Monitoring and Safety Committee. At these interim-analyses all primary and secondary endpoints will be evaluated. It is anticipated, that the Peto stopping rule may be used as a guide by the DMSC but this alone will not provide the only basis for their recommendations. Thus the recommendation to close or continue a trial is essentially a medical one.

17.7 Monitoring Guidelines for Severe Toxicities

Severe toxicities will be monitored continuously and summarised for group members on a 2-monthly basis. Therefore toxic deaths, surgical events, and MAT related events must be reported immediately. The study centre must also be informed within 1 week about all surgical interventions performed and about patients who are going off study due to progression during the induction chemotherapy. This is needed to be able to judge toxicity rates correctly.

17.7.1 Related to Induction Phase

Log-rank test will be used to compare overall survival within 90 days. The interval will start at randomisation. Deaths from any cause within 90 days will be considered as events. Patients will be censored at day 90. For patients, that have been randomised less than 90 days before, the data cut-off, the data cut-off date will be used as censoring-date. If the p-value is smaller than 0.05, the death-rates during induction will be formally reviewed to check if any modification to the induction therapy is required. This evaluation will be done annually, the first evaluation will be done after at least 75 patients have been included in the randomised trial.

17.7.2 Related to Surgery

If the Kaplan Meier estimated surgical event rate exceeds 5 % within 15 days after surgery then the surgical events will be formally reviewed to check if any modification to the surgical procedure is required.

17.7.3 Related to MAT Phase

The absolute severe adverse MAT related event rate observed in each arm of the first randomisation will be compared to a reference rate in order to detect an absolute excess of toxic events. The need for ventilation or haemofiltration in an ICU setting or death are considered adverse MAT related events. If the Kaplan-Meier estimate of the MAT related event rate within 100 days exceeds 10% the information will be forwarded to the DMC. The DMC will then make a recommendation, after consultation with the study co-ordinators and statisticians, as to how the trial should proceed.
18 APPENDIX: Staging Criteria

18.1 Diagnosis of Neuroblastoma

A diagnosis of neuroblastoma is established if:
1. An unequivocal pathological diagnosis is made from tumour tissue by light microscopy (with or without immunohistology, electron microscopy, increased urine or serum catecholamines or metabolites)*
OR
2. Bone marrow trephines contain unequivocal tumour cells *(e.g. syncytia or immunocytoplogically positive clumps of cells) and increased urine or serum catecholamines or metabolites *

Notes
Ψ If histology is equivocal, karyotypic patterns or abnormalities in tumour cells characteristic of other tumours (e.g. t(11;22)), then exclude a diagnosis of neuroblastoma, whereas genetic features characteristic of neuroblastoma (1p deletion, MYCN amplification) would support this diagnosis.
* Catecholamines and metabolites include dopamine, HVA and/or VMA; levels must be > 3 SD above the mean for age (measured in mmol per mmol creatinine)# to be considered increased, and at least two of these metabolites must be measured.
# Timed urine collections are difficult in young children so normalisation per mmol creatinine does away with the necessity for a timed collection and also avoids the problem of false negatives due to dilute urine.

18.2 Assessment of Extent of Disease

<table>
<thead>
<tr>
<th>Tumour Site</th>
<th>Recommended Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary Tumour</strong></td>
<td>CT and/or MRI scan* with 3D measurements. MIBG scan if available†.</td>
</tr>
<tr>
<td><strong>Metastatic Sites</strong></td>
<td></td>
</tr>
<tr>
<td>Bone marrow</td>
<td>Bilateral posterior iliac crest marrow aspirates and trephine (core) bone marrow biopsies required to exclude marrow involvement. A single positive site documents marrow involvement. Core biopsies must contain at least 1 cm of marrow (excluding cartilage) to be considered adequate</td>
</tr>
<tr>
<td>Bone</td>
<td>mIBG† scan; ⁹⁹Tc scan is required if mIBG scan negative or unavailable, and plain radiographs of positive lesions are recommended.</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>Clinical examination (palpable nodes), confirmed histologically. CT scan for non-palpable nodes (3D measurements)</td>
</tr>
<tr>
<td>Abdomen/liver</td>
<td>CT and/or MRI scan* with 3D measurements</td>
</tr>
<tr>
<td>Chest</td>
<td>AP and lateral chest radiographs. CT/MRI necessary if chest radiograph positive, or if abdominal mass/nodes extend into the chest</td>
</tr>
</tbody>
</table>

Notes
* Ultrasound examination considered suboptimal for accurate 3D measurements
† The mIBG scan is applicable to all sites of disease.
18.3 INSS Staging

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

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

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

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

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

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

Notes

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

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

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

18.4 INRC Definition of Response

<table>
<thead>
<tr>
<th>Response</th>
<th>Primary tumour*</th>
<th>Metastatic Sites†‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>No tumour</td>
<td>No tumour; catecholamines normal</td>
</tr>
<tr>
<td>VGPR</td>
<td>Decreased by 90-99%</td>
<td>No tumour; Residual 99Tc bone changes allowed</td>
</tr>
<tr>
<td>PR</td>
<td>Decreased by &gt; 50%</td>
<td>All measurable sites decreased by &gt; 50% Bones and bone marrow: Number of positive sites decreased by &gt; 50%; no more than 1 positive bone marrow site allowed†.</td>
</tr>
<tr>
<td>MR</td>
<td>No new lesions; &gt; 50% reduction of any measurable lesion (primary or metastases) with &lt; 50% reduction in any other; &lt; 25% increase in any existing lesion.</td>
<td></td>
</tr>
<tr>
<td>NR</td>
<td>No new lesions; &lt; 50% reduction but &lt; 25% increase in any existing lesion.</td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>Any new lesion; increase of any measurable lesion by &gt; 25%; previous negative marrow positive for tumour.</td>
<td></td>
</tr>
</tbody>
</table>

Notes
CR Complete Response
VGPR Very Good Partial Response
PR Partial response
MR  Mixed Response  
NR  No Response  
PD  Progressive Disease  
*  Evaluation of primary and metastatic disease as outlined in Table 2  
†  One positive marrow aspirate or biopsy is allowed for PR if this represents a decrease in the number of positive sites at diagnosis.

### 18.5 Trial Specific Response Definitions

<table>
<thead>
<tr>
<th>Response</th>
<th>Metastatic Sites</th>
</tr>
</thead>
</table>
| **Metastatic CR** | • skeletal mIBG negative  
                   • No positive bone marrow biopsy  
                   • Cytomorphological CR in 2 bone marrow aspirates |
| **Metastatic PR** | • > 50% reduction in skeletal mIBG  
                   • No new lesions  
                   • Not more than 3 positive, but improved spots on mIBG  
                   • No positive bone marrow biopsy  
                   • Cytomorphological CR in 2 bone marrow aspirates |

**Notes**  
CR  Complete Response  
PR  Partial response  
*  Evaluation of primary and metastatic disease as outlined in Table 2
19 APPENDIX: Pathology and Biology Guidelines for resectable and unresectable Neuroblastic Tumours and Bone Marrow Examination Guidelines

Gabriele Amann M.D. (Austria, g.amann@akh-wien.ac.at)
Klaus Beiske M.D., Ph.D. (Norway, klaus.beiske@labmed.uio.no)
Emanuele d'Amore M.D. (Italy, emanuele.damore@unipd.it)
Inge M. Ambros, M.D., (Austria, peter.ambros@ccri.at)
Peter F. Ambros, Ph.D. (Austria, inge.ambros@ccri.at)
Jean Benard Ph.D. (France, benard@igr.fr)
Nick Bown (United Kingdom, nick.bown@ncl.ac.uk)
Sue Burchill, Ph.D. (United Kingdom, S.A.Burchill@leeds.ac.uk)
Valerie Combaret Ph.D. (France, COMBARET@lyon.fnclcc.fr)
Maria-Valeria Corrias, Ph.D (Italy, mariavaleriacorrias@ospedale-gaslini.ge.it)
Jerome Couturier M.D. (France, jerome.couturier@curie.net)
Sandro Dallorso, MD (Italy, sandrodallorso@ospedale-gaslini.ge.it)
Raffaella Defferrari (Italy, RaffaellaDefferrari@ospedale-gaslini.ge.it)
Olivier Delattre M.D. (France, Olivier.Delattre@curie.fr)
Claudio Gambini M.D. (Italy, claudiogambini@ospedale-gaslini.ge.it)
Nicole Gross (Switzerland, nicole.gross@chuv.hospvd.ch))
Tim Lammens (Belgium, tim.lammens@ugent.be)
John Lunec Ph.D. (United Kingdom, John.Lunec@newcastle.ac.uk)
Barbara Marques (Portugal, barbara.marques@insa.min-saude.pt)
Tommy Martinsson Ph.D. (Sweden, tommy.martinsson@clingen.gu.se)
Katia Mazzocco PhD (Italy, KatiaMazzocco@ospedale-gaslini.ge.it)
Rosa Noguera (Spain, Rosa.Noguera@uv.es)
Michel Peuchmaur M.D. (France, michel.peuchmaur@rdb.ap-hop-paris.fr)
Gudrun Schleiermacher (France, gudrun.schleiermacher@curie.net)
Frank Speleman Ph.D. (Belgium, franki.speleman@ugent.be)
Gian Paolo Tonini Ph.D. (Italy, gianpaolo.tonini@istge.it)
Deb Tweddle (United Kingdom, d.a.tweddle@newcastle.ac.uk)
Alexander Valent (France, avalent@igr.fr)
Ales Vicha (Czech Republic, avicha@yahoo.com)
Nadine Van Roy Ph.D. (Belgium, nadine.vanroy@ugent.be)
Virginie Viprey, Ph.D (UK, v.viprey@leeds.ac.uk)

19.1 General Remarks

- The resected tumour or the biopsy should be transferred from the operating room to the local pathology department under sterile conditions as quickly as possible – please indicate time of transport!
- Tumour handling and sectioning should always be performed by the pathologist (as soon as possible; the time the tumour samples were taken should be stated) following the scheme presented in these Guidelines. If this is not possible, two tumour samples should be frozen directly in the operating theatre (at -70°C or in liquid nitrogen).
- At least two macroscopically different tumour areas (if present) should be chosen for molecular-genetic/biological analyses.
The patient’s peripheral blood (5-10ml, with EDTA or heparin or according to the requirements of the laboratory) is needed for molecular-biological studies as reference and should be sent to the reference biology laboratory together with the tumour specimens.

The material selected for molecular-genetic/biologic investigations should be sent as quickly as possible to the National Reference Biology Laboratory. Please refer to the Appendix (chapter 19) for contact details specific to your National Group. In case of queries please contact the National group co-ordinator.

The paediatric oncologist in charge and/or the surgeon has to inform in a timely manner the local pathologist and biologist (local and/or national) about the new patient and the material to be expected.

19.2 Pathology guidelines

19.2.1 REMARKS AND RECOMMENDATIONS FOR PATHOLOGY

The handling of the tumour tissue should always be performed by the pathologist who, besides the important task of making morphologic diagnoses and giving prognoses based on histopathologic findings, should choose the relevant tumour areas for molecular-genetic/biological analyses as another major task. This procedure is a sine qua non to enable reliable interpretation of the molecular-genetic results for which the exact tumour cell content of the specimen used for these investigations has to be determined. This is possible only if the pathologist evaluates the specimens adjacent to those used for molecular-genetic/biological analyses (for details see below). In all instances, concerning either tumour resection or biopsies, tumour material from different tumour areas (nodules are of special interest!) ought to be taken for histologic and molecular-genetic/biological examination. The reason for this recommendation is based on the observation of tumour heterogeneities at the genetic level (e.g. for the MYCN and/or the chromosome 1p status) and/or at the histologic level (ganglioneuroblastoma, nodular subtype according to the International Neuroblastoma Classification, INPC,103 both of which have prognostic implications. Close co-operation between pathologists and biologists is therefore strongly recommended. Pathologists should inform the biologists if morphologically unfavorable looking areas are present in the paraffin embedded material but most likely not in the specimens selected for molecular-genetic/biological investigations. These areas should also be specifically analysed using the paraffin material.

In case the tumour pieces selected for molecular-genetic/biological investigations were not appropriate for getting reliable results, MYCN and chromosome 1p36.3 status and ploidy can be determined on the paraffin embedded material.

19.2.2 SECTIONING AND SECURING TUMOUR MATERIAL IN CASE OF RESECTED TUMOURS

The following procedure is recommended (it is necessary for the pathologist to have help from a person, i.e. from a technician or the paediatric oncologist):

Cut the tumour along the largest diameter and take at least two samples from morphologically different-appearing areas (1x1x1cm) if such are present. Tissue from a suspected nodule must always be sampled. Identify the samples specifically with capitals (A, B, etc.), or whatever system is the practice of each laboratory, and cut each of them into four pieces which are marked with numbers (e.g. tumour specimen A 1-4, specimen B 1-4). More material can be processed in the same way (C, D, etc.), but material from two different areas is the minimum. Check carefully for the presence of nodules! (See also Figure 3).

Samples A1 and B1: make 10 touch preparations (at least 5) from a freshly cut surface. The slides are air-dried and unfixed and, if necessary, they can be stored at –20°C (storage of the slides
for one week at room temperature does not adversely affect the following analyses); for fluorescence based in situ hybridisation (FISH) and image cytometry (ICM). **After making the touch preparations, these pieces should be fixed in formalin for routine histologic examination.** This also should include the determination and indication of the tumour cell content versus content of normal cells, such as Schwann cells, lymphocytes, fibrovascular stroma etc.; amount of necrosis should be indicated as well. This information is crucial for the interpretation of the FISH, ICM and cytogenetic results!

**Samples A2,3 and B2,3: snap freeze as soon as possible** in separate vials in liquid nitrogen or at −70°C carbon dioxide. Please indicate the time between extraction of the tumour and freezing. Before using these for further analyses, making cryosections for the determination of the tumour cell content is mandatory.

**Samples A4 and B4: put in sterile culture medium** (RPMI 1640) for preparation of tumour cell suspensions which may serve for evaluation of ploidy, drug sensitivity, etc. Tumour cell content should be checked by immunocytology on the cytospin preparations using appropriate antibodies (see below).

**The samples should be forwarded as soon as possible!** After this procedure, the rest of the surgical specimen can be fixed in formalin and worked-up according to standard guidelines. The whole central 4mm section of the tumour at the plane of the largest diameter should be embedded. A minimum of one tumour section per cm should be embedded from the whole specimen including central and peripheral areas of the tumour. Surgical margins must be reliably and reproducibly identifiable.

**Figure 3.** Tumour samples (at least 1x1x1cm) are designated with the letters A, B, etc. There are two methods of sectioning. From piece 1 touch preparations are made before formalin fixation; in the variant on the right hand side, one of the central sections is designated as piece 1. Pieces 2, 3 (and 4) are snap frozen. Piece 4 is put in RPMI 1640 for classic cytogenetic investigation and/or making cytospin preparations. In case of small specimens, touch preparations and snap freezing are the first priorities.

**19.2.3 Securing Tumour Material in Case of Unresectable Tumours**

The general recommendations given above should be followed. The sectioning procedure depends on the amount of tumour material available for diagnostic histology and molecular-genetic/biological investigations. **Touch preparations can almost always be performed before fixing and embedding the tumour material** (for tru cut and fine needle aspirations see below).

**All the recommendations for open biopsies, tru cut biopsies and fine needle aspirations (e.g. number of different tumour areas) concern only those patients for whom the risk of taking tumour material is considered to be reasonable.** This risk has to be weighed carefully; for example in the case of patients with stage 4s neuroblastomas with coagulation disorders, or patients with large and fragile tumours. It is clear that if the procedure to retrieve material is risky, it should not be performed! One should think about a less dangerous procedure, e.g. biopsy of subcutaneous metastases or bone marrow aspiration, instead of a dangerous biopsy from the primary tumour.
Tru cut biopsies (A, B, etc.), at least 1cm long, 0.1cm thick.

Figure 4. Sectioning of biopsy cylinder for histologic and molecular-genetic/biological investigations. The exact sizes of pieces used for the individual investigations should be discussed between pathologist and biologist.

19.2.3.1 Open Biopsies
In case of open biopsy, **two different areas** of the tumour should be biopsied by the surgeon. Specimen size should equal **at least 1cm³**. In accordance with the **recommendations given above**, the tumour specimens are labelled with capitals (A, B etc.), are cut into 4 pieces, and the procedure given in the paragraph above can be followed.
In case of **smaller specimens**, the material can be cut into only three pieces (piece 1 for touch preparations and histology; piece 2 for snap freezing; piece 3 for RPMI 1640) or into two pieces (piece 1 for touch preparations and histology and piece 2 for snap freezing).

19.2.3.2 Tru cut Biopsies
- **General remarks.**
  If only tru cut biopsies are feasible, preferably four samples (at least two biopsies in case of small lesions) from different areas of the tumour should be obtained. The recommended needle size is 18G. Tru cut biopsies are usually ultrasound-guided and performed by radiologists, but the pathologist should be present during the procedure and immediately assess the quality of the specimens (macroscopically). This is in order to save trouble and frustration due to delivery of mainly necrotic material (see below). It is important that the tumour cell content be determined in the biopsied material used for molecular-genetic/biological analyses.

  - **Handling of tru cut biopsies.**
    The pathologist/radiologist should not try to scrape off the tissue from the biopsy needle using a scalpel or other devices, but put the needle into a tube/glass with sterile RPMI 1640 or Hanks or PBS solution and shake gently until the specimen is released into the fluid. This procedure helps to avoid artefacts due to crushing and squeezing. In addition, needle biopsies tend to stick to each other which may hamper the subsequent division of the material. Therefore, each biopsy should be kept in a separate tube in the above mentioned sterile solution.

  - **Assessment of quality.**
    Specimens should be at least 1 cm long and 0.1 cm thick. Tiny and fragmented material cannot be approved. If there is any doubt regarding the quality of the material, shake the glass with the specimen thoroughly once or twice. If the specimen breaks into pieces, it often indicates tissue necrosis. The pathologist should not hesitate to ask for a new biopsy. It is impossible to judge macroscopically, whether a needle biopsy contains tumour cells or mainly stroma. Therefore, an ideal procedure would be, if feasible, to have tru cut biopsies preceded by fine needle aspiration which (a) informs about the tumour cell content of the elected area after only a few minutes, and (b) produces cell suspensions for ploidy measurement and large numbers of cytological specimens for FISH analyses.

  - **Securing of tru cut biopsies.** Either of two acceptable methods can be recommended for the securing of tru cut biopsies.

    **Method 1)**
    All pieces obtained are identified with capitals (A, B, etc.) and transferred from the tubes into separate Petri dishes, pouring the entire contents of the tube into the dish. Each sample is divided
carefully into two parts using a scalpel (Figure 4). This procedure should be done without using tweezers to avoid crush artefacts. **One part** of each piece is carefully transferred into a tube with **formalin** using a Pasteur pipette (plastic, for single use) to avoid crush artefacts. The **other half** of each piece is **snap frozen**. Put the specimen carefully on the wall of a freezing tube in order to avoid a curved orientation of the piece and put the tube into liquid nitrogen. The pathologist and the biologist should discuss and agree on the size of the parts they need for histology and for the biological analyses. Alternatively, one whole biopsy specimen could be used for histology and the second (third, etc.) specimen can be split. Before the material is transferred to the biologist, the **representativity** (tumour cell content) must **always** be checked and documented by the pathologist either on frozen sections or on touch preparations (immunocytochemistry).

**Method 2)**

If four pieces are available, two pieces are transferred from the isotonic solution or the RPMI 1640 into 4% buffered formalin in separate glasses and embedded in separate paraffin blocks (marked A and B) for morphologic and immunohistochemical analysis. Two pieces are gently transferred by tweezers into separate, flat-bottomed plastic tubes, carefully overlaid by a water-based embedding compound for preparation of frozen tissue sections (try to keep the specimen localised at the bottom level of the vial!) and must be **snap frozen** as soon as possible in liquid nitrogen or at –70°C carbon dioxide for molecular-genetic/biological analyses and immunocytochemistry. Ten touch preparations are made as described below. If only two pieces are available for analysis, one is transferred into formalin and embedded in paraffin and the other piece is snap frozen as described above followed by making 10 touch preparations.

- **Procedure for making touch preparations**

  It is well known that preparations containing whole nuclei (touch or cytospin preparations) are superior for FISH analyses. However, fresh unfixed needle biopsies only rarely yield enough cells when used for touch preparations. This problem can be solved by using the snap frozen material (see above) for making touch preparations. In method 1), the frozen tumour piece can be directly touched on warm glass slides, without thawing the tissue. After this, a frozen section for tumour cell determination should be performed. In method 2) the plug of embedding compound, containing the biopsy material at the bottom, is carefully removed from the tube with a forceps by warming the tube wall between two fingers, and mounted in a microtome at –20°C. Frozen sections are cut starting from the bottom of the frozen plug, stained with H&E and screened in a microscope until sufficient areas of morphologically intact tumour cells are found (representativity). It is important to perform frozen sections prior to the preparation of imprints because the cutting procedure removes any embedding compound which might have covered the tissue, merge with the tumour cells and thus impair the quality of the imprints. The cold cut surface of the plug is used for making 10 touch preparations on warm (room temperature) glass slides, but the plug should be carefully refrozen in time, because complete thawing destroys the morphology and renders the material inappropriate for any in situ-analyses at the single cell level. However, such specimens can still serve as sources for DNA/RNA and protein extraction (Southern blot, Polymerase chain reaction, comparative genome hybridisation, Northern blot, Western blot).

  Tru cut biopsies eventually frozen in plastic tubes without a water based embedding compound, are well suited for investigations based on DNA/RNA and protein extraction. However, it might pose a serious problem to use small, frozen and non-embedded pieces of e.g. stroma-poor, fragile tumour tissue for preparation of imprints. Without the mechanical support of the embedding compound, they thaw rapidly, become crushed or even lost and unavailable for the analysis of morphology and tumour cell content. In this case frozen, non-embedded tru-cut biopsies are transferred into a water-based compound and frozen sections are cut before preparing imprints.
Further comment. If the frozen material does not contain a sufficient number of tumour cells, FISH and ploidy analyses can be assessed on nuclei extracted from the paraffin-embedded biopsy material.

19.2.3.3 Fine Needle Aspiration Cytology (FNAC)

- General remarks.
  In case of unresectable tumours, the pathologists and biologists can also be confronted with tumour material derived from fine needle aspiration (FNA). In general, at least two separate punctures/aspirations should be performed from each tumour. Depending on the size of the tumour, the needle should be moved backward and forward into different areas under constant aspiration in order to sample from more than one region of the lesion (important for representativity!). FNA is also recommended to be done before true cut biopsies are taken (for determination of the representativity of elected tumour area!). The recommended needle size is: external diameter of 0.6-0.7 mm; 22-23 Gauge. The FNA sampling is usually performed in close co-operation between radiologist and cytopathologist. Due to the results of palpation and ultrasound examination of the tumour, FNA is either performed directly by the cytopathologist (palpable tumours) or by means of ultrasound guidance (non-palpable tumours). In this case, the radiologist guides the needle into the lesion, while the cytopathologist aspirates.

- Handling
  It should be considered that the ultrasound gel when aspirated, might ruin the morphology of the cells. Therefore, the skin must be wiped carefully before puncture. An adequate aspiration will often contain $10^4$-$10^6$ cells. The aspirate should always be utilised for preparation of smears and cell suspensions.
  Depending on the amount of aspirate, one droplet is placed on each of at least four slides and ordinary monolayer smears are produced and air-dried. Subsequently, the needle and the rest of aspirate are flushed through with 1 ml sterile phosphate buffered saline and the cell suspension is kept in the Eppendorf vial. One smear from each aspiration is stained by the DiffQuik method which takes 3 min to render the smear light-microscopically evaluable (preferentially done by an assisting cytotechnician!). A preliminary report on adequacy of the sample is given to the clinician and the radiologist, and the cytopathologist decides whether the FNA has to be repeated.

- Recommendations for subsequent analyses
  Smears are well-suited for morphologic evaluation (May-Grünwald-Giemsa stain), immunocytoLOGY, FISH (MYCN and 1p status etc.), and image cytometry, e.g. for static ploidy analysis. Cell suspensions can be used for flow-cytometry analysis of ploidy, tissue culture for e.g. cytogenetics, preparation of cytospins, which are air-dried over night and used for the same purposes as smears.

QUALITY ASSESSMENT

A SERIES OF CYTOSPIN PREPARATIONS OFTEN CONTAINS A MORE EQUAL (I.E. PREDICTABLE) NUMBER OF TUMOUR CELLS AS COMPARED TO A SERIES OF INDIVIDUALLY PRODUCED TOUCH PREPARATIONS AND SMEARS. ONE SMEAR/CYTOSPIN OF EACH SERIES/ASPIRATION HAS TO BE ANALYSED BY MORPHOLOGY/IMMUNOCYTOLoGY IN ORDER TO ESTIMATE THE NUMBER OF TUMOUR CELLS. THIS INFORMATION MUST BE SENT TO THE REFERENCE BIOLOGY LABORATORY. CYTOSPINS, SMEARS AND CELL SUSPENSIONS MAY BE STORED FROZEN FOR FUTURE ANALYSIS.

19.2.3.4 Bone Marrow Aspirations (see below)

In case of a sufficient high tumour cell number infiltrating the bone marrow, these cells can also be used for determination of the genetic composition of the disseminated neuroblastic tumour cells. Lower infiltrations should always be analysed by FISH for evaluating the MYCN and 1p status. In unclear situations, a GD2 staining preceding the FISH is highly recommended.
19.2.4 HISTOLOGY/CYTOMETRY REPORT

**Surgically resected tumours.** Morphologic classification: The tumour should be classified according to the *International Neuroblastoma Classification*. The mitotic rate and calcifications should also be indicated. Surgical margins of resection: There should be a comment regarding whether there are tumour cells infiltrating the resection margins or not, without making any conclusion as to whether the tumour residual is microscopic or macroscopic.

**Histologic report on the specimens A1, B1 etc.:** This report must clearly indicate the estimated percentage of tumour cells, i.e. neuroblastic/ganglionic cells, versus Schwann cells and other normal cells contained in the samples used for the biological studies. A copy of the report should then be submitted to the molecular biologist.

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

**Fine Needle Aspiration Cytology (FNAC).** If only FNAC material is available for primary diagnosis, the report including number, morphology (differentiation status of tumour cells, Schwann cells, necrotic debris) and immunophenotype of the cells analysed must be submitted to the biologist.

19.2.5 TUMOUR MATERIAL OBTAINED AFTER CYTOTOXIC THERAPY

Sectioning of the tumour material in resected tumours or biopsies after cytotoxic therapy can be done following the same guidelines as for tumours resected or biopsied at diagnosis before cytotoxic therapy. However, for sampling, it must be remembered that necrotic areas and also calcifications can be massive. Therefore, it is essential to state exactly the percentage of viable tumour cells versus normal cells (see above), and to indicate the amount of necrosis and calcification. It is known that both chemo- and radiotherapy can induce marked morphologic changes and can also induce cytodifferentiation and maturation (with development of a Schwann cell stroma), but most likely do not change the original genetic characteristics of the tumour. Therefore, assignment to the prognostic subgroups must not be made, although the tumours can be classified morphologically according to the INPC. The statement on pre-operative therapy has to be included in the diagnosis.

19.2.6 REGIONAL LYMPH NODE EXAMINATION

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


19.2.7 IMMUNOHISTOLOGY/-CYTOLOGY

**Differential Diagnosis.** In some cases, i.e. neuroblastomas, undifferentiated subtype according to the INPC, the differential diagnosis can present difficulties. In these instances, the use of the following antibodies is recommended: MIC2 (CD99), actin, desmin, low molecular-weight cytokeratin, leukocyte common antigen (CD45), and vimentin. These antibodies are usually negative in neuroblastic tumours. Positive markers are: CD56 (N-CAM), NB84a, (monoclonal neuron specific enolase (NSE), neurofilament triplet protein (NF), synaptophysin, tyrosine hydroxylase, and the protein gene product 9.5). However, it has to be kept in mind that these markers, although often positive, may also be negative in undifferentiated neuroblastomas. In addition, NB84a also reacts with epithelial cell and endothelial cells (see also below). Although GD2 is positive in virtually all cases of neuroblastic tumours and very useful in for example detection of neuroblastic cells in the bone marrow, anti GD2 staining cannot be recommended for use on paraffin sections (due to very high background staining). Moreover, GD2 expression is not restricted to neuroblastic tumours!

**Lymph nodes.** For differential diagnosis see above. In addition, the morphological result can be controlled by immunohistology using anti-CD56 and anti-NSE antibodies. If the NB84a antibody is applied, its reaction with endothelial cells should be kept in mind.

**Cytologic material.** For detection and quantification of tumour cells in bone marrow and fine needle aspirates, anti-GD2 for bone marrow diagnostics, and anti GD2, NB84a, anti CD56 and anti S-100 for material obtained by fine needle aspiration are recommended. For differential diagnosis see above.

**Determination of the tumour cell content.** See Histology/Cytology Report and Biology Guidelines.

19.2.8 EXACT DETERMINATION OF THE TUMOUR CELL CONTENT

It is mandatory that the tumour cell content is evaluated in all samples used for molecular-genetic/biological investigations and DNA analyses. GD2, NB84a and common leukocyte antigen as well as the use of S-100 for unequivocal detection of Schwann cells is recommended. If the number of tumour cells in the touch preparations is low and obviously not corresponding to the tumour cell content in the paraffin material the imprints originate from, the touch preparations have to be checked by immunocytology for the presence of tumour/stromal cells. This should be done preferably in combination with FISH for MYCN, 1p36.3 or other chromosomal aberrations (for further details see also Biology Guidelines).

19.2.9 PATHOLOGY REVIEW

The SIOP Europe Neuroblastoma Board and Pathology Committee decided that all tumours are to be reviewed by a central review panel. H&E slides from all available paraffin blocks or slides from 2 representative blocks and 10 unstained sections have to be sent to the National reference pathologist. It is especially important to include the H&E slide from the paraffin block adjacent to the tumour specimens used for molecular-genetic/biological investigations (sample A, B. etc. 1). In addition, 10 unstained sections from the most representative paraffin blocks and a copy of the written report including immunohistologic results should also be forwarded to the National reference pathologist.

19.3 Biology Guidelines

**19.3.1 GENERAL REMARKS AND RECOMMENDATIONS FOR BIOLOGY**

In neuroblastomas, investigation of the MYCN gene status, of the chromosomal region 1p36.3 and of the DNA content gives critically important information. The recommended methods for the individual parameters are summarised below. Besides the use of molecular-genetic/biological methods, it has to be stressed that classical cytogenetic analyses frequently give excellent results.
As already pointed out in the Pathology Guidelines, a reliable interpretation of the molecular-genetic results is possible only if the exact tumour cell content of the specimen used for these investigations is determined. This is possible only if the pathologist evaluates the specimens adjacent to those used for molecular-genetic/biological analyses. An exact determination of the tumour cell content and correct tumour sampling are crucial prerequisites and absolutely necessary for obtaining reliable molecular-genetic/biological results (see also above). The slides used for molecular-genetic/biological analyses and the blots should be stored according to national standards. It is recommended that FISH slides be stored in the dark at 4°C.

19.3.2 PROCEDURES FOR THE DETERMINATION OF THE TUMOUR CELL CONTENT
In case of resected tumours and open biopsies (see also Pathology Guidelines), FISH/ICM is performed on touch preparations of piece 1 (see Figure 3). In this case, the tumour cell content is determined on the formalin-fixed paraffin-embedded material of piece 1 and is included in the pathology report. No conclusion about the status of genetic markers should be made in case no numeric and no structural chromosome aberrations are found by FISH and/or the ICM indicates a diploid DNA content. First, the pathologist has to indicate whether the cells analysed are tumour cells or not! Pieces 2 and 3 are snap frozen by the pathologist and are used for immunohistological investigations and DNA extraction. For interpretation of PCR results, the knowledge of the exact tumour cell content is especially important. Therefore, the following procedure is recommended: the snap frozen piece is first used for making touch preparations (avoid complete thawing of the piece) for FISH and ICM. Then, frozen sections, ideally cut from two sides, i.e. from the top and from the bottom, are made before extracting DNA for PCR or other investigations. The frozen sections are especially important for determination and documentation of the tumour cell content. Piece 4 in RPMI 1640 is used for cytogenetic studies or for making cell suspension for FCM and cytospin preparations. Therefore, determination of the tumour cell content cannot be carried out using this specific piece. If there is any doubt about the nature of the cells analysed, due to lack of numeric and structural chromosome aberrations, a GD2, NB84a, S100 and common leukocyte staining is strongly recommended. (to solve this question see also Pathology Guidelines)
In case of tru cut biopsies, the snap frozen material received from the pathologist should be handled as indicated above for pieces 2 and 3.
Smears and cell suspensions from fine needle aspirations are provided by the cytopathologist who should also indicate the tumour cell content.

19.3.3 GENETIC PARAMETERS TO BE ANALYSED

19.3.3.1 MYCN Oncogene

Methods and general remarks. The MYCN copy number can be determined by fluorescence based in situ hybridisation (FISH) or MLPA (multiplex ligation-dependent probe amplification). Polymerase chain reaction (PCR) is not recommended to be used without a second method. It has to be kept in mind that by using MLPA or PCR as the only method for MYCN evaluation, the chance to miss possible heterogeneities in the MYCN status or to misinterpret low amplification is higher (due to the ‘dilution’ effect) as compared to FISH analysis which is done on the single cell level. For aCGH, MLPA and PCR, the tumour cell content has to be over 60 per cent. For FISH, all slides and all areas of the individual slides have to be screened and analysed carefully. 10,000 tumour cells is the minimum cell number which should be available for analysis. At least 200 cells should be counted including all cells (different hybridisation patterns and also cell without signals which give valuable information about the hybridisation efficiency).
Recommendation of DNA probes. For FISH either the Kreatech or Vysis MYCN probe and a chromosome 2 specific probe are recommended. The chromosome 2 specific reference probe must be used in case of more than 3 MYCN signals to be able to discriminate MYCN gains from
chromosome 2 polysomies. PCR studies are only accepted when the PCR data are compared with MLPA or FISH results.

**MYCN copy number.** Irrespective of the method used, the copy number has to be indicated according to the number of chromosomes 2.

For **MYCN definitions and report of results see Table 6.** The terms given in this table should be used for giving the results to the clinicians and for documentation (data base).

**Table 6.** Definitions and report of the results for **MYCN** status determined by fluorescence in situ hybridisation and by polymerase chain reaction.

<table>
<thead>
<tr>
<th>FISH - MYCN</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MYCN amplification</strong> = <strong>over 4 fold</strong> increase of the <strong>MYCN</strong> signal number in relation to the number of chromosomes 2.</td>
</tr>
<tr>
<td><strong>MYCN amplification focal</strong> = more or less focal occurrence of cells (at least 20) showing a <strong>MYCN</strong> amplification surrounded by non-amplified tumour cells</td>
</tr>
<tr>
<td><strong>MYCN gain (=inconclusive)</strong> = n up to 4-fold excess of <strong>MYCN</strong> copies in relation to the reference probe on chromosome 2. <strong>Needs further clarification!</strong></td>
</tr>
<tr>
<td><strong>No MYCN amplification</strong></td>
</tr>
<tr>
<td><strong>No result (please specify)</strong> = Unclear or not interpretable result</td>
</tr>
<tr>
<td>Not enough tumour cells contained in the sample</td>
</tr>
<tr>
<td>No tumour</td>
</tr>
<tr>
<td>Not done</td>
</tr>
</tbody>
</table>

19.3.3.2 Segmental Chromosomal Alterations (SCA)

As reported by a number of European, American and Japanese Groups and recommended by the INRG Biology Group common genomic regions should be analyzed ideally on every neuroblastoma tumour [183]. Therefore, the SIOOPEN Biology Group favours the use of aCGH or MLPA to analyze amplified, gained or lost genes or chromosomal regions. These techniques are validated by interlaboratory, intralaboratory and inter-technique comparisons [184].

19.3.4 DNA CONTENT

**Methods and general remarks.** Two methods, i.e. **Flow Cytometry (FCM)** and **Image Cytometry (ICM)** can be used for the assessment of the tumour cell DNA content. For FCM, reference cells derived from human tissue (peripheral blood lymphocytes) from normal individuals or the same patient should be used.

**Report of the FCM/ICM results.** The report should indicate the method used and specify the number of tumour cells versus normal cells contained in the sample under investigation. The results on the DNA content of the tumour cells should be given in absolute numbers.

19.3.4.1 Expression Studies

A retrospective expression data on neuroblastoma tumours derived from HR patients indicate a prognostic significance of a multigene-expression signature [185] Therefore, it is strongly
recommended to store tumour tissue at -80°C or lower and extract RNA to enable this type of studies.

19.3.4.2 Molecular-genetic/-biologic investigations on tumour material obtained after cytotoxic therapy
This kind of tissue often contains only a low number of tumour cells due to therapy induced regressive changes. The possibly low tumour cell content should be especially taken into account with reference to DNA extracting methods. Yet also, preceding cytotoxic treatment can cause major difficulties in the interpretation of the FISH pattern because of the frequently observed polyploidisation occurring after therapy and the often pronounced centromeric associations. These associations of centromeres can either simulate an equal number between the DNA sequence of interest and the reference probe (i.e. the centromeric sequence) instead of an imbalance or a deletion, or they lead to the impression of a gain of the respective DNA sequence instead of a balanced number. The use of non-centromeric reference probes from the concerned chromosome can be of help in critical cases. Sometimes however, it is not possible to give a reliable result. Altogether, test interpretation should be made with caution considering these biological post-treatment changes and the report should always stress that investigations were performed after cytotoxic treatment.

19.3.5 CENTRAL REVIEW
It is recommended that the molecular-genetic/biological data are reviewed either by a central review panel or by online reviewing (SIOPEN-R-NET).

19.4 Bone Marrow Examination Guidelines

19.4.1 GENERAL REMARKS ON BONE MARROW ASPIRATIONS
The following guidelines have been developed for the purpose of improving initial staging accuracy, treatment response evaluation, and, ultimately, patient care. Bone marrow (BM) aspirates from two sites (left and right) have to be performed. In addition, two trephine biopsies are mandatory. 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 (see section 6.1.7, page 79). 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 of BM is aspirated into the syringe and immediately dropped on a glass slide.

- **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. The syringe should be shaken immediately to allow the anticoagulant to mix with the bone marrow. This procedure is repeated for each puncture site.

To enable a highly sensitive technique for detection and characterisation of rare neuroblastoma cells or tumour cell associated RNA:
- At diagnosis, Rapid COJEC day 40 or modified N7 after cycle 3, end of induction, post TVD (if applicable), post MAT and post immunotherapy, transfer immediately 0.5ml of the drawn 3-5ml BM in heparin from each side into two separate PAXgene™ tubes. Do not pool.
- At diagnosis, Rapid COJEC day 40 or modified N7 after cycle 3, end of induction, post TVD (if applicable), post MAT and post immunotherapy, transfer 4.5ml (remainder) to
National Immunocytology Reference Laboratory for processing of at least $3 \times 10^6$ cells on cytospins by isolating mono nuclear cell suspension and using an adequate cytocentrifuge (e.g. Hettich), ideally 2 times $3 \times 10^6$ 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 provided by the reference laboratory in Leeds (Prof. Sue Burchill or Dr Virginie Viprey. More information and contact details in section 6.1.7 (page 79) and section 31.5 (page 268).

**19.5 Handling of the bone marrow cells in the laboratory**

**19.5.1 SEPARATION OF MNC, PREPARATION AND STORAGE OF CYTOSPIN PREPARATIONS, IMMUNOCYTOTOLOGICAL STAINING, EVALUATION CRITERIA AND REPORTING OF RESULTS.**

The methods for preparation of mononuclear cells (MNC), processing, sending and storage of cytospins, evaluation of immunocytochemical 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 [147, 186] (Swerts et al., (2005) J Histochem Cytochem 53:1433-1440; Beiske et al (2009) BJC 100:1627-1637. Immunocytological staining can also be combined with FISH and evaluated using an automated scanning and relocation system (AIPF) (e.g. Metafer4/RCDetect, MetaSystems, Altlussheim, Germany) [184]. Further detail on SOPs for QRT-PCR studies are described in Viprey et al [187]

**19.5.2 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 $-70^\circ$C or below) is most important. Besides this, it is advisable to store touch preparations and cytospin preparations at $-20^\circ$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 $-20^\circ$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.
**FORM B1. QRT-PCR IN PERIPHERAL BLOOD, BONE MARROW AND PBSC HARVEST REQUEST FORM**

Please send this form together with samples (0.5 ml bone marrow and 2 ml peripheral blood) in PAX tubes frozen at –80°C (dry ice) to your reference laboratory. Peripheral stem cell harvest samples (0.5 ml in a PAX tube) should also be sent frozen to your reference laboratory. **One form for each sample should be completed at the time of sampling.** Samples and completed forms should be sent to:-

**Attention of your reference laboratory:**
Ms Deborah Roebuck, Children’s Cancer Research Group, Cancer Research UK Clinical Centre, Leeds Institute of Molecular Medicine, St. James’s University Hospital, Leeds LS9 7TF, UK.

PLEASE contact Deborah Roebuck before you send the samples; 00 44 113 2064922 or 00 44 113 2064917. email:- d.roebuck@leeds.ac.uk

<table>
<thead>
<tr>
<th>PATIENT NAME:-</th>
<th>DATE OF BIRTH:-</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOSPITAL NUMBER:-</td>
<td>CONTACT CLINICIAN/ NURSE:-</td>
</tr>
<tr>
<td>CENTRE:-</td>
<td>DATE OF SAMPLE:-</td>
</tr>
<tr>
<td></td>
<td><strong><strong><strong>/</strong></strong><em>/</em></strong>____</td>
</tr>
<tr>
<td>HR-NBL-1 REGISTRATION NUMBER:-</td>
<td>SAMPLE TAKEN AT:-</td>
</tr>
<tr>
<td></td>
<td>DAY 0 or DIAGNOSIS……………</td>
</tr>
<tr>
<td>IS THIS SAMPLE (please tick):-</td>
<td>DAY 40 ……………………</td>
</tr>
<tr>
<td>(i) Peripheral blood</td>
<td>DAY 80 (prior to MGT/PBSC) ……</td>
</tr>
<tr>
<td>(ii) Bone marrow aspirate</td>
<td>Post TVD (when applicable) ………</td>
</tr>
<tr>
<td>1.</td>
<td>Post MAT…………………</td>
</tr>
<tr>
<td>2.</td>
<td>Post MRD TREATMENT……………</td>
</tr>
<tr>
<td>(iii) Peripheral stem cell harvest</td>
<td></td>
</tr>
</tbody>
</table>

PLEASE ensure tubes are clearly and permanently labelled with HR-NBL-1 registration number, date of sample and sample type.
19.7 Aphaeretic products
To verify whether the aphaeretic product is free from tumour cells it is advised to use a highly sensitive and specific technique for tumour cell detection. For this type of studies 2ml of the aphaeretic product should be used. Keep cells on ice until further processed. Transfer 0.5ml in PAXgene tube and store at -80°C and send for QRT-PCR to Leeds (Prof. Burchill). The residual 1.5ml are further processed for immunocytological analysis. Lyse cells with Erythrocyte lysis buffer and make cytospin slides with the use of an adequate cytocentrifuge (e.g. Hettich). Analyse cells with anti GD2 antibody and send unfixed and unstained slides for genetic verification to the CCRI.

20 Appendix: $^{123}$I mIBG Imaging Protocol

20.1 Objectives
This appendix provides guidance to support consistency and high standards of paediatric nuclear medicine imaging. mIBG imaging should be restricted to nuclear medicine departments with appropriate facilities and staff experienced in paediatric imaging.

20.2 Indications
Indications for diagnostic $^{123}$I mIBG imaging in neuroblastoma (NBL) include
- Confirm diagnosis in suspected NBL
- Staging
- Assess treatment response
- Routine follow up
- Select patients for high activity mIBG targeted therapy

In this study, the first 2 mIBG scintigraphies are used for both evaluation the extension of the disease at diagnosis and assessment of response to induction chemotherapy: high quality images of the entire skeleton are mandatory to allow proper scoring and clinical decision management. Follow-up examinations may allow early relapse identification and/or residual tumour activity: with respect to those particular issues, we recommend to complete planar images with SPECT (SPECTCT if available).

20.3 Patient Preparation

20.3.1 Information
The child and parents should receive detailed information about the entire procedure. Radiation protection precautions should be explained carefully to the family and ward/clinical staff prior to $^{123}$I mIBG injection. Written information sheets about the procedure help to reinforce verbal advice.

20.3.2 Drug Interaction
Several drug classes such as antidepressants (tricyclics, tetracyclics, serotonin reuptake inhibitors), sympathomimetics including nasal decongestants and bronchodilators, phenothiazines and combined alpha and beta blockers such as labetalol are known to interfere with mIBG uptake and must be withdrawn prior to radiopharmaceutical injection. The optimal withdrawal period is determined by the half life of the drug concerned, but in general, a washout period of 5 – 6 half lives is reasonable. Other drugs such as calcium channel blockers and ACE inhibitors may
theoretically interfere with mIBG uptake and should be withdrawn where feasible. Please refer to
the EANM guideline on mIBG scintigraphy of the EANM available at:
https://www.eanm.org/scientific_info/guidelines/gl_onco_mibg.pdf

20.3.3 THYROID BLOCKADE
Thyroid blockade using potassium iodide, potassium iodate or aqueous iodine oral solution
(Lugol’s iodine) is essential to prevent thyroidal uptake of free radioactive iodide. Ideally,
blockade should commence 24 hours prior to mIBG injection and be continued for 2 days.
Potassium iodate and iodide tablets crushed and mixed with food or sweets are more palatable
than Lugol’s iodine. The following iodine doses are recommended:
Children under 3 years  24 mg daily,  3 to 13 years  48 mg daily, over 13 years 98 mg daily.
For conversion purposes, 60 mg potassium iodide contains 45 mg iodine, 85 mg potassium iodate
contains 50 mg iodine and 1 ml of Aqueous Iodine Oral Solution BP (Lugol’s Iodine) contains
130 mg iodine. Please refer to the EANM guidelines available at:
https://www.eanm.org/scientific_info/guidelines/gl_paed_mibg.pdf

20.3.4 SEDATION
Sedation / general anaesthesia may be required to ensure optimal image quality, particularly where
tomographic imaging requiring a long scan time is planned. This should be assessed at the time of
referral, well in advance of the child’s attendance in the imaging department. As pelvic and
lumbar region may be obscured by a full bladder containing excreted radioactive tracer, urinary
catheter may be required in children examined under sedation or general anaesthesia. Acquisition
of delayed image of the pelvis/abdomen should be considered as a good option.

20.4 Procedure

20.4.1 123I MIBG ADMINISTRATION
The child should be well hydrated prior to and during the next hours after 123I mIBG
administration. It is preferable to inject via a peripheral cannula but indwelling central venous
lines may be used when venous access is difficult. 123I mIBG is given by slow intravenous
injection over 5 minutes followed by a slow 5-10 ml saline flush or glucose 5% (mIBG is more
stable in glucose solution). Rapid injection typically results in adrenergic symptoms including
tachycardia, pallor, vomiting and abdominal pain.
In needle phobic infants/children the use of MEOPA, equimolar mix of nitrous oxide and oxygen,
should be encouraged (see section 20.8, page 177) Long lines should be flushed thoroughly after
injection to avoid image artefacts.

20.4.2 ADMINISTERED ACTIVITY
The activity administered should be the minimum consistent with obtaining a diagnostic result.
The relative radiation risk resulting from 123I mIBG administration must be balanced against the
adverse impact on clinical management of inadequate imaging. Higher activities may, therefore,
be justified according to individual patient circumstances.

The minimum recommended activity varies according to specific guidelines, being 80MBq for the
EANM, and 37 MBq for the Society of Nuclear Medicine. According to the experience of most
members of this SIOPEN-HR-NBL Committee we recommend to use a weight based calculation
of the administered activity of 5 MBq/kg, with a minimum of 37 MBq and a maximum of 370
MBq.
The guidelines of the Society of Nuclear Medicine are available online at: http://pedrad.org/associations/5364/files/Pediatric_dose_consensus_guidelines_Final_2010.pdf

The dosage card and activity calculator of the EANM is available online at: https://www.eanm.org/scientific_info/dosagecard/dosagecard.php?navId=548

20.4.3 IMAGE ACQUISITION
An appropriate imaging room adapted for paediatric use (including for example, toys, audiovisual equipment, decorations etc) encourages cooperation and can reduce movement artefact. Experienced staff are essential to ensure optimal image quality, and parental involvement before and during the procedure is encouraged.

20.4.3.1 Gamma camera settings
Low energy, high resolution collimator(s). Photo peak 159keV with 20% energy window. Medium energy collimators may also be used.

20.4.3.2 Imaging
Delayed planar images acquired 18 to 24 hours post injection are mandatory. Tomographic (SPECT) images can be performed on completion of the planar study, if necessary. The child should be encouraged to void immediately prior to imaging. Nappies/diapers should be changed immediately prior to imaging. Images can be acquired as static overlapping views of the whole body or as a whole body scan from vertex to toes. Although older children may be able to co-operate with lengthy whole body scans, superior resolution images are usually achieved in young patients (up to 5 years of age) using the static overlapping approach. Whole body imaging requires a speed inferior to 10 cm/sec or a minimal total acquisition time of 30 minutes to produce satisfactory images.

A static image of the posterior abdomen immediately or 4 hours after $^{123}$I mIBG injection may be helpful to differentiate between renal excretion of the tracer and tumour uptake. In those cases, a delayed image at 48 hours post injection could be helpful.

20.4.3.3 Overlapping images
10 minutes per view or 250k counts per image are recommended, to deliver optimal image quality within reasonable time constraints. A pixel size of approximately 2mm requires a 256x256 matrix or 128x128 matrix with zoom. The minimum data set includes anterior and posterior views of: skull, thorax, abdomen, pelvis (empty bladder) and lower limbs. The latter should be positioned similarly to bone imaging i.e. toes turned inwards and knees together. The feet must be included. Additional lateral skull images are highly desirable. Images of both arms are mandatory and are usually included on thoracoabdominal images, and on lateral projections of the head and neck (left arm with right lateral, right arm with left lateral). In order to increase the quality of the images, the child should be positioned directly on the collimator of the gamma camera (Figure 12). In case of doubtful lesions, especially after the end of induction chemotherapy, additional images obtained 48 h after mIBG injection may be of great help. These may be at least of 15 minutes.
20.4.3.4 Whole body scan
A scan speed inferior to 10 cm/min or a total acquisition time of 30 minutes is appropriate. Additional static skull views with arms in the field of view are mandatory. Additional spot views of pelvis are acquired after voiding if needed (Figure 13). As counting statistic may be low on whole-body scan, additional anterior view of both legs should be acquired.
20.4.3.5 Single Photon Emission Tomography (SPECT) or SPECT CT
SPECT or SPECT CT images provide additional anatomical clarification and allow separation of structures that appear superimposed on 2 dimensional images. SPECT imaging requires a dual headed camera. Acquisition protocols may vary between centres, but projections should be at least taken in 128 x 128 matrix on 360 degrees (120 projections) with minimal frame duration of 20-30 sec. Reconstruction should be made using OSEM iterative algorithms, or FBP with Hanning 0.55-0.7. In centres where the default cross-sectional imaging modality is CT, a diagnostic quality CT with iv contrast using the CT component of the SPECT CT device should be considered, especially in children who need sedation/general anaesthesia. Where MRI is the default cross-
sectional imaging modality, the addition of a low dose CT to the MIBG scan for co-localization and attenuation correction is questionable on the ground of additional probably unnecessary radiation and additional time for imaging which may require sedation or general anaesthesia in infants.

20.5 Image interpretation

Images should be checked for quality and completeness before the child leaves the Nuclear Medicine department.

Images should be interpreted by experienced nuclear medicine specialists with access to all relevant clinical information and correlative imaging. In addition to conventional reporting, semi-quantitative image analysis using the attached proforma (see section 20.9 – mIBG score sheet) has to be reported for the diagnostic and second evaluation (after end of induction chemotherapy) in order to assess the response. This scoring method should also allow comparison of data between centres and standardise reporting within clinical trials.

20.6 Data archive

It is essential that raw image data are stored digitally or on appropriate media to allow comparison between serial studies and peer review. Where possible, DICOM or Interfile format is recommended to facilitate transfer between centres. JPEGs or TIFF images are not appropriate for central review.

20.7 Quality control

It is essential that the MIBG images are reviewed by the Nuclear Medicine and Physics Committee.

This information may be uploaded to the SIOPEN-R-NET via the Nuclear Medicine & Physics Sub-Study or alternatively sent to the Sponsor in Vienna for central upload within 2 weeks of the scan being done.

All information sent to the Sponsor should be clearly labelled with the patient’s 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. The Sponsor’s address for sending the above information is:

Department of Studies and Statistics (S²IRP)
HR-NBL-1.5/SIOPEN Nuclear Medicine Review
St. Anna Kinderkrebsforschung
Zimmermannplatz 10
A-1090 Wien
Austria

20.8 Additional information


20.9 mIBG score sheet

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Study Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary tumour site</td>
<td>Thorax</td>
</tr>
<tr>
<td>Primary resected</td>
<td>YES/NO</td>
</tr>
</tbody>
</table>

**GENERAL QUALITY OF SCAN**

| Is it complete? | YES/NO |
| Are all images well definite/clear? | YES/NO |
| Are some artefacts/equivocal findings present? | YES/NO |
| Is count rate adequate? | YES/NO |
| Is resolution adequate? | YES/NO |
| Final balance | Full diagnostic scan | Non-diagnostic scan | Partial diagnostic scan |

**Skeleton Score**

<table>
<thead>
<tr>
<th></th>
<th>Pre treatment</th>
<th>Post treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skull and facial bones</td>
<td></td>
<td>Head &amp; neck</td>
</tr>
<tr>
<td>Thoracic cage</td>
<td></td>
<td>Thorax</td>
</tr>
<tr>
<td>Right humerus</td>
<td></td>
<td>Right upper limb</td>
</tr>
<tr>
<td>Left humerus</td>
<td></td>
<td>Left upper limb</td>
</tr>
<tr>
<td>Right forearm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left forearm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spine</td>
<td></td>
<td>Abdomen</td>
</tr>
<tr>
<td>Pelvis</td>
<td></td>
<td>Pelvis</td>
</tr>
<tr>
<td>Right femur</td>
<td></td>
<td>Right lower limb</td>
</tr>
<tr>
<td>Left femur</td>
<td></td>
<td>Left lower limb</td>
</tr>
<tr>
<td>Right tibia/fibula</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left tibia/fibula</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Soft tissue Score**

<table>
<thead>
<tr>
<th></th>
<th>Pre treatment</th>
<th>Post treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**SKELETAL SCORE**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No abnormality</td>
</tr>
<tr>
<td>1</td>
<td>1 focal lesion</td>
</tr>
<tr>
<td>2</td>
<td>2 focal lesions</td>
</tr>
<tr>
<td>3</td>
<td>3 focal lesions</td>
</tr>
<tr>
<td>4</td>
<td>Diffuse &lt; 50% of bone or &gt; 3 focal lesions</td>
</tr>
<tr>
<td>5</td>
<td>Diffuse 50 – 95% of bone</td>
</tr>
<tr>
<td>6</td>
<td>Diffuse involving whole bone</td>
</tr>
<tr>
<td>X</td>
<td>Un-evaluable</td>
</tr>
</tbody>
</table>

**SOFT TISSUE SCORE**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No abnormality</td>
</tr>
<tr>
<td>1</td>
<td>Solitary lesion</td>
</tr>
<tr>
<td>2</td>
<td>Multiple lesions</td>
</tr>
<tr>
<td>X</td>
<td>Un-evaluable</td>
</tr>
</tbody>
</table>

**Uptake**

<table>
<thead>
<tr>
<th>Primary tumour</th>
<th>Pre treatment</th>
<th>Post treatment</th>
</tr>
</thead>
</table>

**Size**

<table>
<thead>
<tr>
<th>Primary tumour</th>
<th>Pre treatment</th>
<th>Post treatment</th>
</tr>
</thead>
</table>

**Intensity**

<table>
<thead>
<tr>
<th>Primary tumour</th>
<th>Pre treatment</th>
<th>Post treatment</th>
</tr>
</thead>
</table>

**TECHNICAL DATA**

| Was sedation/GA used for examination? | YES/NO |
| Whole body scan was performed at 24 hrs post injection? | YES/NO |
| If yes, speed of scan | <10 cm/min / > 10 cm/min |
| Additional static views obtained? | YES/NO |
| If yes, the minimum duration of the acquisition was | <10 min / > 10 min |
| If yes, anteroposterior views of the skull | YES/NO |
| lateral views of the skull including arms: | YES/NO |
| left lateral including right arm | YES/NO |
| right lateral including left arm | YES/NO |
| other, specify | YES/NO |
21 APPENDIX: Monitoring for Ototoxicity

Please note audiometry guidelines in section 11.3 above.

21.1 Brock Grading

Grading system for Cisplatin-induced bilateral high-frequency hearing loss [153, 188-190]

<table>
<thead>
<tr>
<th>BILATERAL HEARING LOSS</th>
<th>GRADE</th>
<th>Designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 40 dB at all frequencies</td>
<td>0</td>
<td>Minimal</td>
</tr>
<tr>
<td>≥ 40 dB at 8,000 Hz only</td>
<td>1</td>
<td>Mild</td>
</tr>
<tr>
<td>≥ 40 dB at 4,000 Hz and above</td>
<td>2</td>
<td>Moderate</td>
</tr>
<tr>
<td>≥ 40 dB at 2,000 Hz and above</td>
<td>3</td>
<td>Marked</td>
</tr>
<tr>
<td>≥ 40 dB at 1,000 Hz and above</td>
<td>4</td>
<td>Severe</td>
</tr>
</tbody>
</table>

21.2 ASHA Criteria

21.2.1 ELEMENTS OF MONITORING
A basic cochleotoxicity monitoring programme requires (a) specific criteria for identification of toxicity, (b) timely identification of at-risk patients, (c) pre-treatment counselling regarding potential cochleotoxic effects, (d) valid baseline measures (pre-treatment or early in treatment), (e) monitoring evaluations at sufficient intervals to document progression of hearing loss or fluctuation in sensitivity, and (f) follow-up evaluations to determine post-treatment effects.

21.2.2 AUDIOMETRIC CRITERIA FOR COCHLEOTOXICITY
Specific criteria for defining drug-induced hearing decrease are controversial [191]. Here we have attempted to construct criteria by which most cases of true ototoxicity will be detected. These criteria are conservative, because the occasional false-positive identification is preferable to methods that may delay detection of the ototoxic process.

In a study of normal test-retest variability of audiometric thresholds normal variability was reflected by independent shifts at random frequencies [192]. Thus, shifts at adjacent test frequencies indicate more systematic change and increase the likelihood of a true decrease in sensitivity [193]. Frequency averaging (i.e. calculating the average of thresholds across some frequency range) has been advocated for detecting decreased sensitivity, and the use of adjacent frequencies is equivalent to averaging over those frequencies. Another fundamental concept is that a decrease, observed on repeated tests, is a valid change (Royster & Royster 1982). Thus, a shift relative to baseline that is seen at least twice is likely to represent a true shift and not normal variation.

Change in hearing sensitivity is always computed relative to baseline measures. Criteria to indicate hearing decrease during ototoxicity monitoring are defined here as (a) 20dB decrease at any one test frequency, (b) 10dB decrease at any two adjacent frequencies, or (c) loss of response at three consecutive test frequencies where responses were previously obtained (the third criterion refers specifically to the highest frequencies tested, where earlier responses are obtained close to the limits of audiometric output and later responses cannot be obtained at the limits of the audiometer). Finally, change must be confirmed by repeat testing.
21.2.3 **PATIENT IDENTIFICATION**
Patients requiring monitoring are those whose treatment includes the administration of a therapeutic drug known or suspected to have cochleotoxic side effects. Once a patient is identified as being treated with a cochleotoxic drug, a monitoring programme should be implemented in a timely manner. Access to a registry of hospitalised patients being treated with potentially cochleotoxic drugs is a critical component of a comprehensive monitoring programme and must often be developed in co-operation with hospital personnel.

21.2.4 **PRETREATMENT COUNSELLING**
Prior to treatment with cochleotoxic drugs, patients should be counselled regarding potential effects on the auditory system. During initial medical treatment counselling, the physician should include information regarding the risks and benefits of drug therapy. The audiologist should counsel patients on signs and symptoms of cochlear damage and potential effects on communication ability. Symptoms such as tinnitus, fullness, loss of balance or changes in hearing sensitivity should be reviewed and the patient instructed to inform health care professionals if they occur. Potential effects such as exposure to noise during or following treatment should be discussed. If the patient lives or works in an environment with high noise levels, the possible synergistic effect of noise and cochleotoxic damage must be considered, and both the patient and family should be made aware of this increased risk.

21.2.5 **BASELINE TESTING**
The purpose of baseline testing is to document the status of hearing prior to treatment. At-risk individuals should receive baseline evaluations that are as complete as possible. Word discrimination should be included in the ideal scope of audiologic practice. The reliability of behaviour responses should be assessed during baseline by repeating selected portions of the evaluation. In addition, results of the first test following baseline should be evaluated for interest reliability.

The optimal timing of baseline testing depends largely on the drug(s) the patient is receiving. For example, animals receiving large bolus doses of kanamycin do not show histologic evidence of cochleotoxicity until after 72 hours (Brummell 1983, Brummell & Fox 1982). Thus, in the absence of more precise data, baseline audiometric evaluation of patients receiving aminoglycosides should be done prior to or within 72 hours of first treatment dose [194]. Cisplatin can cause observable cochleotoxicity following a single course of treatment [195]. Thus, it is important to obtain baseline measures prior to the first dose of Cisplatin [196].

21.2.6 **MONITORING SCHEDULE AND FOLLOW-UP TESTS**
Monitoring tests should be scheduled at intervals that will enable the earliest possible detection (within reason) of cochleotoxic effects. Immediate post treatment testing suggested, to document auditory status at the end of drug treatment. Follow-up testing should be done at intervals appropriate to detect post-treatment cochleotoxicity or to document recovery.

21.2.7 **PHYSIOLOGIC ASSESSMENT**

21.2.7.1 **ABR Testing for Threshold Estimation**

Stimuli: Frequency-specific stimuli (tone bursts of low, mid and high frequency).

Transducer: Insert earphones are recommended, unless contraindicated, for air-conduction testing. A bone-conduction transducer will be needed if air conduction is elevated (i.e. if air-conduction thresholds are greater than 20 dB nHL, bone conduction testing should be completed to assess the type of hearing loss).
Protocol Responses should be attempted down to 20 dB nHI. Definition of threshold should be attempted in at least 10 dB steps. Recording epochs of 20-25 ms are necessary for adequate ABR threshold detection measures in infants, especially when tonal stimuli are used. Many children in this age group can be tested during natural sleep, without sedation, using sleep deprivation with nap and feeding times co-ordinated around the test session. However, active or older infants may require sedation to allow adequate time for acquisition of high-quality recordings and sufficient frequency-specific information.

21.2.8 OAEs

21.2.8.1 Limited
TEOAE: One level (e.g. 80 dB pSPL) click stimulus should be completed. Normal distributions for this condition for normal hearing are documented in the literature [197].

21.2.8.2 Comprehensive
TEOAE: Two levels (e.g. 80 dB pSPL and a lower level) may be completed and/or one level using click and multiple frequencies for stimuli.

21.2.8.3 Acoustic Immitrance Assessment
Probe tones equal to or greater than 660 Hz should be used because of the poor validity of tympanometry when using a low frequency probe tone with this age group and the demonstrated diagnostic value of tympanometry with a high-frequency probe tone.

21.2.8.4 Unresponsive Patients
In non-responsive patients, objective hearing measures (e.g. auditory evoked potentials and evoked otoacoustic emissions) may be the only means to obtain auditory information. Although objective procedures provide only gross information on hearing sensitivity, they are nonetheless capable of detecting ototoxic hearing loss [198-201].

Hearing evaluation in patients unable to provide reliable behavioural responses is a complex issue. Aside from the question of which objective assessment technique to use, there is the medicolegal concern of informed consent. For unresponsiveness patients, proxy consent must be obtained according to the laws of the state and the policies and procedures of the specific institution.

Three objective evaluation procedures have potential for ototoxicity monitoring or unresponsive patients; otoacoustic emissions (OAE), electrocochleography (ECoChG), and auditory brainstem response (ABR). These techniques are in various stages of development for use as objective ototoxicity monitoring tools. Thus, specific guidelines for application of these procedures to ototoxicity monitoring cannot be recommended at this time. Under conditions when no behavioural response is available.

However, use of objective measures is encouraged. Responses obtained in this manner at least document the gross responsiveness of the auditory system. Repeated testing can be informative regarding changes during treatment. At a minimum, the absence of a previously obtained response indicates that gross auditory function has been reduced or loss.

OAE. Three types of OAE measurements have received concentrated attention; spontaneous, transiently evoked, and distortion product. OAE assessment is specifically sensitive to the status of outer hair cells in the cochlea and is a relatively efficient objective test. It has been used to assess cochlear function in patients receiving Cisplatin with promising results [202, 203]. Although OAE testing presents a new and exciting tool for cochleotoxicity monitoring. Its application has not been evaluated sufficiently to enable formulation of specific guidelines.

ECoChG. This evoked potential technique has been used for a number of years to evaluate cochlear and neural responses. The most sensitive ECoChG technique requires transtympanic placement of the electrode and is, therefore, more invasive than OAE or ABR. Tympanic
membrane or ear canal placements are less invasive but also provide less sensitive threshold estimates. Furthermore, the use of these special electrode placements may not be suitable for all patients. In any configuration, ECochG requires a significant amount of time to acquire frequency-specific information. This technique therefore is not appropriate for routine objective auditory monitoring.

**ABR.** ABR is subject to the same limitations as ECochG with respect to length of testing and frequency specificity. The use of acoustic clicks as stimuli, limits response information to frequencies between 1 and 4 kHz and thus decreases its effectiveness in cochleotoxicity monitoring. Studies using high frequency tone-burst stimuli to provide high frequency specific response information however have shown that this ABR technique holds promise as an objective monitoring tool for early detection of ototoxicity [204]. Recent advances in multiple-stimulus ABR [205-207] may shorten the test time when such developments are incorporated into clinical instrumentation.

### 21.3 VRA, ABR and OAE Guidelines

#### 21.3.1 Protocol for Performing Visual Reinforcement Audiometry (VRA)

Great Ormond Street Hospital for Sick Children, Audiology Department, April 2003

Visual Reinforcement Audiometry is suitable for children between the ages of 7 months and 3 years. In certain circumstances, it is also suitable for older developmentally delayed or handicapped children.

##### 21.3.1.1 Test and Room Requirements
- The procedure requires 2 testers, one to present the stimulus and one to distract the baby’s attention.
- The room should be sound proof, with a minimum floor area of 16m² have a small table and a selection of toys available for distraction.
- The ambient noise in the room should not exceed 25 – 30 dBA.
- Baby should sit on parent’s knee facing forward, sitting erect at an angle of 45 - 90 to the reinforcers.

##### 21.3.1.2 Parent should be given the following instructions:
- Support the baby at the waist, sit on the centre of the knee and ensure that there is a gap between the baby and themselves (ie baby is not leaning against parent).
- Do not react to the sounds, keep looking forward as any movement on their part could initiate a response from the baby and invalidate the test.
- Remain quiet throughout the test.

##### 21.3.1.3 Tester 1 (Distracter)
The person at the front should attract and control the baby’s attention using appropriate distraction techniques.
The distraction should not stop when the sound is presented, but should be kept low key.

##### 21.3.1.4 Tester 2 (Stimulus presenter)
The person presenting the sounds should be placed out of the line of sight of the patient. Sound should be warble tone or narrow band noise, NOT pure tone. Begin by presenting a sound of 70dBHL dial setting (Around 80dB(A)), sound field, reinforcing the sound at the same time to
condition the child. Repeat this until the child is conditioned (Turns to sound with no prompt from distracter). Once conditioned, obtain minimal response levels at 1kHz sound field. (See attached standard of procedure.) Once a reliable sound field response is obtained at 1kHz, attempt to obtain ear specific information using insert earphones. (See attached standard of procedure.) When minimal response levels are obtained, assess localization using narrow band noise at a level of 20dB above threshold at 2kHz. (See attached standard of procedure.)

Standard of procedure for Sound Field Audiometry

Begin at 1kHz

Use a dial setting of 70dBHL

response

Decrease dial setting by 20dB

response

no response

Increase dial setting by 20dB

response

no response

Increase dial setting by 10dB

response

no response

Decrease dial setting by 5dB

response

no response

Increase dial setting by 5dB

response

no response

Once 2 ascending presentations are obtained, take this as the minimum response level
Standard of procedure for insert VRA

1. **Obtain reliable sound field at 1kHz**
2. **Insert earphones into baby’s ears**
   - tolerates
   - does not tolerate
3. **Carry out testing using PTA protocol (10dB down, 5dB up)**
4. **Do not force. Stop and carry out sound field testing.**

**Test order**

1kHz ↔ 4kHz ↔ 500Hz ↔ 250Hz ↔ 2kHz

2. **Insert Ear Phone**
   - Ear (1) 1kHz → Ear (2) 1kHz → Ear (2) 4kHz → Ear (1) 4kHz
   - Ear (1) 250Hz ↔ Ear (2) 250Hz ↔ Ear (2) 500Hz ↔ Ear (1) 500Hz
   - Ear (1) 2kHz → Ear (2) 2kHz
21.3.1.5 Comparison of Threshold ABR Protocols (Tone Burst)
The following table summarises the main parameters for threshold tone burst ABR testing and compares the current Great Ormond Street (G.O.S) parameters with the recommended protocol as published on the Neonatal Hearing Screening Website.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>G.O.S</th>
<th>Recommended (Elliott, Lightfoot et al)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Freq Filter (Hz)</td>
<td>30</td>
<td>20 – 30</td>
</tr>
<tr>
<td>High Freq Filter (Hz)</td>
<td>1500</td>
<td>1k – 3k</td>
</tr>
<tr>
<td>Rise / plateau</td>
<td>2-1-2</td>
<td>2-1-2</td>
</tr>
<tr>
<td>Click Polarity</td>
<td></td>
<td>Alternating</td>
</tr>
<tr>
<td>Click Rate (Hz)</td>
<td>39.1</td>
<td>Typically 37</td>
</tr>
<tr>
<td>Sweep Limit</td>
<td>2000</td>
<td>2000 minimum (occasionally 3000 or 4000)</td>
</tr>
<tr>
<td>Window (ms)</td>
<td></td>
<td>20 - 25</td>
</tr>
<tr>
<td>Noise</td>
<td>notch</td>
<td>Not specified</td>
</tr>
<tr>
<td>Noise level</td>
<td>≥ 20dB below stimulus</td>
<td>Not specified</td>
</tr>
</tbody>
</table>

The recommended protocol is essentially the same as the one currently used in G.O.S. The protocol on the UNHS website does not make any recommendations for the use of notched noise to improve frequency specificity.

21.3.2 Comparison of Threshold ABR Protocols (Air Conduction)
The following table summarises the main parameters for air conduction threshold ABR testing and compares the current G.O.S protocol with the recommended protocol as published on the Neonatal Hearing Screening Website.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>G.O.S</th>
<th>Recommended (Elliott, Lightfoot et al)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Freq Filter (Hz)</td>
<td>30</td>
<td>20 – 30</td>
</tr>
<tr>
<td>High Freq Filter (Hz)</td>
<td>3k</td>
<td>1500</td>
</tr>
<tr>
<td>Click Duration (µs)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Click Polarity</td>
<td>Alternating</td>
<td>Alternating</td>
</tr>
<tr>
<td>Click Rate (Hz)</td>
<td>31.1</td>
<td>49.1</td>
</tr>
<tr>
<td>Sweep Limit</td>
<td>1500</td>
<td>2000</td>
</tr>
<tr>
<td>(1024 in protocol)</td>
<td>(min 1500)</td>
<td></td>
</tr>
<tr>
<td>Window (ms)</td>
<td>15</td>
<td>18-20</td>
</tr>
</tbody>
</table>

The main differences between the recommended protocol and the one that we are currently using are minimal and are outlined below:
i) High frequency filter
1500Hz is recommended in order to reduce the effect of electronic noise from the amplifier. There is very little response at frequencies higher than this and so there would be no significant effect on the waveform. In practice, the filter setting regularly has to be changed to 1500 Hz in order to obtain a clear, usable waveform.

ii) Click repetition rate
The increased rate would reduce the test time, which may be advantageous when testing sleeping/sedated babies. There would, however, be an effect on the waveform (probably slightly smaller responses). Therefore, it would be necessary to carry out a few ‘normals’ to determine any correction factor required. These could be done on (e.g. 10) adults as all thresholds are compared to average psycho-acoustic thresholds for normally hearing adults. There is no requirement for normative data for paediatric latencies for threshold determination.

iii) Threshold determination
This method of determining threshold is recommended. The authors suggest using steps of 10dB only and using the system of indicating definite/possible thresholds (++) to identify whether responses are threshold or threshold + 5 dB. I feel that it may be useful to adopt this method. Again it would reduce test time and would increase the likelihood of obtaining threshold measurements on both ears, rather than spending too long trying to obtain a threshold to the nearest 5 dB on one ear and then potentially running out of time on the second. The additional information gained by using steps of 5 dB is minimal.

21.3.3 Comparison of threshold ABR protocols (Bone Conduction)
The following table summarises the main parameters for threshold ABR testing and compares the current G.O.S protocol with the recommended protocol as published on the Neonatal Hearing Screening Website.

<table>
<thead>
<tr>
<th></th>
<th>G.O.S</th>
<th>Recommended (Elliott, Lightfoot et al)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low Freq Filter (Hz)</strong></td>
<td>150</td>
<td>20-30</td>
</tr>
<tr>
<td><strong>High Freq Filter (Hz)</strong></td>
<td>3k</td>
<td>1500</td>
</tr>
<tr>
<td><strong>Click Duration (μs)</strong></td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><strong>Click Polarity</strong></td>
<td>Alternating</td>
<td>Alternating</td>
</tr>
<tr>
<td><strong>Click Rate (Hz)</strong></td>
<td>11.1</td>
<td>49.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19.1 (when recording wave I)</td>
</tr>
<tr>
<td><strong>Sweep Limit</strong></td>
<td>1024</td>
<td>2000 (min 1500)</td>
</tr>
<tr>
<td><strong>Window (ms)</strong></td>
<td>12</td>
<td>18 - 20</td>
</tr>
</tbody>
</table>

Recommended positioning of the bone conductor and electrodes are discussed in the protocol.

The main differences between the recommended protocol and current protocols are minimal and are outlined below:

iv) High frequency filter
1500Hz is recommended in order to reduce the effect of electronic noise from the amplifier. There is very little response at frequencies higher than this and so there would be no significant effect on the waveform. In practice, I have found that the filter setting regularly has to be changed to 1500 Hz in order to obtain a clear, usable waveform.
v) Click repetition rate
The increased rate would reduce the test time, which may be advantageous when testing sleeping/sedated babies. There would, however, be an effect on the waveform (probably slightly smaller responses). Therefore, it would be necessary to carry out a few ‘normals’ to determine any correction factor required. These could be done on (e.g. 10) adults as all thresholds are compared to average psycho-acoustic thresholds for normally hearing adults. Ages correction factors for testing babies are outlined in the protocol. There is no requirement for normative data for paediatric latencies for threshold determination.

vi) Threshold determination
A slightly different method of determining threshold is recommended. The authors suggest using steps of 10dB only and using the system of indicating definite/possible thresholds (++ or +) to identify whether responses are threshold or threshold + 5 dB. This is the recommended method. Again it would reduce test time and would increase the likelihood of obtaining threshold measurements on both ears, rather than spending too long trying to obtain a threshold to the nearest 5 dB on one ear and then potentially running out of time on the second.

The additional information gained by using steps of 5 dB is minimal.

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>Conversion dB (A) to dB SPL</th>
<th>Minimum audible field (Bin) dB SPL from ISO 226</th>
<th>True conversion values dB (A) to dB HL equivalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>+9</td>
<td>+12</td>
<td>-3</td>
</tr>
<tr>
<td>500</td>
<td>+3</td>
<td>+6</td>
<td>-3</td>
</tr>
<tr>
<td>1000</td>
<td>0</td>
<td>-4</td>
<td>.4</td>
</tr>
<tr>
<td>2000</td>
<td>-1</td>
<td>-1</td>
<td>-2</td>
</tr>
<tr>
<td>4000</td>
<td>-1</td>
<td>-4</td>
<td>+3</td>
</tr>
<tr>
<td>8000</td>
<td>-1</td>
<td>+15</td>
<td>-14</td>
</tr>
<tr>
<td>10000</td>
<td>+3</td>
<td>+16</td>
<td>-13</td>
</tr>
</tbody>
</table>

21.3.3.1 Conversion factors from dB (A) to HL equivalent (corrections to be added)

From: Practical Aspects of Audiology, Chapter 4 Behavioural Hearing Tests, 6 months to 3 ½ years, Paediatric Audiology 0-5 years, 2nd Edition, Editor: Barry McCormick

21.3.3.2 OAEs

Limited
- TEOAE: One level (e.g., 80 dB pSPL) click stimulus should be completed. Normal distributions for this condition for normal hearing are documented in the literature [197].
- DPOAE: One level of L1 and L2 65/55 dB SPL at least at four frequencies. Normal distributions for this condition for normal hearing are documented in the literature [208].

Comprehensive
- TEOAE: Two levels (e.g., 80 dB pSPL and a lower level) may be completed and/or one level using click and multiple frequencies for stimuli, or
- DPOAE: Use of three levels (e.g., 65/55 and lower levels, a shown by Kummer, Janssen, & Arnold [209], Kummer, Janssen, Hulin & Arnold [210]) should be completed to obtain DPOAE input-output functions, or at one level for multiple (more than four) frequencies, or
- Comparison of TEOAE (e.g., single level, single stimulus) and DPOAE (e.g., single level): The TEOAE is a better predictor of low frequency and DPOAE of high frequency sensitivity [211, 212].
21.4 Ototoxic Drugs

The following drugs are known to be ototoxic and should be avoided in children treated with cisplatin. If the use of any of these drugs are judged necessary in an individual child on the trial and the drug cannot be substituted with a less toxic alternative the use has to be documented.

Especially aminoglycosides should be avoided (if not impossible) during the study and follow-up. Even the use of aminoglycosides ≤ 12 months prior to start of cisplatin treatment has to be reported.

Amikacin
Aminoglycosides
Aspirin (temporary by causing tinnitus)
Bumetanide
Desferroxamine
Ethacyrinic acid
Erythromycin (give i.v)
Furosemide
Gentamycin
Hexachlorobenzene
Interferon alpha 2 therapy
Kanamycin
4-Mehtylthiobenzoic acid interacts with platinium based medication
Mercury if ingested
Mitomycin (topical)
Neomycin
Norvancomycin
Propythiouracil
Quinine
Streptomycin
Streptidine
Styrene
Super oxides (Paraquat)
Teicoplanin
Tirapazamine
Tylenol
Vancomycin
Vincristine
APPENDIX: Monitoring of Renal Toxicities

22.1 Recommended Procedure for GFR Evaluation

1. Prepare a syringe containing 0.04 MBq of Cr-51-EDTA per kg of patient weight, and using a minimum activity of 0.4 MBq. At the same time prepare an accurately known percentage standard of the EDTA of about 2-4% of the activity to be administered.

2. Administer the EDTA to the patient using the most appropriate iv route, but taking care to thoroughly flush any lines if they are used.

3. Keep the emptied syringe and any other disposable items (e.g. butterfly needle or canula) that have been used in the administration.

4. Take three blood samples of 2-5ml from the patient, using a route of venous access which is different to that used for the administration. The samples should be taken at 1, 2 and 4 hours from administration of the EDTA. The exact timing is not critical but the sampling should not start before 1 hour or extend beyond 4 hours from the time of administration. The time of each sample should be recorded accurately.

5. Spin the blood samples down and transfer 1-2 ml of plasma into tubes suitable for counting on a standard gamma sample counter. Rinse out the contents of the EDTA syringe and any other items saved from the administration of the EDTA into a counting vial. If necessary split this between two or more vials – this is the ‘dead space’. Ensure all of the plasma samples, the dead space and the standard prepared in (1) are of about the same volume by adding water to any that might need it.

6. Count all the blood samples, the standard and the dead space together with an inactive background sample in an automatic gamma sample counter. Samples should be counted long enough to achieve at least 10,000 counts for each sample and more than 1,000 counts for the background.

7. Calculate the GFR for the patient using a single exponential model. This calculation should yield:
   - GFR, ml/min
   - T1/2 of the fitted line
   - The correlation coefficient for the fitted line
   - The volume of distribution projected to the time of administration

8. In order to ensure data from different centres are compatible data should be pooled centrally, using e-mailed spreadsheets, and in addition to the data listed in (7) ask for:
   - Administered activity (MBq)
   - Percentage standard
   - Standard counts per minute
   - For each sample:
     - Time for administration (minutes)
     - Volume (ml)
     - Counts per minute
   - Background counts per minute
9. If any centres prefer to use Tc-99m DTPA then they can either (i) use the above procedure ensuring that the standard is diluted sufficiently so that all samples can be counted simultaneously or (ii) count the plasma samples some time after counting the standard and dead space, and this time interval should also be recorded and communicated.

Remark: The point in the above list is to avoid prescribing exactly how each stage is done. This is to give basic guidelines on minimum requirements to avoid most frequent errors in evaluating GFR rates by the above method.

Recommendation by M.J. Keir BSc, PhD, MIPEM
CONSULTANT Medical Physicist
Head of Royal Victoria Infirmary Unit

Contact addresses:

Royal Victoria Infirmary
Queen Victoria Road
Newcastle upon Tyne, NE1 4 LP

Tel: +44 191 282 4038
Fax: +44 191 233 0351
www.rmpd.org.uk
22.2 Prescription sheet for GFR estimation

ESTIMATION OF GLOMERULAR FILTRATION RATE

PATIENT’S NAME: ..................................... HOSPITAL NUMBER: ............................

ADDRESS. ............................................. DATE OF BIRTH: ..............................

........................................................................ IP / OP Ward: ..............................

........................................................................ HOSPITAL: ..............................

Radiopharmaceutical: Cr – 51 EDTA

Activity of Dose: ...........................MBq Date of Test: ..............................

Volume of Injection: ...........................

Instructions To Ward Staff

1. The EDTA should be administered i.v. and the exact time of injection written below. The administration techniques are outlined overleaf. The method of administration (butterfly or multiple lumen central venous catheter) should be recorded below.

2. Replace the blind hub on the empty EDTA syringe and return it to the Medical Physics Department with the blood samples.

3. Blood samples of 5ml each should be taken at 1,2 and 4 hours and placed in heparinised tubes. These times are not critical, but the exact time of sampling should be noted below. All blood samples should come from a site remote from that used to administer the EDTA, but if one lumen of a central venous catheter is used to administer the EDTA, another lumen may be used to obtain samples.

Any deviation from this protocol should be noted below

Please Record:

Time of injection: .............................. butterfly □ multiple lumen catheter □

Weight (kg): .............................. Height (cm): ..............................

Sampling times: 1) .............................. 2) .............................. 3) ..............................

Notes:
ADMINISTRATION TECHNIQUE

In general, always administer using a butterfly needle.

i) Prime it with saline which can then be used to check the correct siting of the needle.

ii) Administer the EDTA.

iii) Use more saline to flush any EDTA remaining in the butterfly into the patient.

FOR PAEDIATRIC ONCOLOGY PATIENTS

Administration of EDTA down one lumen of a multiple (double or triple) lumen central venous catheter, with blood sampling from a separate lumen, is permitted as long as the following procedure is followed:

1. Establish that both lumens are flushing and sampling satisfactorily.

2. Inject 5ml 0.9% Saline through bung of one lumen using a blue needle. Other sizes of needle (e.g. green) are not adequate since they are the wrong length.

3. Inject EDTA through bung of same lumen using another blue needle.

4. Inject 10ml 0.9% Saline through bung using another blue needle and follow this with a 5ml Hepsal (or Heplock) flush.

5. Do not remove the bung to attach the syringe directly to the catheter hub during procedures 2, 3 or 4 since this does not allow adequate flushing of EDTA.

6. Identify the lumen used for EDTA injection and record this on the front of this form. Do not use again until all sampling complete, unless absolutely necessary for clinical purposes.

7. Sample at 1, 2 and 4 hours from the lumen not used for EDTA injection.

8. Tick the box on the Medical Physics form to indicate that central venous catheter has been used for EDTA administration.
## 22.3 GFR Table

Normal GFR in children and young adults

<table>
<thead>
<tr>
<th>Age (Sex)</th>
<th>Mean GFR ± SD (mL/min/1.73m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 week (males and females)</td>
<td>40.6 ± 14.8</td>
</tr>
<tr>
<td>2-8 weeks (males and females)</td>
<td>65.8 ± 24.8</td>
</tr>
<tr>
<td>&gt; 8 weeks (males and females)</td>
<td>95.7 ± 21.7</td>
</tr>
<tr>
<td>2-12 years (males and females)</td>
<td>133.0 ± 27.0</td>
</tr>
<tr>
<td>13-21 years (males)</td>
<td>140.0 ± 30.0</td>
</tr>
<tr>
<td>13-21 years (females)</td>
<td>126.0 ± 22.0</td>
</tr>
</tbody>
</table>

[http://www.kidney.org/professionals](http://www.kidney.org/professionals)

Data based on three studies:
2. Coulthard (1985) [214]
23  **APPENDIX : Drug Information**

### 23.1 Carboplatin

**Formulation**  Vials containing 50 mg/5 mls, 150 mg/15 mls or 450 mg/45 mls.

**Storage**  At room temperature.

**Reconstitution**  Each vial is reconstituted with water for injection BP, glucose injection 5% or sodium injection 0.9% to a final concentration of 10mg carboplatin/ml.

**Stability**  When reconstituted with water for injection or glucose 5%, 8 hours at room temperature, 24 hours in refrigerator. After dilution in glucose 5% infusion stable for 24 hours in refrigerator.

**Administration**  In this protocol as 1 hour intravenous infusion.

**Supplier**  Commercially available

#### Toxicity Frequencies

<table>
<thead>
<tr>
<th>Frequency and Timing</th>
<th>Common</th>
<th>Occasional</th>
<th>Rare</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immediate:</strong> Within 1-2 days of receiving drug</td>
<td>Nausea, vomiting</td>
<td>Hypersensitivity reactions(^2) (anaphylaxis, bronchospasm, hypotension), constipation, diarrhoea</td>
<td>Metallic taste, rash, mucositis</td>
</tr>
<tr>
<td><strong>Prompt:</strong> Within 2-3 weeks, prior to the next course</td>
<td>Myelosuppression(^1) (anaemia, neutropenia, leukopenia, thrombocytopenia), Electrolyte abnormalities (↓ Na, K, Ca, Mg)</td>
<td>↑ LFT’s (Alk Phos, AST), abdominal pain, Nephrotoxicity (↓ GFR, ↑ Cr and BUN), asthenia</td>
<td>↑ bilirubin</td>
</tr>
<tr>
<td><strong>Delayed:</strong> Any time later during therapy, excluding the above conditions</td>
<td>Ototoxicity (tinnitus, hearing loss)</td>
<td>Peripheral neuropathy with mild paresthesias, diminished sense of vibration, light touch, pinprick, and joint position, alopecia; temporary loss of vision to light and colors</td>
<td></td>
</tr>
<tr>
<td><strong>Late:</strong> Any time after completion of treatment</td>
<td></td>
<td></td>
<td>Secondary leukemia</td>
</tr>
<tr>
<td><strong>Unknown Frequency and Timing</strong></td>
<td>Fetal toxicities and teratogenic effects of carboplatin have been noted in animals and may cause fetal harm when administered to pregnant women. It is unknown whether the drug is excreted in breast milk.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Thrombocytopenia is more severe or dose limiting.

\(^2\) Hypersensitivity reactions are seen more frequently with repeated courses of therapy (after six courses in adults).

(L) Toxicity may also occur later.

### 23.2 Cisplatin

**Formulation**  Amber glass vials containing cisplatin solution 1mg (10, 25, 50 ml). Vials containing lyophilised cisplatin either 10mg or 50 mg.

**Storage**  At room temperature.

**Reconstitution**  Powder reconstituted with water for injection to a final concentration of 1mg/ml cisplatin. Must be further diluted before administration.

**Stability**  Cisplatin is unstable in aqueous vehicles unless chloride ions are present. Minimum concentration of sodium chloride providing an acceptable level of stability is approximately 0.3%w/v. At adequate chloride concentration, stability is unaffected by presence of glucose. Less than 4% degradation after 24hours at 25°C in solutions containing recommended chloride-ion concentration.

**Administration**  In this protocol as continuous 24hour intravenous infusion.

**Supplier**  Commercially available
Toxicity Frequencies

<table>
<thead>
<tr>
<th></th>
<th>Common</th>
<th>Occasional</th>
<th>Rare</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immediate:</strong></td>
<td>Within 1-2 days of receiving</td>
<td>Nausea (L), vomiting (L)</td>
<td>Metallic Taste (L)</td>
</tr>
<tr>
<td></td>
<td>drug</td>
<td></td>
<td>Anaphylactic reaction</td>
</tr>
<tr>
<td><strong>Prompt:</strong></td>
<td>Within 2-3 weeks, prior to</td>
<td>Anorexia (L), myelosuppression, hypomagnesemia (L), high frequency hearing loss (L), nephrotoxicity (L)</td>
<td>Electrolyte disturbances (L)</td>
</tr>
<tr>
<td></td>
<td>the next course</td>
<td></td>
<td>Peripheral neuropathy (L), tinnitus (L), seizure (L), liver toxicity (L)</td>
</tr>
<tr>
<td><strong>Delayed:</strong></td>
<td>Any time later during therapy, excluding the above conditions</td>
<td>Hearing loss in the normal hearing range</td>
<td></td>
</tr>
<tr>
<td><strong>Late:</strong></td>
<td>Any time after completion of treatment</td>
<td></td>
<td>Secondary malignancy</td>
</tr>
</tbody>
</table>

*(L) Toxicity may also occur later.

23.3 Etoposide

**Formulation**  
Vials containing 100 mg etoposide in 5 ml.

**Storage**  
At room temperature.

**Reconstitution**  
Ideally dilute to a concentration of 0.25-0.4 mg/ml in 0.9% sodium chloride or 5% dextrose.

**Stability**  
Vials are stable for 5 years at room temperature. At concentrations of 0.4 mg/ml in 0.9% saline solutions are stable for 96 hours at room temperature in normal fluorescent lighting, in PVA containers. Solution in PVC infusion bags should be used immediately, to avoid leaching out of potential carcinogenic plasticisers.

**Administration**  
During rapid COJEC by intravenous infusion over 4 hours - protected from light. Caution: Anaphylactic reaction usually manifested as severe hypotension may occur if infusion given too rapidly. Avoid extravasation.

**Supplier**  
Commercially available

Toxicity Frequencies

<table>
<thead>
<tr>
<th></th>
<th>Common</th>
<th>Occasional</th>
<th>Rare</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immediate:</strong></td>
<td>Within 1-2 days of receiving</td>
<td>Nausea, vomiting</td>
<td>Transient hypotension during infusion; anaphylaxis (chills, fever, tachycardia, dyspnea, bronchospasm, hypotension)</td>
</tr>
<tr>
<td></td>
<td>drug</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Prompt:</strong></td>
<td>Within 2-3 weeks, prior to</td>
<td>Myelosuppression (anemia, leukopenia), alopecia</td>
<td>Thrombocytopenia, diarrhea, abdominal pain, asthenia, malaise, rashes and urticaria</td>
</tr>
<tr>
<td></td>
<td>the next course</td>
<td></td>
<td>Peripheral neuropathy, Mucositis, hepatotoxicity, chest pain, thrombophlebitis, congestive heart failure, Stevens-Johnson Syndrome, exfoliative dermatitis</td>
</tr>
<tr>
<td><strong>Delayed:</strong></td>
<td>Any time later during therapy, excluding the above conditions</td>
<td>Dystonia, ovarian failure, amenorrhea, anovulatory cycles, hypomenorrhea, onycholysis of nails</td>
<td></td>
</tr>
<tr>
<td><strong>Late:</strong></td>
<td>Any time after completion of treatment</td>
<td></td>
<td>Secondary malignancy (preleukemic or leukemic syndromes)</td>
</tr>
</tbody>
</table>

**Unknown Frequency and Timing:** Fetal toxicities and teratogenic effects of etoposide have been noted in animals at 1/20th of the human dose. It is unknown whether the drug is excreted in breast milk.
23.4 Cyclophosphamide

**Formulation**
100 mg, 200 mg, 500 mg and 1 g vials for reconstitution.

**Storage**
At room temperature.

**Stability**
Unreconstituted vials stable for 5 years at room temperature. A solution of cyclophosphamide appears to be chemically stable for at least 28 days when stored at 4°C. Reconstituted solution (20 mg/ml) should be used within 3 hours when stored at room temperature, unless prepared under strict aseptic conditions, when it may be used within 8 hours.

**Administration**
Cyclophosphamide is given as i.v. bolus followed by post-cyclophosphamide infusion over 24 hours containing mesna. A mesna i.v. bolus is given prior to cyclophosphamide in addition in this protocol. (see individual course details for more information).

**Supplier**
Commercially available

### Toxicity Frequencies

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate: Within 1-2 days of receiving drug</td>
<td>Anorexia (L), nausea (L), vomiting (L)</td>
<td>Happens to 21-100 children out of every 100</td>
</tr>
<tr>
<td>Occasional</td>
<td>Abdominal discomfort, Diarrhoea</td>
<td>Happens to 5-20 children out of every 100</td>
</tr>
<tr>
<td>Rare</td>
<td>Transient blurred vision, nasal stuffiness with rapid administration, arrhythmias (rapid infusion), skin rash, anaphylaxis, SIADH</td>
<td>Happens to &lt;5 children out of every 100</td>
</tr>
<tr>
<td>Prompt: Within 2-3 weeks, prior to the next course</td>
<td>Leukopenia, alopecia, Immune suppression</td>
<td>Cardiac toxicity with high dose (acute CHF hemorrhagic myocarditis, myocardial necrosis) (L), hyperpigmentation, nail changes, impaired wound healing, infection secondary to immune suppression</td>
</tr>
<tr>
<td>Delayed: Any time later during therapy, excluding the above conditions</td>
<td>Gonadal dysfunction: azoospermia or oligospermia (prolonged or permanent) (L)</td>
<td>Amenorrhea&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Late: Any time after completion of treatment</td>
<td>Secondary malignancy (ALL, ANLL, AML), bladder carcinoma (long term use &gt; 2 years), bladder fibrosis</td>
<td></td>
</tr>
</tbody>
</table>

**Unknown Frequency and Timing:** Fetal toxicities and teratogenic effects of cyclophosphamide (alone or in combination with other antineoplastic agents) have been noted in humans. Toxicities include: chromosomal abnormalities, multiple anomalies, pancytopenia, and low birth weight. Cyclophosphamide is excreted into breast milk. Cyclophosphamide is contraindicated during breast feeding because of reported cases of neutropenia in breast fed infants and the potential for serious adverse effects.

<sup>1</sup>Dependent on dose, age, gender and degree of pubertal development at time of treatment

<sup>2</sup>Risk increased with chest radiation and high dose.

(L) Toxicity may also occur later.

23.5 MESNA (sodium 2-mercaptoethanesulfonate)

**Formulation**
Available in 1000mg/10ml multidose vials which contain 10.4mg/ml of benzyl alcohol<sup>8</sup> as a preservative, or in 200mg/2ml ampoules without preservatives for neonates and infants or patients with hypersensitivity to benzyl alcohol.

**Source and Pharmacology:** MESNA is a thiol compound with the capacity of inhibiting the urotoxicity of the oxazaphosphorines, ifosfamide and cyclophosphamide. Within 1 hour of administration, MESNA is completely oxidised to DiMESNA, a totally inert compound. After an 800mg dose the t½ for MESNA and DiMESNA is 0.36 hours and 1.17 hours, respectively. There is little or no tissue penetration.
Following glomerular filtration DiMESNA is rapidly reduced in the renal tubules back to MESNA which inactivates acrolein and the oxazaphosphamides, thus preventing bladder toxicity. Young children receiving high doses of benzyl alcohol (> 99 mg/kg/day) may develop the gasping syndrome manifested by gasping, metabolic acidosis and multiple organ system failure. Benzyl alcohol is the preservative in multidose vials of MESNA.

**Storage**
MESNA is not light-sensitive, but is oxidised to DiMESNA when exposed to oxygen. Non-preserved ampoules should be used immediately after opening, while benzyl alcohol-preserved vials may be stored and used for 8 days.

**Stability**
After further dilution for administration, either product is chemically stable for at least 24 hours. Lack of an antimicrobial preservative suggests that the non-preserved product should be used within 6-8 hours after diluted for administration. For IV administration, dilute to 20 mg/ml with any of the following fluids: 5% dextrose, 5% dextrose in 0.45% sodium chloride, 0.9% sodium chloride or Lactated Ringer's. MESNA may be mixed with ifosfamide or cyclophosphamide.

**Administration**
IV: Can be given orally but has a foul taste. Total dose is usually 60% of the oxazaphosphorine dose given in divided doses. Higher doses or continuous infusions are used with high dose ifosfamide or cyclophosphamide, or in patients with a history of hemorrhagic cystitis.

**Toxicity**
The package insert for MESNA states that multidose vials contain benzyl alcohol 10.4mg/ml (1%) as a preservative, should not be used in neonates or infants, and should be used with caution in older paediatric patients. A 200mg/2ml ampoule remains available free of charge for paediatric patients less than 2 years old and for patients with hypersensitivity to benzyl alcohol. It may be obtained in the U.S. by calling Bristol-Myers Squibb Oncology at 1-800-437-0994. The medical literature includes reports of gasping syndrome in premature infants receiving saline flushes with benzyl alcohol at benzyl alcohol doses greater than 99 mg/kg/day. There is also a report of metabolic acidosis occurring in a 5 year old girl receiving continuous infusion diazepam which contained 180 mg/kg/day of benzyl alcohol [216]. The syndrome includes gasping respiration, severe metabolic acidosis, and multiple organ system failure. It results from inability to adequately conjugate benzoic acid with glycine, a metabolic pathway poorly developed under 8 weeks of age. Even if the amount of benzyl alcohol in MESNA is not enough to cause problems in a patient, a number of other drug products contain benzyl alcohol and therefore could add to the dose a patient is receiving. Your pharmacist can check product contents and calculate the dose of benzyl alcohol any patient is receiving.

**Supplier**
Commercially available

**Toxicity Frequencies**

<table>
<thead>
<tr>
<th></th>
<th>Common</th>
<th>Occasional</th>
<th>Rare</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Happens to 21 - 100 children out of 100</td>
<td>Happens to 5-20 children out of 100</td>
<td>Happens to &lt;5 children out of 100</td>
</tr>
<tr>
<td><strong>Immediate:</strong></td>
<td>Within 1-2 days of receiving drug</td>
<td>Bad taste with oral use</td>
<td>Headache, pain in arms, legs, and joints; fatigue, rash, transient hypotension, allergy</td>
</tr>
<tr>
<td><strong>Prompt:</strong></td>
<td>Within 2-3 weeks, prior to the next course</td>
<td>Nausea, vomiting, stomach pain</td>
<td>Diarrhoea</td>
</tr>
<tr>
<td><strong>Delayed:</strong></td>
<td>Any time later during therapy, excluding the above conditions</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Late:</strong></td>
<td>Any time after completion of treatment</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
23.6 Vincristine

**Formulation**
1 mg, 2 mg, 5 mg vials with 10 mls diluent. 1 ml, 2 ml, 5 ml vials of solution 1 mg/ml.
Also available in pre-filled syringes containing 1 mg in 1 ml, 2 mg in 2 mls unpreserved.

**Storage**
At 2-8°C in refrigerator.

**Stability**
Depends on formulation: Lyophilised powder (0-6°C) 3 years. Solution (0-6°C) 2 years. Reconstituted injection is stable for 14 days (2-8°C). Diluted infusion (in 0.9% saline, 5% dextrose or Ringer's lactate) is stable for 24 hours at 20 μg/ml.

**Administration:** By bolus intravenous injection. Ensure that needle is well into the vein to avoid extravasation. It is strongly recommended that all vincristine injections are labelled "FOR INTRAVENOUS USE ONLY".

**Supplier**
Commercially available

### Toxicity Frequencies

<table>
<thead>
<tr>
<th>Immediate: Within 1-2 days of receiving drug</th>
<th>Occasional: Happens to 5-20 children out of every 100</th>
<th>Rare: Happens to &lt;5 children out of every 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hair loss, constipation</td>
<td>Weakness, abdominal pain, mild brief myelosuppression (leukopenia, thrombocytopenia, anemia)</td>
<td>Paralytic ileus, ptosis, diplopia, night blindness, hoarseness, vocal cord paralysis, SIADH, seizure, defective sweating</td>
</tr>
</tbody>
</table>

**Delayed:** Any time later during therapy, excluding the above conditions

| Loss of deep tendon reflexes | Peripheral paresthesias (including numbness, tingling and pain); clumsiness; wrist drop, foot drop; abnormal gait | Difficulty walking or inability to walk, sinusoidal obstruction syndrome (SOS, formerly VOD) (in combination), blindness, optic atrophy, urinary tract disorders (including bladder atony, dysuria, polyuria, nocturia, urinary retention), autonomic neuropathy with postural hypotension; 8th cranial nerve damage with dizziness, nystagmus, vertigo and hearing loss |

**Late:** Any time after the completion of treatment

**Unknown Frequency and Timing:** Fetal toxicities and teratogenic effects of vincristine (either alone or in combination with other antineoplastic agents) have been noted in humans. The toxicities include: chromosome abnormalities, malformation, pancytopenia, and low birth weight. It is unknown whether the drug is excreted in breast milk.

23.7 Topotecan

**Nomenclature and structure**
Generic names: topotecan (topotecan for injection contains topotecan hydrochloride) SK&F 104864
Commercial name: Hycamtin
Chemical name: (S)-9-dimethyl-aminomethyl-10-hydroxy-20(s)-CPT
Molecular formula: C_{23}H_{23}N_{3}O_{5}
Molecular weight: 457.92
**Availability** Topotecan for injection is supplied by SmithKlineBeecham in vials containing 1mg and 4 mg of topotecan. Each vial contains mannitol, 48 mg, and tartaric acid, 20 g. Topotecan is also available in oral formulation for investigational use only.

**Storage** Vials should be stored between 20°C-25°C and protected from light.

**Administration** Topotecan for injection should be reconstituted for i.v. use by adding 1.1 ml of sterile water for injection (10% overage) to the 1mg vial or 4.4 ml sterile water to the 4mg vial. The resulting yellow or yellow-green solution will contain 1 mg per ml. An appropriate volume of the injection should be further diluted with 5% dextrose injection or 0.9% sodium chloride injection. Final concentrations and volumes of 20 to 50 microgrammes per ml in 50 to 100 ml of diluent are recommended. Because topotecan contains no antimicrobial agent, it should be used immediately. However, the reconstituted injection may be stored in a refrigerator for up to 24 hours if necessary. After further dilution for i.v. infusion, the product is stable for up to 24 hours when stored between 20° and 25°C.

**Toxicity Frequencies**

<table>
<thead>
<tr>
<th></th>
<th>Common</th>
<th>Occasional</th>
<th>Rare</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Happens to 21-100 children out of every 100</td>
<td>Happens to 5-20 children out of every 100</td>
<td>Happens to &lt;5 children out of every 100</td>
</tr>
<tr>
<td><strong>Immediate:</strong></td>
<td>Nausea, vomiting, Diarrhea (L), constipation, Fever, pain (abdominal, skeletal, back pain)</td>
<td>Anorexia, headache, asthenia, rash (urticaria, pruritis, bullous eruption) (L), asymptomatic hypotension, Dyspnea</td>
<td>Anaphylaxis, angioedema, chest pain, rigors</td>
</tr>
<tr>
<td><strong>Prompt:</strong></td>
<td>Myelosuppression, Fatigue, Febrile neutropenia</td>
<td>Stomatitis/mucositis, Increased AST/ALT/ALK PHOS, Sepsis</td>
<td>Elevated Bilirubin, paresthesias, Myalgia, arthralgia, intratumoral bleeding</td>
</tr>
<tr>
<td><strong>Delayed:</strong></td>
<td>Alopecia</td>
<td></td>
<td>Microscopic proteinuria</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Late:</strong></td>
<td>Anytime after completion of therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Unknown Frequency and Timing:</strong></td>
<td>Teratogenic effects of topotecan have been noted in animal models at doses ≤ to those used in humans. It is not known if topotecan is excreted into human breast milk.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*(L) Toxicity may also occur later.*

### 23.8 Doxorubicin

**Formulation** Doxorubicin is supplied in vials containing 10mg and 50mg freeze dried powder. These should be reconstituted with water for injection or sodium chloride 0.9% injection adding 5ml to the 10mg vial and 25ml to the 50mg vial to give a 2mg/ml solution. It is also available as vials containing 10mg and 50mg as a 2mg/ml solution in 0.9% sodium chloride.

**Dilution** Doxorubicin can be diluted in 0.9% saline or in 5% dextrose

**Stability** A large body of information is available on the stability of doxorubicin in solution. Doxorubicin is compatible with polypropylene, polyvinyl chloride (PVC), glass, ethylene vinylacetate (EVA) and polyisoprene containers. Solutions should be protected from light during storage and administration unless the solution is freshly prepared and the concentration is greater than or equal to 0.5mg/ml. In addition doxorubicin appears to be chemically stable in polypropylene, PVC, or EVA containers for at least 7 days, when refrigerated or stored at room temperature, protected from light, and diluted in sodium chloride 0.9% at concentrations of 0.1mg/ml to 2mg/ml.
Administration A baseline echocardiogram must be done prior to treatment. This should be repeated prior to alternate courses of doxorubicin up to a total cumulative dose of 300mg/m², and before each course thereafter. Due to its extremely vesicant nature doxorubicin should be administered via an indwelling central venous catheter.

Supplier Commercially available

Toxicity Frequencies

<table>
<thead>
<tr>
<th>Immediate: Within 1-2 days of receiving drug</th>
<th>Common Happens to 21-100 children out of every 100</th>
<th>Occasional Happens to 5-20 children out of every 100</th>
<th>Rare Happens to &lt;5 children out of every 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea, vomiting, pink or red color to urine, sweat, tears and saliva</td>
<td>Hyperuricemia, facial flushing, sclerosis of the vein</td>
<td>Diarrhea, anorexia, erythematous streaking of the vein (flare reaction), extravasation (rare) but if occurs = local ulceration, anaphylaxis, fever, chills, urticaria, acute arrhythmias</td>
<td></td>
</tr>
</tbody>
</table>

Prompt: Within 2-3 weeks, prior to next course

- Myelosuppression (leukopenia, thrombocytopenia, anemia), alopecia
- Mucositis (stomatitis and esophagitis), hepatotoxicity
- Radiation recall reactions, conjunctivitis and lacrimation

Delayed: Anytime later during therapy

- Cardiomyopathy\(^1\) (CHF occurs in 5-20% @ cumulative doses ≥450mg/m²)(L)
- Cardiomyopathy\(^2\) (CHF occurs in <5% @ cumulative doses ≤400mg/m²) (L), ulceration and necrosis of colon, hyper-pigmentation of nail bed and dermal crease, onycholysis

Late: Anytime after completion of therapy

- Subclinical cardiac dysfunction
- CHF (on long term follow up in pediatric patients)
- Secondary malignancy (in combination regimens)

Unknown Frequency and Timing: Fetal and teratogenic toxicities. Carcinogenic and mutagenic effects of doxorubicin have been noted in animal models. Doxorubicin is excreted into breast milk in humans.

\(^1\) Risk increases with chest radiation, exposure at a young or advanced age. (L) Toxicity may also occur later.

23.9 Busulfan

23.9.1 I.V. BUSULFAN (BUSILVEX ®)

Summary of product characteristics

Name of the medicinal product
Busilvex ® 6 mg/ml concentrate for solution for infusion

Qualitative and quantitative composition
1 ml of concentrate contains 6 mg of busulfan (60 mg in 10 ml).
After dilution: 1 ml of solution contains 0.5 mg of busulfan
For excipients see “Pharmaceutical Particulars/List of excipients”

Pharmaceutical form
Concentrate for solution for infusion.
Clear, colourless solution.
Clinical particulars

Therapeutic indications
Busilvex ® followed by cyclophosphamide (BuCy2) is indicated as conditioning treatment prior to conventional haematopoietic progenitor cell transplantation (HPCT) in adult patients when the combination is considered the best available option.
Busilvex ® followed by cyclophosphamide (BuCy4) or melphalan (BuMel) is indicated as conditioning treatment prior to conventional haematopoietic progenitor cell transplantation in paediatric patients.

Posology and method of administration
Busilvex ® administration should be supervised by a physician experienced in conditioning treatment prior to haematopoietic progenitor cell transplantation.

Dosage in adults
When followed by 2 cycles of 60 mg/kg body weight (BW) cyclophosphamide the recommended dosage and schedule of administration is 0.8 mg/kg BW of busulfan as a two-hour infusion every 6 hours over 4 consecutive days for a total of 16 doses prior to cyclophosphamide and conventional haematopoietic progenitor cell transplantation (HPCT).

It is recommended that cyclophosphamide dosing should not be initiated for at least 24 hours following the 16th dose of Busilvex ®.

Dosage in new-born infants children and adolescents
The recommended dose of Busilvex ® is as follows:

<table>
<thead>
<tr>
<th>Actual body weight (kg)</th>
<th>Busilvex® dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;9</td>
<td>1.0</td>
</tr>
<tr>
<td>9 to &lt; 16</td>
<td>1.2</td>
</tr>
<tr>
<td>16 to 23</td>
<td>1.1</td>
</tr>
<tr>
<td>&gt; 23 to 34</td>
<td>0.95</td>
</tr>
<tr>
<td>&gt; 34</td>
<td>0.8</td>
</tr>
</tbody>
</table>

followed by 4 cycles of 50 mg/kg body weight (BW) cyclophosphamide (BuCy4) or by one administration of 140 mg/m² melphalan (BuMel).
Busilvex® is administered as a two-hour infusion every 6 hours over 4 (or 5) consecutive days for a total of 16 doses prior to cyclophosphamide or melphalan and conventional haematopoietic progenitor cell transplantation (HPCT).

It is recommended that melphalan administration should not be initiated for at least 24 hours following the 16th dose of Busilvex ®.

Administration
Busilvex® must be diluted prior to administration. A final concentration of approximately 0.5 mg/ml busulfan should be achieved. Busilvex® should be administered by intravenous infusion via central venous catheter.
Busilvex® should not be given by rapid intravenous, bolus or peripheral injection.

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®.
In adults all studied patients received phenytoin. There is no experience with other anticonvulsant agents such as benzodiazepines. In children studied patients received either phenytoin or benzodiazepines.

Antiemetics should be administered prior to the first dose of Busilvex® and continued on a fixed schedule according to local practice through its administration.

**Obese patients**

**In adults**
For obese patients, dosing based on adjusted ideal body weight (AIBW) should be considered.

Ideal body weight (IBW) is calculated as follows:

- IBW men (kg) = 50 + 0.91 x (height in cm - 152);
- IBW women (kg) = 45 + 0.91 x (height in cm - 152).

Adjusted ideal body weight (AIBW) is calculated as follows:

\[ \text{AIBW} = \text{IBW} + 0.25 \times (\text{actual body weight} - \text{IBW}) \]

**In new-born infants, children and adolescents**
There is no experience in obese children and adolescents with body mass index Weight (kg) / (m)^2 > 30 kg/m^2.

**Renally impaired patient:**
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. However, caution is recommended.

**Hepatically impaired patient:**
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.

**Contraindications**
Hypersensitivity to the active substance or to any of the excipients
Pregnancy and lactation.

**Special warnings and special precautions for use**
The consequence of treatment with Busilvex® at the recommended dose and schedule is profound myelosuppression, occurring in all patients. Severe granulocytopenia, thrombocytopenia, anaemia, or any combination thereof may develop. Frequent complete blood counts, including differential white blood cell counts, and platelet counts should be monitored during the treatment and until recovery is achieved.

Prophylactic or empiric use of anti-infectives (bacterial, fungal, viral) should be considered for the prevention and management of infections during the neutropenic period. Platelet and red blood cell support, as well as the use of growth factors such as G-CSF, should be employed as medically indicated.

In adults, absolute neutrophil counts < 0.5x10^9/l at a median of 4 days post transplant occurred in 100% of patients and recovered at median day 10 and 13 days following autologous and allogeneic transplant respectively (median neutropenic period of 6 and 9 days respectively). Thrombocytopenia (< 25,000/mm^3 or requiring platelet transfusion) occurred at a median of 5-6 days in 98% of patients. Anaemia (haemoglobin < 8.0 g/dl) occurred in 69% of patients.
In paediatric patients, absolute neutrophil counts < 0.5x10^9/l at a median of 3 days post transplant occurred in 100% of patients and lasted 5 and 18.5 days in autologous and allogeneic transplant respectively. In children, thrombocytopenia (< 25,000/mm3 or requiring platelet transfusion) occurred in 100% of patients. Anemia (haemoglobin< 8.0 g/dl) occurred in 100% of patients.

The Fanconi anemia cells have hypersensitivity to cross-linking agents. There is few clinical experience of the use of busulfan as component of conditioning regimen prior to HSCT in children with Fanconi anemia. Therefore Busilvex® should be used with caution in this type of patients. Busilvex® as well as busulfan has not been studied in patients with hepatic impairment. Since busulfan is mainly metabolized through the liver, caution should be observed when Busilvex® is used in patients with pre-existing impairment of liver function, especially in those with severe hepatic impairment. It is recommended when treating these patients that serum transaminase, alkaline phosphatase, and bilirubin should be monitored regularly 28 days following transplant for early detection of hepatotoxicity.

Hepatic veno-occlusive disease is a major complication that can occur during treatment with Busilvex®. Patients who have received prior radiation therapy, greater than or equal to three cycles of chemotherapy, or prior progenitor cell transplant may be at an increased risk.

Caution should be exercised when using paracetamol prior to (less than 72 hours) or concurrently with Busilvex® due to a possible decrease in the metabolism of busulfan.

As documented in clinical studies, no treated patients experienced cardiac tamponade or other specific cardiac toxicities related to Busilvex®. However cardiac function should be monitored regularly in patients receiving Busilvex®.

Occurrence of acute respiratory distress syndrome with subsequent respiratory failure associated with interstitial pulmonary fibrosis was reported in Busilvex® studies in one patient who died, although, no clear etiology was identified. In addition, busulfan might induce pulmonary toxicity that may be additive to the effects produced by other cytotoxic agents. Therefore, attention should be paid to this pulmonary issue in patients with prior history of mediastinal or pulmonary radiation.

Periodic monitoring of renal function should be considered during therapy with Busilvex®.

Seizures have been reported with high dose busulfan treatment. Special caution should be exercised when administering the recommended dose of Busilvex® to patients with a history of seizures. Patients should receive adequate anti-convulsant prophylaxis. In adults, all data with Busilvex® were obtained using phenytoin. There are no data available on the use of other anticonvulsant agents such as benzodiazepines. Thus, the effect of anticonvulsant agents (other than phenytoin) on busulfan pharmacokinetics is not known.

In paediatric patients, data with Busilvex® were obtained using benzodiazepines or phenytoin.

The increased risk of a second malignancy should be explained to the patient. On the basis of human data, busulfan has been classified by the International Agency for Research on Cancer (IARC) as a human carcinogen. The World Health Association has concluded that there is a causal relationship between busulfan exposure and cancer. Leukaemia patients treated with busulfan developed many different cytological abnormalities, and some developed carcinomas. Busulfan is thought to be leukemogenic.

Fertility: busulfan can impair fertility. Therefore, men treated with Busilvex® are advised not to father a child during and up to 6 months after treatment and to seek advice on cryo-conservation of sperm prior to treatment because of the possibility of irreversible infertility due to therapy with Busilvex. Ovarian suppression and amenorrhoea with menopausal symptoms commonly occur in
pre-menopausal patients. Busulfan treatment in a pre-adolescent girl prevented the onset of puberty due to ovarian failure. Impotence, sterility, azoospermia, and testicular atrophy have been reported in male patients. The solvent dimethylacetamide (DMA) may also impair fertility. DMA decreases fertility in male and female rodents.

**Interaction with other medicinal products and other forms of interaction**

No specific clinical trial was carried out to assess drug-drug interaction between i.v. busulfan and itraconazole. From published studies, in adults administration of itraconazole to patients receiving high-dose busulfan may result in reduced busulfan clearance. Patients should be monitored for signs of busulfan toxicity when itraconazole is used as an antifungal prophylaxis with i.v. busulfan.

Published studies in adults described that ketobemidone (analgesic) might be associated with high levels of plasma busulfan. Therefore special care is recommended when combining these two drugs.

In adults, for the BuCy2 regimen it has been reported that the time interval between the last oral busulfan administration and the first cyclophosphamide administration may influence the development of toxicities. A reduced incidence of Hepatic Veino Occlusive Disease (HVOD) and other regimen related toxicity have been observed in patients when the lag time between the last dose of oral busulfan and the first dose of cyclophosphamide is > 24 hours.

In paediatric patients, for the BuMel regimen it has been reported that the administration of melphalan less than 24 hours after the last oral busulfan administration may influence the development of toxicities.

Paracetamol is described to decrease glutathione levels in blood and tissues, and may therefore decrease busulfan clearance when used in combination.

Phenytoin or benzodiazepines were administered for seizure prophylaxis in all patients in the clinical trials conducted with i.v. busulfan. The concomitant systemic administration of phenytoin to patients receiving high-dose busulfan has been reported to increase busulfan clearance, due to induction of glutathion-S-transferase. However no evidence of this effect has been seen in i.v. data.

No interaction has been reported when benzodiazepines such as diazepam, clonazepam or lorazepam have been used to prevent seizures with high-dose busulfan.

No interaction was observed when busulfan was combined with fluconazole (antifungal agent) or 5-HT3 antiemetics such as ondansetron or granisetron.

**Pregnancy and lactation**

**Pregnancy**

HPCT is contraindicated in pregnant women; therefore, Busilvex® is contraindicated during pregnancy. Busulfan has caused embryofetal lethality and malformations in pre-clinical studies. There are no adequate and well-controlled studies of either busulfan or DMA in pregnant woman. A few cases of congenital abnormalities have been reported with low-dose oral busulfan, not necessarily attributable to the drug, and third trimester exposure may be associated with impaired intrauterine growth.

Women of childbearing potential have to use effective contraception during and up to 6 months after treatment.

**Lactation**

Patients who are taking Busilvex® should not breast-feed. It is not known whether busulfan and DMA are excreted in human milk. Because of the potential for tumorigenicity shown for busulfan in human and animal studies, breast-feeding should be discontinued at the start of therapy.

**Effects on ability to drive and use machines**

Not relevant
Undesirable effects

Undesirable effects in adults

Adverse events information are derived from two clinical trials (n=103) of Busilvex®. Serious toxicities involving the hematologic, hepatic and respiratory systems were considered as expected consequences of the conditioning regimen and transplant process. These include infection and Graft-versus-host disease (GVHD) which although not directly related, were the major causes of morbidity and mortality, especially in allogeneic HPCT.

Blood and the lymphatic system disorders:
Myelo-suppression and immuno-suppression were the desired therapeutic effects of the conditioning regimen. Therefore all patients experienced profound cytopenia: leukopenia 96%, thrombocytopenia 94%, and anemia 88%. The median time to neutropenia was 4 days for both autologous and allogeneic patients. The median duration of neutropenia was 6 days and 9 days for autologous and allogeneic patients.

Immune system disorders:
The incidence of acute graft versus host disease (a-GVHD) data was collected in OMC-BUS-4 study (allogeneic)(n=61). A total of 11 patients (18%) experienced a-GVHD. The incidence of a-GVHD grades I-II was 13% (8/61), while the incidence of grade III-IV was 5% (3/61). Acute GVHD was rated as serious in 3 patients. Chronic GVHD (c-GVHD) was reported if serious or the cause of death, and was reported as the cause of death in 3 patients.

Infections and infestations:
39% of patients (40/103) experienced one or more episodes of infection, of which 83% (33/40) were rated as mild or moderate. Pneumonia was fatal in 1% (1/103) and life-threatening in 3% of patients. Other infections were considered severe in 3% of patients. Fever was reported in 87% of patients and graded as mild/moderate in 84% and severe in 3%. 47% of patients experienced chills which were mild/moderate in 46% and severe in 1%.

Hepato-biliary disorders:
15% of SAEs involved liver toxicity. HVOD is a recognized potential complication of conditioning therapy post-transplant. Six of 103 patients (6%) experienced HVOD. HVOD occurred in: 8.2% (5/61) allogeneic patients (fatal in 2 patients) and 2.5% (1/42) of autologous patients. Elevated bilirubin (n=3) and elevated AST (n=1) were also observed. Two of the above four patients with serious serum hepatotoxicity were among patients with diagnosed HVOD.

Respiratory, thoracic and mediastinal disorders:
One patient experienced a fatal case of acute respiratory distress syndrome with subsequent respiratory failure associated with interstitial pulmonary fibrosis in the Busilvex® studies. In addition the literature review reports alterations of cornea and lens of the eye with oral busulfan.

Adverse events in new-born infants, children and adolescents:
Adverse events information are derived from the clinical study in paediatrics (n=55). Serious toxicities involving the hepatic and respiratory systems were considered as expected consequences of the conditioning regimen and transplant process.

Immune system disorders:
The incidence of acute graft versus host disease (a-GVHD) data was collected in allogeneic patients (n=28). A total of 14 patients (50%) experienced a-GVHD. The incidence of a-GVHD grades I-II was 46.4% (13/28), while the incidence of grade III-IV was 3.6% (1/28). Chronic GVHD was reported only if it is the cause of death: one patient died 13 months post-transplant.
Infections and infestations:
Infections (documented and non documented febrile neutropenia) were experienced in 89% of patients (49/55). Mild/moderate fever was reported in 76% of patients.

Hepato-biliary disorders:
Grade 3 elevated transaminases were reported in 24% of patients. VOD was reported in 15% (4/27) and 7% (2/28) of the autologous and allogenic transplant respectively. VOD observed were neither fatal nor severe and resolved in all cases.

Toxicity frequency listed by organ class:
Adverse reactions reported both in adults and paediatric patients as more than an isolated case are listed below, by system organ class and by frequency. Frequencies are defined as: very common (> 1/10), common (> 1/100, < 1/10), uncommon (> 1/1,000, < 1/100).

<table>
<thead>
<tr>
<th>System organ class</th>
<th>Very common</th>
<th>Common</th>
<th>Uncommon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood and lymphatic system disorders</td>
<td>Neutropenia</td>
<td></td>
<td>Delirium</td>
</tr>
<tr>
<td></td>
<td>Thrombocytopenia</td>
<td></td>
<td>Nervousness</td>
</tr>
<tr>
<td></td>
<td>Anaemia</td>
<td></td>
<td>Hallucination</td>
</tr>
<tr>
<td></td>
<td>Pancytopenia</td>
<td></td>
<td>Agitation</td>
</tr>
<tr>
<td></td>
<td>Febrile neutropenia</td>
<td></td>
<td>Encephalopathy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cerebral haemorrhage</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Seizure</td>
</tr>
<tr>
<td>Nervous system disorders</td>
<td>Insomnia</td>
<td>Confusion</td>
<td>Delirium</td>
</tr>
<tr>
<td></td>
<td>Dizziness</td>
<td></td>
<td>Nervousness</td>
</tr>
<tr>
<td></td>
<td>Depression</td>
<td></td>
<td>Hallucination</td>
</tr>
<tr>
<td>Metabolism and nutrition disorders</td>
<td>Anorexia</td>
<td>Hyponatraemia</td>
<td>Agitation</td>
</tr>
<tr>
<td></td>
<td>Hyperglycaemia</td>
<td></td>
<td>Encephalopathy</td>
</tr>
<tr>
<td></td>
<td>Hypomagnesaemia</td>
<td></td>
<td>Cerebral haemorrhage</td>
</tr>
<tr>
<td></td>
<td>Hypokalaemia</td>
<td></td>
<td>Seizure</td>
</tr>
<tr>
<td></td>
<td>Hypocalcaemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hypophosphatemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oedema</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychiatric disorders</td>
<td>Anxiety</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac disorders</td>
<td>Tachycardia</td>
<td>Arrhythmia</td>
<td>Femoral artery thrombosis</td>
</tr>
<tr>
<td></td>
<td>Hypertension</td>
<td>Atrial fibrillation</td>
<td>Ventricular extrasystoles</td>
</tr>
<tr>
<td></td>
<td>Hypotension</td>
<td>Cardiomegaly</td>
<td>Bradycardia</td>
</tr>
<tr>
<td></td>
<td>Vasodilatation</td>
<td>Pericardial effusion</td>
<td>Capillary leak syndrome</td>
</tr>
<tr>
<td></td>
<td>Thrombosis</td>
<td>Pericarditis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decrease ejection fraction</td>
<td></td>
</tr>
<tr>
<td>System organ class</td>
<td>Very common</td>
<td>Common</td>
<td>Uncommon</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>------------------------------------------------------</td>
<td>---------------------------------</td>
<td>---------------------------------</td>
</tr>
</tbody>
</table>
| Respiratory thoracic and mediastinal disorders | Dyspnoea  
Rhinitis  
Pharyngitis  
Cough  
Hiccup  
Epistaxis  
Abnormal breath sounds | Hyperventilation  
Respiratory failure  
Alveolar haemorrhages  
Asthma  
Atelectasis  
Pleural effusion | Hypoxia |
| Gastrointestinal disorders         | Nausea  
Stomatitis  
Vomiting  
Diarrhoea  
Constipation  
Dyspepsia  
Anus discomfort  
Abdominal pain  
Ascites | Oesophagitis  
Ileus  
Haematemesis | Gastrointestinal haemorrhage |
| Hepato-biliary disorders           | Hepatomegaly  
Jaundice | | |
| Skin and subcutaneous tissue disorders | Rash  
Pruritis  
Alopecia | Erythema  
Pigmentation disorder  
Skin desquamation | |
| Musculoskeletal and connective tissue disorders | Back pain  
Myalgia  
Arthralgia | | |
| Renal and urinary disorders        | Creatinine elevated  
Dysuria  
Oligurea | Bun increase  
Haematuria  
Moderate renal insufficiency | |
| General disorders and administration site conditions | Fever  
Headache  
Asthenia  
Chills  
Pain  
Allergic reaction  
Oedema general  
Pain or inflammation at injection site  
Chest pain  
Mucositis | | |
| Investigations                     | Transaminases increased  
Bilirubin increased  
GGT increased  
Weight increased  
Alkaline phosphatases increased | | |
**Overdose**

The principal toxic effect is profound myeloablation and pancytopenia but the central nervous system, liver, lungs, and gastrointestinal tract may also be affected. There is no known antidote to Busilvex® other than haematopoietic progenitor cell transplantation. In the absence of haematopoietic progenitor cell transplantation, the recommended dosage of Busilvex® would constitute an overdose of busulfan. The haematologic status should be closely monitored and vigorous supportive measures instituted as medically indicated. There have been two reports that busulfan is dialyzable, thus dialysis should be considered in the case of an overdose. Since, busulfan is metabolized through conjugation with glutathione, administration of glutathione might be considered. It must be considered that overdose of Busilvex® will also increase exposure to DMA. In humans the principal toxic effects were hepatotoxicity and central nervous system effects. CNS changes precede any of the more severe side effects. No specific antidote for DMA overdose is known. In case of overdose, management would include general supportive care.

**Pharmacological properties**

**Pharmacodynamic properties**

Pharmacotherapeutic group: Cytotoxic agents (alkylating agents).
ATC code: L01AB01

Busulfan is a potent cytotoxic agent and a bifunctional alkylating agent. In aqueous media, release of the methanesulphonate groups produces carbonium ions which can alkylate DNA, thought to be an important biological mechanism for its cytotoxic effect.

**Clinical trials in adults**

Documentation of the safety and efficacy of Busilvex® in combination with cyclophosphamide in the BuCy2 regimen prior to conventional allogeneic and/or autologous HPCT derive from two clinical trials (OMC-BUS-4 and OMC-BUS-3).

Two prospective, single arm, open-label, uncontrolled phase II studies were conducted in patients with haematological disease, the majority of whom had advanced disease.

Diseases included were acute leukemia past first remission, in first or subsequent relapse, in first remission (high risk), or induction failures; chronic myelogenous leukemia in chronic or advanced phase; primary refractory or resistant relapsed Hodgkin’s disease or non-Hodgkin’s lymphoma, and myelodysplastic syndrome.

Patients received doses of 0.8 mg/kg busulfan every 6 hours infusion for a total 16 doses followed by cyclophosphamide at 60 mg/kg once per day for two days (BuCy2 regimen). The primary efficacy parameters in these studies were myeloablation, engraftment, relapse, and survival.

In both studies, all patients received a 16/16 dose regimen of Busilvex®. No patients were discontinued from treatment due to adverse reactions related to Busilvex®.

All patients experienced a profound myelosuppression. The time to Absolute Neutrophil Count (ANC) greater than 0.5 x 10⁶/l was 13 days (range 9-29 days) in allogenic patients (OMC-BUS 4), and 10 days (range 8-19 days) in autologous patients (OMC-BUS 3). Overall mortality and non-relapse mortality at more than 100 days post-transplant was (8/61) 13% and (6/61) 10% in allotransplanted patients, respectively. During the same period there was no death in autologous recipients.
Clinical trials in paediatric patients
Documentation of the safety and efficacy of Busilvex® in combination with cyclophosphamide in the BuCy4 or with melphalan in the BuMel regimen prior to conventional allogeneic and/or autologous HPCT derives from clinical trial F60002 IN 101 G0.

All patients experienced a profound myelosuppression. The time to Absolute Neutrophil Count (ANC) greater than 0.5x10^6/l was 21 days (range 12-47 days) in allogenic patients, and 11 days (range 10-15 days) in autologous patients. 93% of allogeneic patients showed complete chimerism. There was no regimen-related death through the first 100-day post-transplant and up to one year post-transplant.

Pharmacokinetic properties

The pharmacokinetics of Busilvex® has been investigated. The information presented on metabolism and elimination is based on oral busulfan.

Absorption
The pharmacokinetics of i.v. busulfan was studied in 124 evaluable patients following a 2-hour intravenous infusion for a total of 16 doses over four days. Immediate and complete availability of the dose is obtained after intravenous infusion of busulfan. Similar blood exposure was observed when comparing plasma concentrations in patients receiving oral and i.v. busulfan at 1 mg/kg and 0.8 mg/kg respectively. Low inter (CV=21%) and intra (CV=12%) patient variability on drug exposure was demonstrated through a population pharmacokinetic analysis, performed on 102 patients.

Distribution
Terminal volume of distribution Vz ranged between 0.62 and 0.85 l/kg. Busulfan concentrations in the cerebrospinal fluid are comparable to those in plasma although these concentrations are probably insufficient for anti-neoplastic activity. Reversible binding to plasma proteins was around 7% while irreversible binding, primarily to albumin, was about 32%.

Metabolism
Busulfan is metabolised mainly through conjugation with glutathione (spontaneous and glutathione-Transferase mediated). The glutathione conjugate is then further metabolised in the liver by oxidation.

None of the metabolites is thought to contribute significantly to either efficacy or toxicity.

Elimination
Total clearance in plasma ranged 2.25 - 2.74 ml/minute/kg. The terminal half-life ranged from 2.8 to 3.9 hours.
Approximately 30% of the administered dose is excreted into the urine over 48 hours with 1% as unchanged drug. Elimination in faeces is negligible. Irreversible protein binding may explain the incomplete recovery. Contribution of long-lasting metabolites is not excluded.

Pharmacokinetic linearity
The dose proportional increase of drug exposure was demonstrated following intravenous busulfan up to 1 mg/kg.

Pharmacokinetic/pharmacodynamic relationships
The literature on busulfan suggests a therapeutic window between 900 and 1500 μMol.minute for AUC. During clinical trials with i.v. busulfan, 90% of patients AUCs were below the upper AUC limit (1500 μMol.minute) and at least 80 % were within the targeted therapeutic window (900-1500 μMol.minute).
Special populations
The effects of renal dysfunction on i.v. busulfan disposition have not been assessed.
The effects of hepatic dysfunction on i.v. busulfan disposition have not been assessed.
Nevertheless the risk of liver toxicity may be increased in this population.
No age effect on busulfan clearance was evidenced from available i.v. busulfan data in patients
over 60 years.

Pharmacokinetics in paediatric patients
A continuous variation of clearance ranging from 2.49 to 3.92 ml/minute/kg has been established
in children from < 6 months up to 17 years old. The terminal half life ranged from 2.26 to 2.52 h.
The dosing recommended on page 177 allows the achievement of a similar AUC whatever the
children's age, the targeted range of AUCs being the one used for adults. Inter and intra patient
variabilities in plasma exposure were lower than 20% and 10%, respectively.

Pharmacokinetic/pharmacodynamic relationships:
The successful engraftment achieved in all patients during phase II trials suggests the
appropriateness of the targeted AUCs. Occurrence of VOD was not related to overexposure.
PK/PD relationship was observed between stomatitis and AUCs in autologous patients and
between bilirubin increase and AUCs in a combined autologous and allogeneic patient analysis.

Preclinical safety data
Busulfan is mutagenic and clastogenic. Busulfan was mutagenic in Salmonella typhimurium,
Drosophila melanogaster and barley. Busulfan induced chromosomal aberrations in vitro (rodent
and human cell) and in vivo (rodents and humans). Various chromosome aberrations have been
observed in cells from patients receiving oral busulfan.

Busulfan belongs to a class of substances which are potentially carcinogenic based on their
mechanism of action. On the basis of human data, busulfan has been classified by the IARC as a
human carcinogen. WHO has concluded that there is a causal relationship between busulfan
exposure and cancer. The available data in animals support the carcinogenic potential of busulfan.
Intravenous administration of busulfan to mice significantly increased the incidences of thymic
and ovarian tumours.

Busulfan is teratogenic in rats, mice and rabbits. Malformations and anomalies included significant
alterations in the musculoskeletal system, body weight gain, and size. In pregnant rats, busulfan
produced sterility in both male and female offspring due to the absence of germinal cells in testes
and ovaries. Busulfan was shown to cause sterility in rodents. Busulfan depleted oocytes of female
rats, and induced sterility in male rats and hamster.

Repeated doses of DMA produced signs of liver toxicity, the first being increases in serum clinical
enzymes followed by histopathological changes in the hepatocytes. Higher doses can produce
hepatic necrosis and liver damage can be seen following single high exposures.

DMA is teratogenic in rats. Doses of 400 mg/kg/day DMA administered during organogenesis
caused significant developmental anomalies. The malformations included serious heart and/or
major vessels anomalies: a common truncus arteriosus and no ductus arteriosus, coarctation of the
pulmonary trunk and the pulmonary arteries, intraventricular defects of the heart. Other frequent
anomalies included cleft palate, anasarca and skeletal anomalies of the vertebrae and ribs. DMA
decreases fertility in male and female rodents. A single s.c. dose of 2.2 g/kg administered on
gestation day 4 terminated pregnancy in 100% of tested hamster. In rats, a DMA daily dose of 450
mg/kg given to rats for nine days caused inactive spermatogenesis.
Pharmaceutical particulars

List of excipients
Dimethylacetamide, macrogol 400.

Incompatibilities
In the absence of compatibility studies, this medicinal product must not be mixed with other medicinal products except those mentioned under the preparation instructions.

Do not use polycarbonate syringes with Busilvex®.

Shelf life
Ampoules: 2 years
Diluted solution
Chemical and physical in-use stability after dilution has been demonstrated for:

- 8 hours (including infusion time) after dilution in glucose 5% or sodium chloride 9 mg/ml (0.9%) solution for injection, stored at 20 °C ± 5 °C

- 12 hours after dilution in sodium chloride 9 mg/ml (0.9%) solution for injection stored at 2 °C-8 °C followed by 3 hours stored at 20 °C ± 5 °C (including infusion time).

From a microbiological point of view, the product should be used immediately after dilution. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would normally not be longer than the above mentioned conditions when dilution has taken place in controlled and validated aseptic conditions.

Special precautions for storage
Stored at 2 °C-8 °C (in a refrigerator).
Do not freeze.

Nature and contents of container
10 ml of concentrate for solution for infusion in clear glass ampoules (type I)
Pack size: 8 ampoules per box

Instructions for use and handling and disposal

Preparation of Busilvex®

Procedures for proper handling and disposal of anticancer drugs should be considered.
All transfer procedures require strict adherence to aseptic techniques, preferably employing a vertical laminar flow safety hood
As with other cytotoxic compounds, caution should be exercised in handling and preparing the Busilvex® solution:
- The use of gloves and protective clothing is recommended.
- If Busilvex® or diluted Busilvex® solution contacts the skin or mucosa, wash them thoroughly with water immediately.
Calculation of the quantity of Busilvex® to be diluted and of the diluent
Busilvex® must be diluted prior to use with either sodium chloride 9 mg/ml (0.9%) solution for injection or glucose solution for injection 5%.
The quantity of the diluent must be 10 times the volume of Busilvex® ensuring the final concentration of busulfan remains at approximately 0.5 mg/ml. By example:

The amount of Busilvex® and diluent to be administered would be calculated as follows:
for a patient with a Y kg body weight:

- Quantity of Busilvex®:

\[ \frac{Y \text{ (kg)} \times 0.8 \text{ (mg/kg)}}{6 \text{ (mg/ml)}} = A \text{ ml of Busilvex® to be diluted} \]

Y: body weight of the patient in kg

- Quantity of diluent:

\[(A \text{ ml Busilvex®}) \times (10) = B \text{ ml of diluent} \]

To prepare the final solution for infusion, add (A) ml of Busilvex® to (B) ml of diluent (sodium chloride 9 mg/ml (0.9%) solution for injection or glucose solution for injection 5%).

Preparation of the solution for infusion

- Using sterile transfer techniques, break off the top of the ampoule.
- Using a non polycarbonate syringe fitted with a needle:
  - remove the calculated volume of Busilvex® from the ampoule.
  - dispense the contents of the syringe into an intravenous bag (or syringe) which already contains the calculated amount of the selected diluent. Always add Busilvex® to the diluent, not the diluent to Busilvex®. Do not put Busilvex® into an intravenous bag that does not contain sodium chloride 9 mg/ml (0.9%) solution for injection or glucose solution for injection 5%.
  - Mix thoroughly by inverting several times

After dilution, 1 ml of solution for infusion contains 0.5 mg of busulfan.

Diluted Busilvex® is a clear colourless solution.

Instructions for use
Prior to and following each infusion, flush the indwelling catheter line with approximately 5 ml of sodium chloride 9 mg/ml (0.9%) solution for injection or glucose (5%) solution for injection.

Do not flush residual drug in the administration tubing as rapid infusion of Busilvex® has not been tested and is not recommended.

The entire prescribed Busilvex® dose should be delivered over two hours.

Small volumes may be administered over 2 hours using electric syringes. In this case infusion sets with minimal priming space should be used (i.e 0.3-0.6 ml), primed with drug solution prior to
beginning the actual Busilvex® infusion and then flushed with sodium chloride 9 mg/ml (0.9%) solution for injection or glucose (5%) solution for injection.
Do not infuse concomitantly with another intravenous solution.
Do not use polycarbonate syringes with Busilvex®.
For single use only; only a clear solution without any particles should be used.
Any unused product or waste should be disposed of in accordance with local requirements for cytotoxic drugs.

**Marketing authorisation holder**

Pierre Fabre Médicament
45, Place Abel Gance
F-92654 Boulogne Billancourt Cedex
France

**Marketing authorisation number(s)**

EU/1/03/254/001

**Date of first authorisation/renewal of the authorisation**

July 11 2003

**Manufacturing authorisation holder responsible for batch release**

Name and address of the manufacturer responsible for batch release
Pierre Fabre Médicament Production
Site Aquitaine Pharm International
Avenue du Béarn -Idron
F-64320
France

**Conditions of the marketing authorisation**

CONDITIONS OR RESTRICTIONS REGARDING SUPPLY AND USE IMPOSED ON THE MARKETING AUTHORISATION HOLDER
Medicinal product subject to restricted medical prescription.

**OTHER CONDITIONS**
The holder of this marketing authorisation must inform the European Commission about the marketing plans for the medicinal product authorised by this decision.

**6. FURTHER INFORMATION**
This leaflet was last approved on:
---------------------------------------------------------------------------------------------------------------------

The following information is intended for medical or healthcare professionals only

**PREPARATION GUIDE**

**Busilvex® 6 mg/ml concentrate for solution for infusion**
Busulfan

Read this guide prior to the preparation and administration of Busilvex®.
PRESENTATION
Busilvex® is supplied as a clear colorless solution in 10 ml clear glass ampoules (type I). Busilvex® must be diluted prior to administration.

RECOMMENDATION FOR SAFE HANDLING
Procedures for proper handling and disposal of anticancer drugs should be considered.
All transfer procedures require strict adherence to aseptic techniques, preferably employing a vertical laminar flow safety hood
As with other cytotoxic compounds, caution should be exercised in handling and preparing the Busilvex® solution:
- The use of gloves and protective clothing is recommended.
- If Busilvex® or diluted Busilvex® solution contacts the skin or mucosa, wash them thoroughly with water immediately.

Calculation of the quantity of Busilvex® to be diluted and of the diluent
Busilvex® must be diluted prior to use with either sodium chloride 9 mg/ml (0.9%) solution for injection or glucose solution for injection 5%.
The quantity of the diluent must be 10 times the volume of Busilvex® ensuring the final concentration of busulfan remains at approximately 0.5 mg/ml.
The amount of Busilvex® and diluent to be administered would be calculated as follows:
for a patient with a Y kg body weight:
- Quantity of Busilvex®:

\[
\frac{Y \times 0.8 \text{ (mg/kg)}}{6 \text{ (mg/ml)}} = \text{A ml of Busilvex® to be diluted}
\]

Y: body weight of the patient in kg
- Quantity of diluent:

\[
(A \text{ ml Busilvex®}) \times 10 = \text{B ml of diluent}
\]

To prepare the final solution for infusion, add (A) ml of Busilvex® to (B) ml of diluent (sodium chloride 9 mg/ml (0.9%) solution for injection or glucose solution for injection 5%).

Preparation of the solution for infusion
- Using sterile transfer techniques, break off the top of the ampoule.
- Using a non polycarbonate syringe fitted with a needle:
  - remove the calculated volume of Busilvex® from the ampoule.
  - dispense the contents of the syringe into an intravenous bag (or syringe) which already contains the calculated amount of the selected diluent. Always add Busilvex® to the diluent, not the diluent to Busilvex®. Do not put Busilvex® into an intravenous bag that does not contain sodium chloride 9 mg/ml (0.9%) solution for injection or glucose solution for injection 5%.
  - Mix thoroughly by inverting several times

After dilution, 1 ml of solution for infusion contains 0.5 mg of busulfan.

Diluted Busilvex® is a clear colourless solution.
Instructions for use
Prior to and following each infusion, flush the indwelling catheter line with approximately 5 ml of sodium chloride 9 mg/ml (0.9%) solution for injection or glucose (5%) solution for injection.

Do not flush residual drug in the administration tubing as rapid infusion of Busilvex® has not been tested and is not recommended.

The entire prescribed Busilvex® dose should be delivered over two hours.

Small volumes may be administered over 2 hours using electric syringes. In this case infusion sets with minimal priming space should be used (i.e 0.3-0.6 ml), primed with drug solution prior to beginning the actual Busilvex® infusion and then flushed with sodium chloride 9 mg/ml (0.9%) solution for injection or glucose (5%) solution for injection.

Do not infuse concomitantly with another intravenous solution.
Do not use polycarbonate syringes with Busilvex®.

For single use only; only a clear solution without any particles should be used.

PROCEDURE FOR PROPER DISPOSAL
Any unused product or waste should be disposed of in accordance with local requirements for cytotoxic drugs.

Distribution Logistics
Since the closure of the R1 randomisation Busilvex® is no longer supplied free of charge. Busilvex® is commercially available.

23.9.2 ORAL BUSULFAN

<table>
<thead>
<tr>
<th>Formulation</th>
<th>2mg coated tablets.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage</td>
<td>2mg coated tablets – keep dry and store &lt; 25°C.</td>
</tr>
<tr>
<td>Stability</td>
<td>Three years from manufacture.</td>
</tr>
<tr>
<td>Administration</td>
<td>For oral administration.</td>
</tr>
<tr>
<td>Supplier</td>
<td>Commercially available.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Common</th>
<th>Occasional</th>
<th>Rare</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate</td>
<td>Myelosuppression, thrombocytopenia</td>
<td>Nausea, vomiting, diarrhoea</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Within 1-2 days of receiving drug</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prompt</td>
<td>Mucositis, veno-occlusive disease, hyperpigmentation</td>
<td>Anorexia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Within 2-3 weeks, prior to next course</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delayed</td>
<td>Anytime later during therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late</td>
<td>Anytime after completion of therapy</td>
<td></td>
<td>Interstitial pulmonary fibrosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
23.10 Melphalan

Formulation 20 mg vials containing 50 mg anhydrous melphalan hydrochloride with 10 ml buffered diluent.

Storage Below 30°C protect from light

Reconstitution Add 10 ml diluent to 50 mg vial, shake vigorously until dissolution complete. This gives a solution of 5 mg/ml which should be used immediately, or diluted further in 0.9% saline and infused within 2 hours. Dextrose solutions are incompatible with melphalan.

Administration As IV bolus into fast running infusion via a central venous line, or as an infusion over 1-2 hours.

Supplier Commercially available

Toxicity Frequencies

<table>
<thead>
<tr>
<th></th>
<th>Common</th>
<th>Occasional</th>
<th>Rare</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Happens to 21-100 children out of every 100</td>
<td>Happens to 5-20 children out of every 100</td>
<td>Happens to &lt;5 children out of every 100</td>
</tr>
<tr>
<td>Immediate: Within 1-2 days of receiving drug</td>
<td>Anorexia, ulceration if extravasated, nausea and vomiting</td>
<td></td>
<td>Hypotension, diaphoresis, hypersensitivity reaction</td>
</tr>
<tr>
<td>Prompt: Within 2-3 weeks, prior to the next course</td>
<td>Myelosuppression (L), mucositis, diarrhoea, alopecia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delayed: Any time later during therapy, excluding the above conditions</td>
<td></td>
<td>Inanition</td>
<td></td>
</tr>
<tr>
<td>Late: Any time after Completion of treatment</td>
<td></td>
<td></td>
<td>Pulmonary fibrosis, sterility, secondary malignancy</td>
</tr>
</tbody>
</table>

(L) Toxicity may also occur later.

23.11 Granulocyte Colony Stimulating Factor

(r-metHuG-CSF, G-CSF) NSC #614629

Source and Pharmacology: r-metHuG-CSF (produced in E. coli by recombinant DNA technology) stimulates the production of neutrophils in the bone marrow and selected end-cell activation. The 175 amino acid protein (M.W. of 18,800 daltons) differs from the natural protein in that the N-terminal amino acid is a methionine and it is not o-glycosylated. 3.45 μg to 11.5 μg of G-CSF administered subcutaneously resulted in a maximum serum concentration of 4 ng/ml to 49 ng/ml within 2 to 8 hours. The elimination half-life is similar for SQ and IV, approximately 3.5 hours.
## Toxicty Frequencies

<table>
<thead>
<tr>
<th></th>
<th>Common</th>
<th>Occasional</th>
<th>Rare</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Happens to 21–100 children out of every 100</td>
<td>Happens to 5-20 children out of every 100</td>
<td>Happens to &lt;5 children out of every 100</td>
</tr>
<tr>
<td><strong>Immediate:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within 1-2 days of receiving drug</td>
<td>Local irritation at the injection site, headache</td>
<td></td>
<td>Allergic reaction (more common with IV administration than subq): skin (rash, urticaria, facial edema), respiratory (wheezing, dyspnea) and cardiovascular (hypotension, tachycardia), low grade fever</td>
</tr>
<tr>
<td><strong>Prompt:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within 2-3 weeks, prior to the next course</td>
<td>Mild to moderate medullary bone pain,</td>
<td>Increased: alkaline phosphatase, lactate dehydrogenase, uric acid, thrombocytopenia</td>
<td>Splenomegaly, splenic rupture, exacerbation of pre-existing skin rashes, sickle cell crises in patients with SCD, excessive leukocytosis</td>
</tr>
<tr>
<td><strong>Delayed:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anytime later during therapy, excluding the above conditions</td>
<td></td>
<td></td>
<td>Cutaneous vasculitis, ARDS</td>
</tr>
<tr>
<td><strong>Late:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anytime after Completion of treatment</td>
<td></td>
<td></td>
<td>MDS or AML (confined to patients with severe chronic neutropenia and long term administration)</td>
</tr>
</tbody>
</table>

**Fetal toxicities and teratogenic effects of filgrastim in humans are unknown. Conflicting data exist in animal studies and filgrastim is known to pass the placental barrier. It is unknown whether the drug is excreted in breast milk.**

### Formulation and Stability

Supplied as a sterile, clear, colourless and preservative-free solution in pre-filled syringes or vials (1ml or 1.6ml). Vials are preservative free and are intended to be single-use vials; G-CSF must be stored between 2° and 8° C. Stability has been demonstrated for at least 24 months when stored under these conditions. Do not use if discoloured or if there is particulate matter.

### G-CSF Administration

Administer once daily, subcutaneously without dilution. For IV use, dilute in D5W to concentrations ≥ 15 μg/ml; G-CSF is incompatible with normal saline. At dilutions from 5 μg/ml to 14 μg/ml, add human serum albumin to a final albumin concentration of 2 mg/ml to protect against absorption of the G-CSF to container walls (glass or plastic). G-CSF, when diluted as described above, is compatible with a number of plastics commonly used in the manufacture of syringes, IV bags, infusion sets, and IV pump cassettes. These include polyvinyl chloride, polyolefin, and polypropylene. Diluted G-CSF should be stored at 2° to 8° C and used within 24 hours. Do not shake or freeze.

The suggested starting dose is 5 μg/kg. Although guidelines are not well documented in the literature, POG protocols typically recommend stopping G-CSF if the following occurs:

- ANC > 0.5 x 10⁹/L after the nadir is reached (usually 10–14 days) or ANC >> 0.5 x 10⁹/L on 2 consecutive days after nadir is reached
- Generally, the ANC decreases by 50% in 24-48 hours G-CSF should be stopped 48 hours before restarting chemotherapy.

### Supplier

Commercially available. See package insert for further information.
23.12 13-Cis-Retinoic Acid (Isotretinoin®, Roaccutane®)

**Source and Pharmacology:** The exact mechanism of RA-induced maturation of tumour cells is not known. It has been observed that cyclic AMP-inducing agents have a synergistic effect on RA-induced differentiation of the HL-60 promyelocytic leukemic cell line. The observers have hypothesised that RA may induce increased levels of a cAMP-dependent protein kinase whose activity is potentiated by increased intracellular cAMP levels. The role of cAMP-dependent protein kinases in cellular differentiation has been documented. RA also appears to enhance normal haematopoietic differentiation by increasing the responsiveness of myeloid and erythroid progenitor cells to the action of myeloid colony stimulating activity and erythropoietin, respectively.

**Metabolism:** RA is 99.9% bound in plasma (almost entirely to albumin) and has a half-life of 10-20 hours. The major metabolite is 4-oxoisotretinoin, and excretion is in the urine and feces. A single oral dose of 100mg/m² isotretinoin (13-cis-RA) will produce peak plasma levels of 1-2mM. The mean peak-time was 3.2 hours after 80mg orally, with a terminal t½ of 10 to 20 hours.

**Guidelines for cutting Isotretinoin capsules:**
Isotretinoin (13-cis-RA) will be provided in 5mg and 20mg capsules depending on the total daily dose required. All capsules are blister packed. The following guidelines have been developed to maximise the amount of drug recovered from the capsule and to minimise the risk of skin contamination especially to women of childbearing age.

Gloves must be worn for this procedure.

1. Remove capsule from blister pack and transfer required number of capsules for each dose to the plastic medicine pot.
2. Assemble equipment:
   - 1 pair non-sterile gloves
   - small pair sharp clean scissors (to be used only for this purpose)
   - 1 dessert spoon
   - 1 teaspoon
   - 1 small tray
   - small portion of ice cream/yoghurt
   - kitchen roll – kept just for this purpose
   - cytotoxic waste bin
3. Put on gloves.
4. Place dessert spoon on clean surface.
5. Take a capsule between finger and thumb and hold upright firmly. With the scissors snip the tip off the capsule into the tray to avoid any possible harm to the eyes.
6. Carefully squeeze the contents of the capsule onto the dessert spoon.
7. Discard empty capsule in cytotoxic waste bin.
8. Use kitchen roll to wipe any drug from gloves then dispose of kitchen roll immediately in the cytotoxic waste bin.
9. Repeat steps 5 to 8 for each capsule needed.
10. After all the required capsules have been snipped, use the teaspoon to place some soft ice cream or yoghurt onto the dessert spoon.
11. Using the teaspoon mix the ice cream/yoghurt and medicine together.
12. Give medicine to child.
13. Clean surface with kitchen roll and wash all equipment, including scissors, in hot soapy water.
14. Dispose of gloves in cytotoxic waste bin.
15. Wash hands thoroughly.
16. Return cytotoxic waste bin to hospital when full.
### Toxicity:

<table>
<thead>
<tr>
<th>Immediate: Within 1-2 days of receiving drug</th>
<th>Common</th>
<th>Occasional</th>
<th>Rare</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Happens to 21-100 children out of every 100</td>
<td>Happens to 5-20 children out of every 100</td>
<td>Happens to &lt;5 children out of every 100</td>
</tr>
<tr>
<td></td>
<td>Nausea and vomiting</td>
<td>Anaphylaxis, bronchospasm</td>
<td></td>
</tr>
</tbody>
</table>

**Prompt: Within 2-3 weeks, prior to the next course**
- Dry skin (L), dry mucosa (L), Epistaxis, cheilitis (L), Photosensitivity, Elevated ESR, back pain (L), Arthralgias (L), Triglyceride elevation (L), Hypercalcemia (L)
- Rash (L), conjunctivitis (L), headache (L), decrease in high density lipoproteins (L), cholesterol elevations (L), transaminase elevations (L), anemia (L)
- Alopecia, appetite disturbances, Hyperglycemia, Hyper or hypo skin pigmentation, nail changes, eruptive xanthomas, seizures, dizziness, Pseudotumor-cerebri (papilledema, headache, Nausea, vomiting, visual disturbances), Psychiatric disorders (depression, aggressive and/or violent behaviors, suicidal ideation, suicide, dream disturbances), insomnia, lethargy, malaise, nervousness, paresthesias, Weight loss, myelosuppression, elevated platelet counts, anaphylaxis, Hyper or hypo skin pigmentation, nail changes, eruptive xanthomas, seizures, dizziness, Psychiatric disorders (depression, aggressive and/or violent behaviors, suicidal ideation, suicide, dream disturbances), insomnia, lethargy, malaise, nervousness, paresthesias, weight loss, myelosuppression, elevated platelet counts, anaphylaxis.

**Delayed: Any time later during therapy, excluding the above conditions**
- Skeletal hyperostosis
- Osteoporosis, bone fractures or delayed healing, premature epiphyseal closure, Rabdomyolysis, abnormal menses, renal disturbances (WBC in urine, proteinuria, hematuria, renal calculi), calcification of tendon and ligaments

**Late: Any time after the completion of treatment**
- Major human fetal abnormalities related to isotretinoin administration in females have been documented. There is an increased risk of spontaneous abortion. In addition, premature births have been reported. Documented external abnormalities include: skull abnormality; ear abnormalities (including anotia, micropinna, small or absent external auditory canals); eye abnormalities (including microphthalmia); facial dysmorphia and cleft palate. Documented internal abnormalities include: CNS abnormalities (including cerebral abnormalities, cerebellar malformation, hydrocephalus, microcephaly, cranial nerve deficit); cardiovascular abnormalities; thymus gland abnormality; parathyroid hormone deficiency. In some cases death has occurred with certain of the abnormalities previously noted. Cases of IQ scores less than 85 with or without obvious CNS abnormalities have also been reported. It is not known whether this drug is excreted in human milk. Because of the potential for adverse effects, nursing mothers should not receive isotretinoin.

*(L) Toxicity may also occur later.*

**Supplier**

Commercially available
23.13 Chimeric 14.18 anti-GD2 monoclonal antibody (ch 14.18)
Human-mouse chimeric with the human IgG1-Fc part and the specificity for the ganglioside GD2 on human neuroblastoma cells

Manufacturer: POLYMUN Scientific Immunbiologische Forschung GmbH, Vienna, Austria

Effect: the complement dependent (CDC) and antibody dependent cellular cytotoxicity (ADCC) is induced after bondage to neuroblastoma cells

Identification of the product
Aqueous solution containing recombinant chimeric antibody ch14.18, sodium phosphates and sodium chloride. Until now two batches of the drug have been produced (Lot T651204-A and Lot T900310-A.
Lot T651204-A contains 4.3 ml (4.6 mg/ml)
Lot T900310-A contains 4.5 ml (4.5 mg/ml)

Receipt and Storage
Vials will be received on liquid ice (4°C) and must be stored at 2-8 °C until immediately before use.

Supplier
Supplied by the sponsor for patients who are R2 randomised only

Precipitate formation
The formation of a precipitate may be seen. The precipitate appeared after filling the glass vials. The stability data demonstrate that the precipitate had no impact on antibody concentration or any of the other product specifications. The precipitate consists of only a few, hardly visible particles.

Preparation guide
The antibody must be prepared under sterile conditions. The appropriate volume of ch14.18/CHO antibody should be withdrawn from the vials. It is recommended that the antibody solution is filtered (0.2 to 1.2 μm) before injection into the patient either by using an in-line filter during infusion (as some centres do routinely) or by filtering the solution with a particle filter (e.g. filter Nr. MF1830, Impromediform, Germany). The volume of the antibody is added to an infusion bag containing 100 ml NaCl 0.9 % and 5 ml human albumin 20%.
If applicable, the remaining content of the vial should be used for the preparation of the infusion solution on the following day. Partially used vials should continue to be stored at 2 – 8 °C.

Calculation of the quantity of ch14.18/CHO to be diluted
The amount of ch14.18/CHO to be administered is calculated as follows:
Dosage: 20 mg/m²/day, day 1-5, as 8hr infusion. Weeks 4, 8, 12, 16, and 20 after starting of isotretinoin therapy.

Example calculation:
If a patient has a BSA of 0.7, he needs 14 mg (20 x 0.7) per day, or 70 mg for five days (one cycle).
One vial contains 20 mg. Institutions will receive 4 vials for one cycle of treatment for this patient, and 20 vials for the whole treatment (five cycles).
The following table relates to Lot Nr. T900310-A (C6F12).
### Method of administration

Ch14.18/CHO is given as an 8 hour intravenous infusion. All patients must commence with a morphine infusion 2 hrs before the antibody is administered which must be continued throughout. Further analgesics are available in the protocol if pain is not managed with morphine alone. Antihistamine premedication should be given before each ch14.18/HCO infusion (if not clinically indicated avoid steroids).

- Admit to hospital on the day/or the evening before starting ch14.18/CHO infusion
- Preferably the central venous line will still be in place
- G-CSF should not be given prior to any ch14.18/CHO cycle

### Dose/Cycle

<table>
<thead>
<tr>
<th>Surface Area Square Metres</th>
<th>mg ch14.18/CHO needed per day</th>
<th>ml ch14.18/CHO needed per day T651204</th>
<th>ml ch14.18/CHO needed per day T900310</th>
<th>mg ch14.18/CHO needed per course (5 days)</th>
<th>Number of vials sent per course (5 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
<td>0.6</td>
<td>0.7</td>
<td>0.8</td>
<td>0.9</td>
</tr>
</tbody>
</table>

### Administration

- **Hydration**
  - 2000ml/m² Gluc 5%
  - With additional NaCl and KCl according to institutional guidelines

### Toxicity Frequencies

<table>
<thead>
<tr>
<th>Immediate: Within 1-2 days of receiving drug</th>
<th>Common</th>
<th>Occasional</th>
<th>Rare</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain (abdomen -cramps, back, limb, joint, neuralgia, pelvis) Paresthesia, mild hypertension, Mild hypotension, tachycardia, Urticaria, pruritis, fever, nausea, Mild hyponatremia, mild hypokalemia, anorexia</td>
<td>Moderate hypertension, moderate hypotension, emesis, diarrhea, Moderate hyponatremia, moderate hypokalemia, somnolence, Hypoalbuminemia, weight loss, Elevated creatinine, elevated transaminase (mild and transient),</td>
<td>Severe hypertension, severe hypotension, bronchospasm, Wheezing, dyspnea, hypoxia Anaphylaxis, angioedema, Seizure, atrial fibrillation, depressed level of consciousness, cardiac ischemia, cardiac or respiratory arrest, acute vascular leak syndrome, weight gain</td>
<td></td>
</tr>
</tbody>
</table>
**Prompt:** Within 2-3 weeks, prior to the next course

<table>
<thead>
<tr>
<th>Serum sickness</th>
<th>Rash with desquamation, DIC, Neuropathy motor and sensory (dysesthesia, hyperaesthesia, paresthesia)(L), central venous catheter thrombosis, Thrombocytopenia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Delayed:</strong> Any time later during therapy, excluding the above conditions</td>
<td>Impaired accommodation of the eye, photophobia, ptosis, papilledema, Optic atrophy</td>
</tr>
<tr>
<td><strong>Unknown frequency and timing:</strong></td>
<td>The effect of ch14.18 on embryogenesis, reproduction, and spermatogenesis are unknown. It is well known that IgG antibody can pass the placenta and secrete into milk.</td>
</tr>
</tbody>
</table>

(L) Toxicity may also occur later.

**Shelf life**
As of February 1st, 2011, only Lot T900310-A (C6F12) is distributed. This lot has a shelf-life until February 28th, 2015.

**Vials:** Lot T900310-A (C6F12) until February, 28th, 2015
If stored at 2-8 °C (in a refrigerator)

**Diluted solution**
8 hours (including infusion time) after dilution in 100 ml of 0.9% NaCl plus 5 ml 20% human albumin stored at 20 °C ± 5 °C

From a microbiological point of view, the product should be used immediately after dilution. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would normally not be longer than the above mentioned conditions when dilution has taken place in controlled and validated aseptic conditions.

**Instructions for handling and disposal**

**Hazards identification**
No hazardous product as specified in Directive 1999/45/EC.

**First aid measures**
After skin contact: wash off with plenty of water, remove contaminated clothing.
After eye contact: rinse out with plenty of water with the eyelid held wide open.
After swallowing: consult doctor if feeling unwell.
In case of unintentional parenteral uptake (injection under the skin, in tissue, artery or veins) consult physician immediately (contains hormonal active substance).

**Accidental release measures**
Person-related precautionary measures: Do not inhale aerosols.
Environmental-protection measures: Do not allow to enter sewerage system.
Procedures for cleaning / absorption: Take up with liquid absorbent material (e.g. Chemizorb, paper towels); forward for disposal. Clean up affected area. Avoid skin contact

**Disposal considerations**
Must be disposed of in compliance with the respective national regulations
Handling and storage

Handling
Do not handle in syringes with needles (danger of unintentional parenteral administration).
Avoid generation of aerosols.

Storage
Tightly closed
At 2 – 8 °C, as specified on the label
Protected from light

Exposure controls / personal protection

Occupational exposure controls
Personal protective equipment
Respiratory protection: not normally required for normal use.
Eye protection: safety glasses.
Hand protection: nitril rubber or latex gloves.

Physical and chemical properties

Form: liquid
Colour: colourless
Odour: odourless
pH value: 7.0 ± 0.5
Melting point: like water
Boiling point: like water
Flash point: not flammable
Combustibility: not flammable
Explosion limit: not applicable
Relative density (water = 1): approx. 1.00
Solubility in water: miscible with water Pharmaceutical form

Fire-fighting measures
Not flammable (aqueous solution).

Distribution Logistics

Drug supply will not start before
1) approval of the Ethics Committee obtained
2) study notified to the local Health Authorities
3) Co-Sponsorship Agreement signed
⇒ Please, send all 3 documents to the international data centre: St. Anna Kinderkrebsforschung, Dr. Ruth Ladenstein, Zimmermannplatz 10, 1090 Wien, Austria, Tel.: +43 1 40470 4750, Fax: +43 1 40470 7430

R2 randomisation
If a patient is randomised in the database a new window appears, showing the "ch14.18/CHO drug request form" (see the form below) where the registering person must enter the information needed to supply local centre with ch14.18/CHO that include: actual body weight, and body surface area of the patient, complete contact data of the responsible physician as well as pharmacist. The “drug request form” should be sent to the International data centre in Vienna by E-mail (ingrid.pribill@ccri.at, alisa.alspach@ccri.at, or claudia.zeiner@ccri.at) or by fax (Fax number: +43 1 40470 7430).
Drug Request
Antibody ch14.18 AntiGD2

To: St. Anna Children’s Cancer Research Institute (CCRI)
Ingrid Pribill, PhD / Alisa Alspach, BSc / Claudia Zeiner, MSc
Address: Zimmervammplatz 10, 1090 Vienna, Austria
Phone: +43 1 40470 - 4960 / - 4985
Fax: +43 1 40470 - 7430
E-mail: ingrid.pribill@ccri.at, alisa.alspach@ccri.at, claudia.zeiner@ccri.at
Pages: 1 (incl. cover sheet)
Study: HRNB1/SIOPEN – R2 Randomisation

PLEASE FILL IN INFORMATION IN BLOCK LETTERS
Patient Code in Study: [____-____-____-____-____-____-____-____-____]
Body Surface Area: [____ m²]
Start of antibody treatment: [__/__/____ (DD/MM/YY)]
Name of Treating Physician: ________________________________

Delivery Details:

Name of Institute: ________________________________

Pharmacy contact person:

_________________________________________________________

Street: __________________________________________________

Postcode-City/ ________________________________ Country:

Phone: __________________________________________________

Fax: _____________________________________________________

E-Mail: ___________________________________________________

To be completed by the person ordering the antibody
Date: ________ Name: ________________________________

To be completed by the study centre (CCRI)
Number of vials to be delivered: ________________________________
Date of dispatch: [__/__/____ (DD/MM/YY)]
CCRI approval [ ] Yes [ ] No
Date: ________ Name: ________________________________ Signature: __________________
**Delivery of the antibody**
To assure timely delivery of ch14.18/CHO it is mandatory that the R2 randomisation is done as soon as possible after completion of restaging following MAT (between day 60 and 120 post PBSCR). Once the ch14.18/CHO request template is sent to CCRI, it takes at least another week before the drug is delivered at the local centre per FedEx. All orders received within one week will be forwarded to Polymun and dispatched the following Monday.

After receiving the drug at your local pharmacy, it is mandatory to
- Return the receipt form that comes with the drug to Polymun per Fax
- Return the temperature logger in the pre-addressed envelope provided to Polymun as soon as possible. The local centre receiving the antibody is responsible for the costs of the return.

The RDE-system automatically recognises that a certain patient has to be R2 randomised and sends an email-reminder to the person who had registered the patient for the trial.

**Ch14.18/CHO Drug Accountability**
In order to comply with the GCP requirements for investigational agents, the “Drug Accountability Form” must be completed after the patient has finished treatment with the antibody. The completed form has to be sent to the CCRI per email (ingrid.pribill@ccri.at, alisa.alspach@ccri.at, or claudia.zeiner@ccri.at)
Drug Accountability Summary
Antibody ch14.18 AntiGD2

To: St. Anna Children’s Cancer Research Institute (CCRI)
Ingrid Pribill, PhD / Alisa Alspach, BSc / Claudia Zeiner, MSc
Address: Zimmermannplatz 10, 1090 Vienna, Austria
Phone: +43 1 40470 - 4960 / - 4985
Fax: +43 1 40470 - 7430
E-mail: ingrid.pribill@ccri.at, alisa.alspach@ccri.at, claudia.zeiner@ccri.at
Pages: 1 (incl. cover sheet)
Study: HRNBL1/SIOPEN – R2 Randomisation

To be completed by the pharmacist. This form should be faxed to the CCRI within two weeks of the end of antibody treatment.

PLEASE FILL IN INFORMATION IN BLOCK LETTERS

Patient Study Number: _________

Name of Institute: ____________________________________________________________

Name of Pharmacist: _________________________________________________________

<table>
<thead>
<tr>
<th>Lot number*</th>
<th>T651204-A (C6F12)</th>
<th>Lot T900310-A (C6F12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of vials received:</td>
<td>__________________</td>
<td>__________________</td>
</tr>
<tr>
<td>Total number of vials administered:</td>
<td>__________________</td>
<td>__________________</td>
</tr>
<tr>
<td>Total number of vials remaining:</td>
<td>__________________</td>
<td>__________________</td>
</tr>
<tr>
<td>If the number of vials administered is not equal to the number of vials received, please give reason (e.g. dose reduction, broken vial, etc)</td>
<td>__________________</td>
<td>__________________</td>
</tr>
</tbody>
</table>

*If this patient received antibody from different lot numbers, please give the required information for each lot number.

Course number | Start date | End date |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Date: __________________ Signature: ____________________________________________

Ch14.18/CHO drug accountability summary: Version 10, 11th January 2011
23.14 Aldesleukin (IL-2, Interleukin-2, Proleukin®)

Source and Pharmacology: Aldesleukin for injection, a human recombinant interleukin-2 product, is a highly purified protein with a molecular weight of approximately 15,300 daltons, Aldesleukin, a lymphokine, is produced by recombinant DNA technology using a genetically engineered E. coli strain containing an analog of the human interleukin-2 gene. In vitro studies performed on human cell lines demonstrate the immunoregulatory properties of Aldesleukin, including: a) enhancement of lymphocyte mitogenesis and stimulation of long-term growth of human interleukin-2 dependent cell lines; b) enhancement of lymphocyte cytotoxicity; c) induction of killer cell (lymphokine-activated (LAK) and natural (NK) activity); and d) induction of interferon-gamma production. The in vivo administration of Aldesleukin in animals and humans produces multiple immunological effects in a dose dependent manner. These effects include activation of cellular immunity with profound lymphocytosis, eosinophilia, and thrombocytopenia, and the production of cytokines including tumor necrosis factor, IL-1 and gamma interferon. In vivo experiments in murine tumor models have shown inhibition of tumor growth. The exact mechanism by which Aldesleukin mediates its antitumor activity in animals and humans is unknown.

If used intravenously: The drug is rapidly distributed into extravascular space following IV administration. After completing an infusion, approximately 30% of the dose can be found in plasma. Following a 5 minute infusion, the distribution and elimination half-life is 13 and 85 minutes, respectively. Aldesleukin is eliminated by metabolism in the kidneys through both glomerular filtration and peritubular extraction with little or no active protein excreted in the urine. Greater than 80% of the amount of Aldesleukin distributed to plasma, cleared from the circulation and presented to the kidney is metabolized to amino acids in the cells lining the proximal convoluted tubules. In humans, the mean clearance rate in cancer patients is 268 mL/min. Clearance is preserved in patients with rising serum creatinine values.

<table>
<thead>
<tr>
<th>Toxicity Frequencies (for IV administration)</th>
<th>Common</th>
<th>Occasional</th>
<th>Rare</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate: Within 1-2 days of receiving drug</td>
<td>Fever, chills, malaise, fatigue, Flu-like syndrome, diarrhea, Rash, pruritus, hypotension, Peripheral edema, oliguria</td>
<td>Capillary leak syndrome, Tachycardia, arrhythmia, acidosis with compensatory alkalosis, Vomiting, nausea, Hypomagnesemia, Hypocalcemia, Hypophosphatemia, dizziness, Cough, rhinitis, insomnia, Confusion, somnolence, anxiety, Lymphopenia</td>
<td>Hypersensitivity reactions, stupor, Coma, apnea, dyspnea, Hypotension (Grade 4), seizures, Myocardial infarction, angina, Sudden death, myocarditis, Headache, paranoid reaction, Psychosis,</td>
</tr>
<tr>
<td>Prompt: Within 2-3 weeks, prior to next course</td>
<td>Asthenia, anorexia, Eosinophilia, leucocytosis, Anemia, thrombocytopenia, Elevated bilirubin, elevated creatinine, ↑SGOT,</td>
<td>Stomatitis, infection (including sepsis), pain, abdominal pain, Abdominal enlargement, weight gain, fibromyalgia Exfoliative dermatitis, ↑alkaline phosphatase</td>
<td>Coagulation disorder, anuria, Acute kidney failure, Pulmonary abnormalities, Erythema, hemorrhage, Pancreatitis, colitis, intestinal perforation, hyperglycemia, Blurred vision</td>
</tr>
<tr>
<td>Delayed: Anytime later during therapy</td>
<td></td>
<td></td>
<td>Exacerbation of auto-immune disease, thyroid disorders</td>
</tr>
<tr>
<td>Unknown Frequency and Timing:</td>
<td>Aldesleukin has been shown to have embryolethal effects in rats when given in doses at 27 to 36 times the human dose (scaled by body weight). Significant maternal toxicities were observed in pregnant rats administered IV doses 2.1 to 36 times higher than the human dose during the critical period of organogenesis. No evidence of teratogenicity was observed other than that attributed to maternal toxicity. It is not known whether this drug is excreted in human milk.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
If used subcutaneously: From animal studies it has been shown that aldesleukin (IL-2) administered subcutaneously distributes preferentially in the lymph, where it achieves an area under the curve (AUC) comparable to intravenous administration and is biologically active (Chiron data on file). The fraction of the subcutaneous dose absorbed into the systemic circulation can be estimated by comparing the normalized AUC for subcutaneous and intravenous administration. For subcutaneous aldesleukin (IL-2) the fraction is approximately 35%, which suggests that in order to achieve comparable systemic exposure, subcutaneous doses of aldesleukin (IL-2) would have to be given at 2.9-fold higher levels than intravenous doses. However, it is important to note that systemic exposure may not account for the complete pharmacologic profile of aldesleukin (IL-2) administered subcutaneously. A substantial proportion of aldesleukin (IL-2) administered subcutaneously distributes in the lymph, where it may be active. Subcutaneous dosing at levels required to achieve systemic exposure comparable to intravenous administration is therefore considered unnecessary.

Based on the early phase I/II trials it is postulated that rest periods between cycles of aldesleukin (IL-2) could be important for several reasons:

1. improved patient tolerance to treatment [217],
2. better bioavailability of aldesleukin (IL-2) for effector cells after return of circulating soluble aldesleukin (IL-2) receptors [218].

Subcutaneous administration is generally safer and better tolerated than intravenous administration. A study performed by Palmer et al [219] comparing the continuous intravenous infusion of aldesleukin (IL-2) alone versus subcutaneous administration plus α-interferon in patients with advanced renal cell carcinoma, highlights the advantage of subcutaneous administration. The overall response rate was not significantly different. However, there was an important shift in the toxicity profile. The subcutaneous regimen did not induce a clinically detectable vascular leak syndrome, which was the dose limiting toxicity for continuous intravenous regimen. In patients receiving subcutaneous aldesleukin (IL-2) site reactions are common, sometimes with necrosis. These effects can be reduced by changing the injection site.

The SIOPEN group has conducted a phase II trial to establish a safe dose of subcutaneous recombinant interleukin-2 (rIL-2), which sustains an increase of natural killer cells (NKCs), in an outpatient setting for stage 4 neuroblastoma patients after MAT and autologous SCR. Between August 1997 and June 2002, 33 patients with stage 4 neuroblastoma entered the study from 6 countries after receiving MAT/ASCR according to national protocols. Median age at registration was 4.1 years (range 1.8-7.4). Dose levels of 3, 6 and 9x10^6 IU rIL-2/m² were given subcutaneously in six 5-day cycles every 2 weeks. Median follow up time was 5 years (range 4-9.8). Repeated increase of NKCs was achieved in 93% of courses with >100% increase over baseline and/or >1g/L in 58%. On the basis of efficacy and moderate toxicity profile, dose level 2 was chosen for the confirmation phase. At dose level 2 the median increase of absolute NKCs was 1.38 g/L in 93% of all 87 cycles, corresponding to a median relative NKC increase over baseline of 721%. Fever was frequent, but controllable with adequate supportive care. Only 6.5% of patients where hospitalized. Localised pain was moderate and acceptable. Simultaneous radiation during rIL-2 treatment blocked completely NKC increase. Event free and overall survival rates at 5 years were 45% (±9) and 48% (±9) respectively. Therefore, the use of 6x10^6 IU/m² rIL-2 s.c. is feasible in outpatients and efficacious. The low toxicity profile allows integration in therapeutic settings aiming for immunomodulation. Based on these observations, the aim of this protocol is to address the question of whether there is a survival benefit for patients receiving aldesleukin (IL-2) in addition to isotretinoin (13-cis-RA) therapy and ch14.18/CHO.
**Formulation and Stability:** Aldesleukin is supplied as a sterile, white to off-white, lyophilized cake in single-use vials. Each vial contains 22 million international units (MIU). When reconstituted with 1.2 mL Sterile Water for Injection, USP, each mL contains 18 million IU (1.1 mg) Aldesleukin, 50 mg mannitol, and 0.18 mg sodium dodecyl sulfate, buffered with approximately 0.17 mg monobasic and 0.89 mg dibasic sodium phosphate to a pH of 7.5 (range 7.2 to 7.8). The manufacturing process for Aldesleukin involves fermentation in a defined medium containing tetracycline hydrochloride. The presence of the antibiotic is not detectable in the final product. Aldesleukin contains no preservatives. Store refrigerated at 2° to 8°C (36° to 46°F).

Reconstitute the 22 MIU vial with 1.2 mL of sterile water **without preservative** for Injection, USP, to achieve a final concentration of 18 MIU/mL or 1.1mg/mL. Diluent should be directed against the side of the vial to avoid excess foaming. Swirl contents gently until completely dissolved. Do not shake. After reconstitution and dilution, store in a refrigerator at 2° to 8°C (36° to 46°F). Do not freeze. Administer aldesleukin within 48 hours of reconstitution. The solution should be brought to room temperature prior to administration to the patient.

**Supplier** Commercially available
24  **APPENDIX: Pharmacology Studies**

24.1 **Pharmacodynamics and pharmacokinetics of high-dose busulfan and melphalan**

24.1.1 **RATIONALE**

1. High-dose busulfan and melphalan combination is effective in high-risk neuroblastoma, but induces a significant level of multi-organ toxicity, especially liver toxicity [220, 221]. It has been suggested that pharmacokinetically – guided dose adjustment may improve high-dose chemotherapy regimens, by reducing the inter-patient variability in systemic exposure to toxic alkylating agents [222-224].

2. A PK/PD relationship between a high AUC of busulfan after oral administration and risk of severe toxicity has been documented by several teams when busulfan is combined with cyclophosphamide [225, 226]. However, no such correlation has been found when oral busulfan is combined with melphalan in neuroblastoma and Ewing sarcoma. This suggests that drug interaction may occur between busulfan and melphalan, and/or that exposure to both drugs should be considered to better evaluate PK/PD relationships and to develop appropriate dose adjustment.

3. Pharmacodynamics of high-dose melphalan has been reported in children [227].

4. Both drugs are alkylating agents, sharing the same metabolic pathway through conjugation to glutathione by glutathione-S-transferases.[228-231] Genetic polymorphism have been reported for this metabolic pathway and may be involved in liver toxicity of high-dose busulfan and melphalan [232, 233]. Genotyping can be easily performed using new molecular technologies.

5. Pharmacokinetics of oral busulfan and melphalan has been extensively studied in children [227, 234]. Limited sampling strategies using bayesian population methodologies have been defined for both drugs [235-237] enabling prospective, multicentre studies with a small number of blood samples needed for each patient.

24.1.2 **OBJECTIVES**

24.1.2.1 Primary objective

- To study whether a high AUC of busulfan and/or melphalan is associated with an increased risk of severe liver or renal toxicity in children with liver or renal impairment in patients receiving I.V. Busulfan (Busilvex®) and I.V. melphalan as consolidation therapy for high-risk neuroblastoma.

24.1.2.2 Secondary objectives

- To describe concomitantly the pharmacokinetics of busulfan and melphalan using population PK models and to identify possible drug interactions.

- To study the PK/PD relationships between exposure to busulfan and melphalan and severe toxicities other than VOD.
- To genotype patients for major detoxifying enzymes (mainly glutathione-transferases) involved in alkylating agents metabolic pathway and to study the risk of severe toxicity with regard to systemic exposure to both drugs, and genotype.

- To identify the AUCs of busulfan and melphalan that could be prospectively targeted to reduce the risk of severe toxicity in high-dose combined chemotherapy regimens including I.V. Busulfan (Busilvex®) and melphalan.

24.1.3 TYPE OF STUDY

- This is multi-institution pharmacological study exploring pharmacokinetics, pharmacodynamics and pharmacogenetics of two anticancer drugs using limited blood sampling strategies and NONMEM programmes.
- This study will be conducted according to Good Laboratory Practice.

24.1.4 BUSULFAN – BLOOD SAMPLING/ ASSAYS

For all blood samples (2 ml), plasma will be separated at 0°C and stored at –20°C until sample shipment. Samples should be sent to the reference laboratory as soon as possible following collection to avoid issues regarding sample stability.

Busulfan plasma levels will be assayed after doses 1 and 9, with three blood samples withdrawn following these two doses (before administration and at 2.5 and 6 hours after the START of infusion). In addition, plasma levels will be measured after dose 13, with a single sample being taken 6 hours after the START of infusion.

Busulfan will be assayed by gas-chromatography with mass spectrometry in the laboratory of Dr Angelo Paci (IGR, France).
Briefly, 0.2 ml (heparin) plasma is required for the analysis. Internal standard (1,5-bis(methansulfonoxy)pentane) or Deuterium busulfan) is added along with 1 ml sodium iodide (8M) and 0.4 ml n-heptane. A micro magnet is added to the screw cap tube. The reaction is carried on at 70 °C for 45 min under continuous magnet stirring. Then, the organic phase (n-heptane) is taken to analysis in the GC system.

24.1.5 MELPHALAN – BLOOD SAMPLING/ ASSAYS

Melphalan plasma levels will be assayed after a single – iv infusion and four blood samples will be withdrawn (before administration and at 5, 23 and 90 minutes after the END of infusion).

Melphalan will be assayed by HPLC with UV detection in the laboratory of Dr Gareth Veal (Newcastle, UK) as previously described [237].

24.1.6 PHARMACOKINETIC MODELING

Pharmacokinetic parameters will be established according to validated and reported bayesian population (NONMEM program) for busulfan and melphalan [227].

24.1.7 GENOTYPING

Total blood sample (5 ml) will be collected on EDTA before treatment for genotyping. Gene sequence for GSTs and other detoxifying enzymes will be performed using Taqman technology. Other polymorphisms will be studied later on using micro-arrays.
24.1.8 NUMBER OF PATIENTS

80 - 100 patients over the accrual period of the protocol.

24.1.9 TOTAL AMOUNT OF BLOOD

Overall the study requires 5 ml for genotyping and 11 samples (2 ml) over the 5 days of treatment, i.e. a total amount of 27 ml.

24.1.10 REFERENCE LABORATORIES

- Dr Gareth Veal, Northern Institute for Cancer Research, Newcastle University, UK, for melphalan assay.
- Dr Angelo Paci, Institut Gustave Roussy, Villejuif for busulfan assay

24.1.11 TRANSPORT OF SAMPLES

All plasma samples should be batched, packed on dry ice in an insulated container and sent by overnight courier to the respective reference laboratory (see section 24.1.10) at the end of sampling. The reference laboratory should be notified on the day the samples are sent. The cost of transporting the samples is the responsibility of the sending site.

24.1.12 OPERATION OF THE STUDY

A Pharmacology committee composed of the study coordinators, a representative of each laboratory of the network, a bio-mathematician and two clinicians involved in the European controlled trial study monitoring committee, will meet twice a year.

24.2 Proposed study to investigate the relationship between isotretinoin (13-cis-RA) pharmacokinetics and metabolism and pharmacogenetic variation in drug metabolizing enzymes

24.2.1 AIMS

The proposed limited sampling study is designed to:

- Investigate the influence of genetic variation in cytochrome P450 enzymes on the metabolism of isotretinoin (13-cis-RA).
- Relate inter-individual variability in 13-cis-retinoic acid metabolism and genetic variation to clinical response and toxicity observed in patients.
- Obtain pharmacokinetic/pharmacogenetic information which could be used in future studies to optimize the treatment of high-risk neuroblastoma.

24.2.2 BACKGROUND

Since the publication of results by Matthay et al. [23], showing the benefit of isotretinoin (13-cis-RA) in the treatment of patients with neuroblastoma, isotretinoin (13-cis-RA) has become an integral part of the standard treatment of high-risk disease. However, despite these initial
successes, the majority of children with high-risk neuroblastoma still succumb to the disease. Optimisation of the clinical use of isotretinoin (13-cis-RA) could lead to a substantial improvement in outcome for this disease, as emphasised by the fact that a European Neuroblastoma Study Group trial (ENSG 4), showed no survival advantage of low dose oral isotretinoin (13-cis-RA) (0-75mg/kg/day; 22.5mg/m²/day), given as continuous treatment for 4 years after myeloablative therapy [238]. Isotretinoin (13-cis-RA) is administered orally and reliable administration depends on the practicalities of administration and on compliance. When absorbed, the drug may be subject to first-pass metabolism and subsequent plasma (and tumour) concentrations will depend on the rate of metabolism to the inactive 4-oxo metabolite. A number of cytochrome P450 (CYP) enzymes have been identified as playing a role in the metabolism of isotretinoin (13-cis-RA) [239, 240]. CYP2C8 is most important in terms of activity and level of expression in the liver. Isotretinoin (13-cis-RA) impairs the metabolism of the CYP2C8 substrate paclitaxel both in vitro and in patients [241]. Genetic polymorphisms in the CYP2C8 gene have been described [242] which result in a lower rate of paclitaxel metabolism and are commonly seen in a Caucasian population [242, 243]. Thus, genetic variation in CYP2C8 activity could underlie individual differences in isotretinoin (13-cis-RA) metabolism and bioavailability. The foetal isoform CYP3A7, which is expressed post-natally in a significant number of individuals, also metabolizes isotretinoin (13-cis-RA) [240]. The expression of CYP3A7, and thus the contribution of this isoform to isotretinoin (13-cis-RA) metabolism, may be predicted by genotyping [244, 245] in a paediatric population. A pilot study recently completed in the UK was designed to investigate the extent of inter-patient variation in isotretinoin (13-cis-RA) pharmacokinetics and metabolism in patients with high-risk neuroblastoma. Preliminary data from this study indicated that plasma concentrations of 4-oxo-13-cis-RA can accumulate to exceed those of the parent compound during a 14 day course of treatment [246]. These data also indicated an approximate 10-fold variation in 4-oxo-13-cis-RA peak plasma concentrations between neuroblastoma patients receiving a standard dose of isotretinoin (13-cis-RA). As this metabolite is a retinoid breakdown product with little or no activity, this level of metabolism in vivo may lead to a diminished efficacy of isotretinoin (13-cis-RA). Preliminary data from this study suggest that extensive metabolism of isotretinoin (13-cis-RA) to its oxo-metabolite may impact on the incidence of disease relapse. It has previously been proposed that the lowering of retinoid plasma levels due to induced metabolism leads to the development of resistance to ATRA in leukaemia patients [247].

24.2.3 RATIONALE FOR STUDY

The proposed study is designed to build on data currently being obtained in a CCLG Study to determine inter-individual variability of isotretinoin (13-cis-RA) pharmacokinetics and drug metabolism in high-risk neuroblastoma patients. In addition, based on data obtained from the ongoing study, the influence of genetic variation in cytochrome P450 enzymes on the metabolism of isotretinoin (13-cis-RA) will be investigated. This will allow potential relationships between inter-individual pharmacokinetic variability, genetic variation in enzyme activity and clinical response (3 year event-free survival) and toxicity (CTC Grade 3/4 skin and hepatic toxicity) to be determined. Results from this study will provide an insight into whether modulation of isotretinoin (13-cis-RA) dosing according to systemic exposure and/or genotype, could be used in future studies to optimize the treatment of high-risk neuroblastoma.

24.2.4 PHARMACOKINETIC SAMPLING AND ANALYSIS

Day 14: isotretinoin (13-cis-RA) 80mg/m² dose given orally twice daily

Samples to be taken on day 14 of a single course of isotretinoin (13-cis-RA) treatment. 5 ml blood samples will be taken and immediately transferred to foil wrapped heparinised tubes at pretreatment and at 4h after oral administration of isotretinoin (13-cis-RA). Additional samples
may be taken at 1, 2 and 6h after oral administration. The times that the samples are taken should be recorded on the Isotretinoin (13-cis-RA) pharmacokinetic study sampling sheet provided. Blood samples will be centrifuged at a speed of 2,000 rpm at 4°C for 5 minutes and the plasma separated, transferred to foil wrapped tubes and stored protected from the light at -20°C prior to transportation to Newcastle upon Tyne, UK, for analysis (see section 24.2.6). Plasma concentrations of isotretinoin (13-cis-RA), ATRA and the metabolite 4-oxo-13-cis-RA, will be determined by HPLC analysis as previously described [248].

24.2.5 PHARMACOGENETICS OF ISOTRETINOIN (13-CIS-RA)

A single 5ml blood sample will be taken from each patient prior to the first course of isotretinoin (13-cis-RA) treatment, transferred to an EDTA tube and stored at –20°C. DNA obtained from this sample will be genotyped for metabolising enzymes thought to be involved in the metabolism of isotretinoin (13-cis-RA), such as CYP2C8 and CYP3A7, using PCR techniques established at the Northern Institute for Cancer Research.

24.2.6 TRANSPORT OF SAMPLES FOR PHARMACOKINETIC ANALYSIS

Samples should be sent to Newcastle upon Tyne, UK, by overnight courier, packed on dry ice in an insulated container, following completion of pharmacokinetic sampling. The Northern Institute for Cancer Research should be notified on the day that the samples are sent (Gareth Veal - Tel: +44 191 246 4332). The cost of transporting the samples is the responsibility of the sending site.
Melphalan Sampling Sheet

DAY OF STUDY

DATE INITIALS HR-NBL-1.5 STUDY No.

WEIGHT S.A GFR

MELPHALAN DOSE (mg):

INFUSION TIME START: FINISH:
(melphalan)

Samples to be taken for melphalan analysis at the specified times from the END of drug infusion.
Label all tubes with the patient initials, date of study and time of sample or sample number

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Time from END Melphalan</th>
<th>Time due</th>
<th>Time taken</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pre-treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>23 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>90 min</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5 ml of blood to be taken in a heparinised tube and centrifuged for 5 min at 1000g and 4°C. Remove plasma and freeze at -20°C. SAMPLES MUST BE SENT TO NEWCASTLE FOR ANALYSIS AS SOON AS POSSIBLE UPON COMPLETION OF SAMPLING DUE TO DRUG STABILITY ISSUES.

COMMENTS:

Address for delivery:
Gareth Veal / Alan Boddy
Northern Institute for Cancer Research
University of Newcastle
Paul O’Gorman Building
North Terrace
Newcastle upon Tyne
NE2 4AD
UNITED KINGDOM

Contact numbers:
Gareth Veal : 0191 246 4332
Alan Boddy : 0191 246 4412
Fax : 0191 246 4301
e mail : G.J.Veal@newcastle.ac.uk
Alan.Boddy@newcastle.ac.uk
**Busilvex® (iv Busulfan) Sampling Sheet**

**DAY OF STUDY**

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Day of busulfan treatment</th>
<th>Time from START busulfan</th>
<th>Time due</th>
<th>Time taken</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Day 1</td>
<td>Pre-treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Day 1</td>
<td>2.5 hr post-dose 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Day 1</td>
<td>6 hr post-dose 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Day 3</td>
<td>Pre-dose 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Day 3</td>
<td>2.5 hr post-dose 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Day 3</td>
<td>6 hr post-dose 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Day 4</td>
<td>6 hr post-dose 13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2 ml of blood to be taken in a **heparinised** tube, centrifuge for 5 min at 2000rpm and 4°C. Remove plasma and freeze at -20°C.

**COMMENTS:**

Samples to be sent by overnight courier, packed on dry ice in an insulated container. Address for delivery :-

Angelo Paci
Service interdépartemental de Pharmacologie & d’Analyse du Médicament (SiPAM)
Plateau des Laboratoires - ERP
Institut de cancérologie Gustave Roussy
114, rue Edouard Vaillant
94805 Villejuif cedex
FRANCE

Contact details :-
Phone: +33 1 42 11 47 30
Fax: +33 1 42 11 52 77
Email: angelo.paci@igr.fr
Isotretinoin (13-cis-RA) pharmacokinetic study

HR-NBL-1 Study No.

Date / / Patient initials Hospital

Weight S.A. GFR (if available)

Isotretinoin (13-cis-RA)
dose (mg/m²)

Administration of isotretinoin (13-cis-RA):
Capsules swallowed with milk OR Capsules snipped / mixed with ice-cream

Other (please specify)

Study day 1 14 (circle answer) Treatment course 1, 2, 3, 4, 5, 6 (circle answer)

Sample for genetic analysis (to be taken prior to 1st course of isotretinoin (13-cis-RA) treatment):

<table>
<thead>
<tr>
<th>Sample date</th>
<th>Time from start 13-cis-RA (hr)</th>
<th>Sample volume (ml)</th>
<th>Time taken</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment</td>
<td>5 ml</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SAMPLE TRANSFERRED TO EDTA TUBE AND STORED AT –20°C PRIOR TO TRANSPORTATION TO NEWCASTLE

Pharmacokinetic sample analysis (sample 1 and 4 mandatory, samples 2, 3, and 5 optional):

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Time from start 13-cis-RA</th>
<th>Sample volume (ml)</th>
<th>Time due</th>
<th>Time taken</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pretreatment</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1 hr</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2 hr</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4 hr</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>6 hr</td>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sampling for isotretinoin (13-cis-RA)

DAY 14: 80MG/M² DOSE GIVEN ORALLY TWICE DAILY (SAMPLING ON FIRST DOSE ONLY)
Samples (5 ml) to be collected in heparinised tubes and wrapped immediately in aluminium foil. Plasma separated by centrifugation in the dark for 5 min at 2 000 g, plasma transferred to second foil wrapped tube (ideally such that plasma sample nearly fills the tube) and frozen at –20°C prior to transportation.

Samples to be sent packed on dry ice in an insulated container.

Address for delivery :-

Dr Gareth Veal
Northern Institute for Cancer Research
University of Newcastle
Paul O’Gorman Building
North Terrace
Newcastle upon Tyne NE2 4AD
United Kingdom

Contact numbers :-

Gareth Veal : +44 191 246 4332
Fax : +44 191 246 4301
E mail : G.J.Veal@newcastle.ac.uk
25.1 Paediatric apheresis procedure

25.1.1 General Principles in Technique
Although operating procedures differ for different 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 when the peripheral CD34+ count is > 20 cells/µl. The paediatric apheresis procedure should be performed by an experienced paediatric team. The team must be familiar with the paediatric basic life support, advanced life support and the typical age dependent problems of children. The parents or a reliable person for the children should be present during the whole procedure.

25.1.2 Apheresis Machine
The Cobe Spectra or the Fenwal CS 3000+ is recommended because the continuous flow centrifugation devices are better tolerated than 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.
- Blood Priming
  Priming of the machine prior to collection should be with ACD and saline according to manufacturer's directions. In haemodynamic 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, 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.
- Procedural Support
  Use of a Cobe in-line blood warmer on the return line will be used for the Cobe machine. A standard blood warmer device can be used with the Fenwal machine. If patients platelet count is <30,000, one may consider to transfuse with platelet prior to apheresis procedure. However, there is evidence in the literature that apheresis procedure could also be performed in children with platelet counts below 20 x 10⁹/l. [249]
- 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. Hypocalcaemia is a well-recognised side effect of citrate. To prevent hypocalcaemia a prophylactic calcium fluconate 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.
- 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)*
2-5 years (15-20 kg)  25-40 ml/min
>5 years   35-50 ml/min

- may be increased to 25-30 ml/min by ratio ramping

**Collection Goals**
During each leukapheresis procedure, the volume of whole blood processed should be approximately 240 to 480 ml/kg (3 - 6 blood volumes). The total time necessary for the whole apheresis procedure should not exceed 5 h. Optimal collection goal (total for all collections) is more than 3 x 10^6 CD 34+ cells/kg for unpurged PBSC with a rescue of 2 x 10^6 CD 34+ cells/kg. The targeted number of cells can usually be obtained in 1-2 collection days.

**Patient Monitoring**
Patients should be observed continuously during the collection. Vital signs should be obtained q 1 hour.

**Laboratory Studies**
For patients < 25 kg, a type and cross for PRBC 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.

**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.

### 25.2 Cryopreservation of PBSC Products
Each collection should be processed and cryopreserved on the day of collection using 10% dimethyl sulfoxide 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.

**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 total 3000 ml/m²/24 hours.

**Premedication**
The DMSO cryoprotectant may cause a histamine-like reaction when infused into the patient. Therefore premedication with Antihistamines (i.e. Benadryl) is recommended.

**Thawing of PBSC**
PBSC are thawed in a 37°C water bath which is monitored with a mercury thermometer to ensure temperature does not rise above 40°C. 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.
PBSC Infusion

Thawed PBSC should be infused as rapidly as tolerated through a central venous catheter. The PBSC product should NEVER be irradiated prior to infusion. Only a 170 micron blood filter should be used during reinfusion. No WBC filter should be used. The unit may be infused by gravity, or the cells may be drawn up into a syringe and pushed by trained personnel. Microaggregate filters and leukodepletion filters MUST NOT be used for infusion of PBSC. If a thawed unit appears clumpy or stringy and these particles cannot be dispersed with gentle kneading, the PBSC product could be infused through a standard 170 micron blood filter.

- **Possible Symptoms during Infusion**
  
<table>
<thead>
<tr>
<th>Precipitating Factor</th>
<th>Possible Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>haemolysed red cells</td>
<td>fever, chills, haemoglobinuria</td>
</tr>
<tr>
<td>cellular clumps and debris</td>
<td>chest pain, hypoxia, hypertension</td>
</tr>
<tr>
<td>cold 10% DMSO</td>
<td>nausea, headache</td>
</tr>
<tr>
<td>microbial contamination</td>
<td>fever, chills, hypotension</td>
</tr>
<tr>
<td>plasma proteins</td>
<td>urticaria</td>
</tr>
</tbody>
</table>

26 **APPENDIX: Performance Scales**

26.1 Lansky Play-Performance Scale (for patients ≤ 16 years)

- 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.

26.2 Karnofsky Performance Scale (for patients > 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.
27 APPENDIX: Guidelines for Reporting Toxicity/Serious Adverse Events

27.1 Definitions and reporting methods
The processing of Serious Adverse Events in a timely manner is a regulatory requirement. It is also vital in order to safeguard the safety of patients entered into any trial. The information in this section has been taken from EU Directive 2001/20/EC (4 April 2001) and the Detailed guidance on the collection, verification and presentation of adverse reaction reports arising from clinical trials on medicinal products for human use (Revision 2, April 2006).

I. Definitions of Events

a. Adverse Event (AE)
Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.
That is, any unfavourable and unintended sign (including abnormal laboratory findings), symptom, or disease temporally associated with the use of a medicinal product, whether or not it is considered related to the medicinal product.

Please also note the following tips:
Adverse Events include:
- Exacerbation/worsening of a pre-existing condition.
- Increased frequency or intensity of a pre-existing episodic event or condition.
- Conditions diagnosed after the start of the study even though they were possibly present prior to the start of the study.

Adverse Events do not include:
- Medical or surgical procedures (the indication leading to the procedure is an AE).
- Pre-existing diseases and conditions that do not change.
- The disease being studied (e.g. disease progression).
- Death (death is an outcome; the underlying cause is an event).

b. Adverse Reaction (AR)
Any untoward and unintended medical occurrence in a patient or clinical trial subject administered a medicinal product for which a causal relationship is suspected.
That is, all adverse events judged by the reporting physician as having a reasonable causal relationship to the medicinal product qualify as adverse reactions. The expression “reasonable causal relationship” is defined as the case where there is proof or argument to suggest a causal relationship.

c. Unexpected Adverse Reaction (UAR)
Any adverse drug reaction where the nature or severity is not consistent with the applicable product information.
That is, any expected event with a more severe outcome or increased rate of occurrence. Or an event judged by the reporting physician as having a reasonable causal relationship to the medicinal product, but is not documented in the applicable product information.

d. Serious Adverse Events (SAE) or Reaction (SAR)
Any adverse event or reaction that:
- results in death,
- is life threatening (at the time of the event),
- requires in-patient hospitalisation or prolongation of existing hospitalisation,
- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect.

e. Suspected Expected Serious Adverse Reaction (SESAR)
Any serious adverse reaction that is documented in the applicable product information.

f. Suspected Unexpected Serious Adverse Reaction (SUSAR)
Any serious adverse reaction where a causal relationship can not be excluded and the nature or severity is not consistent with the applicable product information.

II. SAEs requiring expedited reporting
Only SUSARs will require expedited (immediate) reporting. Expedited is defined as within 24 hours of knowledge of the event, see part V for more information. Exemptions are detailed in section III.

III. Exemptions from expedited reporting
Expected SAEs i.e. those consistent with the profile of expected toxicities in this trial (section 27.2), unless in the opinion of the local investigator, are unexpectedly severe, do not need reporting expeditely.

The following events are also exempt from expedited reporting:
- Relapse
- Signs and symptoms of disease progression
- Death from disease progression
- Secondary malignancy

All the exempted events must still be reported via the appropriate toxicity forms (i.e. Toxicities should first be reported in the toxicity case report form (CRF) for the appropriate reporting period. If the toxicity does not appear in the toxicity CRF then report in the SAE CRF specifying that it is an expected event.

IV. Documentation of events
Events, regardless of cause, can occur from the time the informed consent is signed up until 30 days after the last day of treatment on the HR-NBL-1.5. Patients who withdraw (for any reason) are also included within this time frame.

The evaluation of the severity of toxicities is based on Common Toxicity Criteria (CTC). For the definition of the seriousness of the toxicity see part I.d.

Adverse events (AEs) and serious adverse events (SAEs) must be documented on the appropriate case report form (CRF). Some SAEs are required to be reported within 24 hours (expedited) of knowledge of the event. See section II for a list of SAEs that require expedited reporting.

V. Reporting procedures and time limits

a. Local Investigator Responsibilities
The local investigator must report all serious adverse events (SAEs) requiring expedited reporting (see part II) within 24 hours of knowledge of the event. The local investigator is responsible for providing an assessment of the seriousness, severity and causality (see Part V.(c)) of the SAE.
The SAE form should be completed as much as possible; the local investigator should not wait for full details prior to making the initial report. The initial report should be promptly followed up with the full details, so that the sponsor can report the event to the regulatory authorities within the required time periods (see sponsor responsibilities for more details).

For reported deaths, the local investigator should promptly supply the sponsor and the IRB/IEC with any additional requested information (e.g. autopsy reports and terminal record reports).

The local investigator must also comply with local requirements related to the reporting of SAEs to the regulatory authorities and IRB/IEC. The local investigator is responsible for the distribution of any information supplied by the sponsor the appropriate regulatory authorities and local IRB/IEC, as required by local laws.

The local investigator must ensure that there is a clearly documented audit trail of the reporting to the sponsor and local regulatory authorities.

b. Sponsor Responsibilities

The sponsor is responsible for the ongoing safety evaluation of the trial. All SAEs received by the sponsor must be reviewed in a timely manner to ensure that the requirements for the onward reporting can be completed in a timely manner. In the case of a SUSAR it must be determined whether or not the event is fatal or life-threatening. Reporting requirements are different for those events which are fatal or life-threatening to those which are not.

SUSARs which are fatal or life-threatening qualify for rapid reporting. The appropriate regulatory authorities and investigators must be notified within seven calendar days after first knowledge by the sponsor that the event qualifies as a SUSAR. A complete report must be supplied within a further eight calendar days.

SUSARs which are not fatal or life-threatening must be reported to the appropriate regulatory authorities and investigators within 15 calendar days after first knowledge by the sponsor that the event qualifies as a SUSAR.

SESARs must be reported together with the annual report to the IRB/IEC yearly.

The sponsor is responsible for notifying all concerned investigators and regulatory authorities of findings that could adversely affect the safety of patients, impact the conduct of the trial, or alter the IRB/IEC opinion to continue the approval of the trial.

If the SAE is considered a major cause of concern in relation to other, or future, patients on a trial, it maybe necessary to issue an urgent safety notice to all investigators pending a formal amendment. The urgent safety notice must be sent from the sponsor and contain a clear explanation of the event and any treatment recommendations.

The sponsor must keep a clearly documented audit trail of receipt of SAEs, clinical review conducted and outcome, and onward reporting.
c. Causality Assessment

<table>
<thead>
<tr>
<th>Relationship</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unrelated</td>
<td>There is no evidence of any causal relationship.</td>
</tr>
<tr>
<td>Unlikely</td>
<td>There is little evidence to suggest there is a casual relationship (e.g. the event did not occur with a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the patient’s clinical condition, other concomitant treatments).</td>
</tr>
<tr>
<td>Possible</td>
<td>There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patient’s clinical condition, other concomitant treatments).</td>
</tr>
<tr>
<td>Probable</td>
<td>There is evidence to suggest a casual relationship and the influence of other factors is unlikely.</td>
</tr>
<tr>
<td>Definitely</td>
<td>There is clear evidence to suggest a casual relationship and other possible contributing factors can be ruled out.</td>
</tr>
<tr>
<td>Not Assessable*</td>
<td>There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.</td>
</tr>
</tbody>
</table>

* In cases where the relationship is assigned to be “not assessable” a precautionary approach will be taken and these cases will be treated as related events. They will be listed in the Annual Safety Report.

27.2 Profile of expected toxicities in this trial

a. Requirement of in-patient hospitalisation or prolongation of existing hospitalisation: Prolongations of existing hospitalisations for application of the study medication are expected within the frame of this intense treatment concept to treat expected secondary treatment effects, i.e. infections and complementary supportive care measures. This includes transfer to ICU (intensive care units).

b. Definition of toxicities (CTC Toxicity Score) as applied in the HR-NBl-1/SIOPEN study for severe, but expected events within this intensive regimen

- **GENERAL CONDITION:** Grade 4- ICU, very sick
- **HAEMATOLOGICAL TOXICITY:** Grade 4
- **SKIN:** Grade 4
  - **exfoliative dermatitis/necrosis** requiring surgical intervention
  - **anaphylaxis**
- **GASTRO-INTESTINAL TRACT**
  - **Nausea/Vomiting:** Grade 4 - intractable vomiting
  - **Constipation:** Grade 4 - ileus >96h
  - **Stomatitis:** Grade 4 – total parenteral nutrition required
  - **Diarrhoea:** Grade 4- leading to dehydration
  - **Liver:** Grade 4 (bilirubin >3 x N) and SGOT/SGPT > 20xN or Bearman Toxicity’s Grade III –VOD: Severe hepatic dysfunction with bilirubin < 20 mg%; or hepatic encephalopathy; or ascites comprising respiratory dysfunction
• **Urinary Tract**
  - **Renal Toxicity**
    - Any Grade 4
    - All GFR rates ≥ Grade 2 ➔ patient ineligible for R1
      Since recovery of GFR rate is observed in particular post surgery at least two GFR rates need to be evaluated post surgery if the first post-surgical one shows a GFR grade 2 to 4 prior to day 150 (with at least a two weeks interval but ideally 4 weeks if feasible)
  - **Hemorrhagic cystitis (Bearman Grade III):**
    Macroscopic haematuria with frank blood, necessitating invasive local intervention with installation of sclerosing agents, nephrostomys or other surgical procedures

• **Cardiac:** any Grade 4

• **Respiratory Tract**
  - **Pulmonary Toxicity (Bearman Toxicity’s Grade III)**
    Interstitial changes requiring mechanical support or >50% oxygen on mask and not caused by infection or CHF

• **Neurological**
  - **Central Neurotoxicity:** Grade 3 Somnolence >50% of time (severe disorientation and hallucinations) and Grade 4- Coma-Seizures
  - **Peripheral Neurotoxicity:** Grade 4 paralysis
  - **Ototoxicity:** Brock grade 3 and 4: ➔ patient is not recommended to receive CEM. Evaluation of Ototoxicity has to be performed before and after MAT; follow up of ototoxicity is required

• **Infections: Grade 3 to 4**
  - **Major infection**
    - septicemia alone (controlled infection)
    - pneumonia alone
    - urinary infection alone
    - severe soft tissue infection
    - minor localised fungal infection (stool culture pos only, diaper rash and/or mouth – candida pos)
  - **Life threatening with hypotension:**
    - septic shock
    - proven systemic fungal infection
      blood culture positive or biopsy positive or unequivocal imaging
      (for prevention of fungal infections please adhere to UK guidelines)

**Note:** Events with lesser grades than those mentioned in the above list are also exempt from expediated reporting (e.g. Haematological toxicities grades 1-3 do not require expedited reporting).
### 28 APPENDIX: Toxicity Grading and Pain Assessment

#### 28.1 TOXICITY AFTER HIGH DOSE CHEMOTHERAPY (BEARMAN)[250]

<table>
<thead>
<tr>
<th>Grade I</th>
<th>Grade II</th>
<th>Grade III</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cardiac</strong></td>
<td>Mild ECG abnormality, not requiring medical intervention; or noted heart enlargement on CXR with no clinical symptoms</td>
<td>Moderate ECG abnormalities requiring and responding to medical intervention; or requiring continuous monitoring without treatment, or congestive heart failure responsive to digitalis or diuretics.</td>
</tr>
<tr>
<td><strong>Bladder</strong></td>
<td>Macroscopic haematuria after 2 days from last chemotherapy dose with no subjective symptoms of cystitis and not caused by infection.</td>
<td>Macroscopic haematuria after 7 days from last chemotherapy dose not caused by infection; or haematuria after 2 days with subjective symptoms of cystitis not caused by infection.</td>
</tr>
<tr>
<td><strong>Renal</strong></td>
<td>Increase in creatinine up to twice the baseline value (usually the last recorded before start of conditioning).</td>
<td>Increase in creatinine above twice baseline but not requiring dialysis.</td>
</tr>
<tr>
<td><strong>Pulmonary</strong></td>
<td>Dyspnea without CXR changes not caused by infection or congestive heart failure; or CXR showing isolated infiltrate or mild interstitial changes without symptoms not caused by infection or congestive heart failure.</td>
<td>CXR with extensive localised infiltrate or moderate interstitial changes combined with dyspnea and not caused by infection or CHF; or decrease of PO₂ (&gt;10% from baseline) but not requiring mechanical ventilation or &gt;50% O₂ on mask and not caused by infection or CHF.</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td>Mild hepatic dysfunction with 2.0 mg% ≤ bilirubin ≤ 6.0mg%; or weight gain &gt;2.5% and &lt;5% from baseline, of noncardiac origin; or SGOT increase more than 2-fold but less than 5-fold from lowest preconditioning.</td>
<td>Moderate hepatic dysfunction with bilirubin &gt;6mg% &lt;20mg%, or SGOT increase &gt;5-fold from preconditioning ; or clinical ascites or image documented ascites &gt;100ml, or weight gain &gt;5% from baseline of noncardiac origin.</td>
</tr>
<tr>
<td><strong>CNS</strong></td>
<td>Somnolence but the patient is easily arousable and orientated after arousal.</td>
<td>Somnolence with confusion after arousal, or other new objective CNS symptoms with no loss of consciousness not more easily explained by other medication, bleeding, or CNS infection.</td>
</tr>
<tr>
<td><strong>Stomatitis</strong></td>
<td>Pain and/or ulceration not requiring a continuous IV narcotic drug.</td>
<td>Pain and/or ulceration requiring a continuous IV narcotic drug (morphine drip).</td>
</tr>
<tr>
<td><strong>Digestive</strong></td>
<td>Watery stools &gt; 500ml but &lt;2,000ml every day not related to infection.</td>
<td>Watery stools &gt;2,000ml every day not related to infection; or macroscopic hemorrhagic stools with no effect on cardiovascular status not caused by infection; or subileus not related to infection.</td>
</tr>
</tbody>
</table>
## RECOMMENDATIONS FOR GRADING OF TOXIC EFFECTS

<table>
<thead>
<tr>
<th>Site</th>
<th>Grade 0</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>H</strong></td>
<td>Haematological</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1</td>
<td>Haemoglobin (g/100 ml)</td>
<td>WNL</td>
<td>&gt; 10.0</td>
<td>8.0-9.9</td>
<td>6.5-7.9</td>
</tr>
<tr>
<td>H2</td>
<td>Leukocytes (1000/mm³)</td>
<td>&lt;4.0</td>
<td>3.0-3.9</td>
<td>2.0-2.9</td>
<td>1.0-1.9</td>
</tr>
<tr>
<td>H3</td>
<td>Granulocytes (1000/mm³)</td>
<td>&lt; 2.0</td>
<td>1.5-1.9</td>
<td>1.0-1.4</td>
<td>0.5-0.9</td>
</tr>
<tr>
<td>H4</td>
<td>Platelets (1000 ’000/mm³)</td>
<td>WNL</td>
<td>&gt; 75</td>
<td>50-75</td>
<td>25-50</td>
</tr>
<tr>
<td>H5</td>
<td>Haemorrhage</td>
<td>None</td>
<td>Petechiae</td>
<td>Mild blood loss</td>
<td>Needing blood transfusion or fundal haemorrhages</td>
</tr>
</tbody>
</table>

| **D** | Digestive | | | | |
| D1 | Bilirubin | WNL | < 1.5 x N | 1.5 - 3 x N | > 3 x N | 3 x N |
| D2 | Transaminases (SGOT/SGPT) | > 1.25 x N | 1.26-2.5 x N | 2.6 - 5 x N | 5.1 - 20 x N | > 20 x N |
| D3 | Alkaline phosphatase | < 1.25 x N | 1.26-2.5 x N | 2.6 - 5 x N | 5.1 - 20 x N | > 20 x N |
| D4 | Amylase | WNL | < 1.5 x N | 1.6 - 2 x N | 2.1 - 5 x N | > 5 x N |
| D5 | Stomatitis | No change | Mild soreness, erythema | Painful oedema, ulcers but can eat solids | Ulcerated lesions, requiring liquid diet only | Oral alimentation not possible |
| D6 | Nausea/vomiting | None | Nausea | Transient vomiting < 5 episodes in 24 hrs | Transient vomiting > 5 episodes in 24 hrs | Intractable vomiting >10 episodes in 24 hrs |
| D7 | Diarrhoea | None | Transient < 2 days | Tolerable but > 2 days | Intolerable requiring therapy | Leading to dehydration |
| D8 | Constipation | None or no change | Mild | Moderate | Severe; abdominal distension | Ileus >96 hrs; distension & vomiting |

| **M** | Metabolic/Renal | | | | |
| M1 | Blood creatinine | WNL | < 1.5 x N | 1.5 - 3 x N | 3.1 - 6 x N | > 6 x N |
| M2 | Proteinuria | No change | 1 or < 3 g/l | 2-3 + or 3-10 g/l | 4+ or > 10 g/l | Nephrotic syndrome |
| M3 | Haematuria | No change | Microscopic | Gross no clots | Gross + clots | Requires transfusion |
| M4 | Na + mmol/L | 135-145 | 146-149/130-134 | 150-155/125/129 | 156-164/116/124 | > 165 < 115 |
| M5 | K+ mmol/L | 3.5-5.4 | 5.5-5.9/3.1-3.4 | 6-6.4/2.6-3 | 6.5-6.9/2.1-2.5 | > 7/ < 2 |
| M6 | Ca + mmol/L | 2.15-2.59 | 2.6-2.89/1.9-2.1 | 2.9-3.09/1.7-1.89 | 3.1-3.3/1.5-1.69 | > 3.3/ < 1.5 |
| M7 | Mg++ mmol/L | 1.5-2.0 | 1.2-1.4 | 0.9-1.1 | 0.6-0.8 | < 0.6 |

| **P** | Pulmonary | | | | |
| P1 | PA O2 | > 90 | 80-89 | 65-79 | 50-64 | < 49 |
| P2 | DL CO | 100-75% | 74-65% | 64-55% | 54-40% | < 40% |
| P3 | CV | 100-75% | 74-65% | 64-55% | 54-40% | < 40% |
| P4 | Function | No change | Mild symptoms | Exertional dyspnoea | Dyspnoea at normal levels of exertion | Dyspnoea at rest |
# Recommendations for Grading of Toxic Effects

<table>
<thead>
<tr>
<th>Site</th>
<th>Grade 0</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Allergy</td>
<td>No change</td>
<td>Oedema, transient rash</td>
<td>Mild bronchospasm, urticaria, no parenteral therapy needed</td>
<td>Bronchospasm, parenteral therapy required</td>
</tr>
<tr>
<td>S</td>
<td>Skin</td>
<td>No change</td>
<td>Macular, papular eruption, erythema, asymptomatic</td>
<td>Dry desquamation, vesiculation, pruritus</td>
<td>General symptomatic macular, papular or vesicular eruption or ulceration</td>
</tr>
<tr>
<td>I</td>
<td>Infection</td>
<td>None or no change</td>
<td>Changes requiring no therapy</td>
<td>Requires therapy but no hospitalisation</td>
<td>Requires therapy</td>
</tr>
<tr>
<td>C</td>
<td>Cardiac</td>
<td>None</td>
<td>Asymptomatic, transient, requiring no therapy</td>
<td>Recurrent/persistent requiring no therapy</td>
<td>Requires treatment</td>
</tr>
<tr>
<td>C1</td>
<td>Rhythm</td>
<td>None</td>
<td>Asymptomatic</td>
<td>Asymptomatic, decline of resting EF &gt; 20% of baseline</td>
<td>Mild congestive heart failure responsive to therapy</td>
</tr>
<tr>
<td>C2</td>
<td>Function</td>
<td>No change</td>
<td>Non specific T wave flattening</td>
<td>Asymptomatic ST + T wave changes suggesting ischaemia</td>
<td>Angina without evidence of infarction</td>
</tr>
<tr>
<td>C3</td>
<td>Ischaemia</td>
<td>&gt; 30%</td>
<td>&gt; 25% and &lt; 30%</td>
<td>&gt; 20% and &lt; 25%</td>
<td>&gt; 15% and &lt; 20%</td>
</tr>
<tr>
<td>C4</td>
<td>Echocardiography (FS)</td>
<td>&gt; 30%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C5</td>
<td>Hypotension</td>
<td>None or no change</td>
<td>Changes requiring no therapy</td>
<td>Requires therapy but no hospitalisation</td>
<td>Requires therapy</td>
</tr>
<tr>
<td>C6</td>
<td>Hypertension</td>
<td>None or no change</td>
<td>Asymptomatic, increase &lt; 20 mmHg or &lt; 150/100 if previous WNL. No treatment required</td>
<td>Recurrent/persistent increase &gt; 20 mmHg or &gt; 150/100 if previous WNL. N6 treatment required</td>
<td>Requires therapy</td>
</tr>
<tr>
<td>N</td>
<td>Neurological</td>
<td>Seizures</td>
<td>Seizures related to:</td>
<td></td>
<td>Syndrome related to:</td>
</tr>
<tr>
<td>N1</td>
<td></td>
<td></td>
<td>1= metabolic disorder, 2= sinus thrombosis, 3= fever, 4= other, 5= unknown</td>
<td></td>
<td>1= metabolic disorder, 2= sinus thrombosis, 3= fever, 4= other, 5= unknown</td>
</tr>
<tr>
<td>N2</td>
<td>Cerebellar</td>
<td>None or no change</td>
<td>Mild somnolence and agitation</td>
<td>Moderate somnolence (&lt; 50% waking hours) or agitation</td>
<td>Severe somnolence, agitation, confusion, disorientation</td>
</tr>
<tr>
<td>N3</td>
<td>Sensory</td>
<td>None or no change</td>
<td>Mild paraesthesia and/or loss of deep tendon reflexes</td>
<td>Intention tremor, dysmetria, slurred speech, nystagmus</td>
<td>Locomotor ataxia</td>
</tr>
<tr>
<td>N4</td>
<td>Motor</td>
<td>None or no change</td>
<td>Subjective weakness, no objective findings</td>
<td>Mild or moderate objective sensory loss; moderate paraesthesia</td>
<td>Severe objective sensory loss, or paraesthesia interfering with function</td>
</tr>
<tr>
<td>N5</td>
<td>Vision</td>
<td>None or no change</td>
<td></td>
<td>Mild objective weakness, without significant impairment</td>
<td>Objective weakness with significant impairment of function</td>
</tr>
<tr>
<td>N6</td>
<td>Pain - Other</td>
<td>None</td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
</tbody>
</table>

Specify site

---

**Recommended for use per:** 01.06.2011
28.3 Pain Assessment

Self-report Pain Assessment Tools

GENERAL INSTRUCTIONS
- Routinely assess pain every 4 hours – increase frequency if score is 4 or above
- Post-op: score hourly for 6 hours then routinely as above

WONG-BAKER FACES

0  NO HURT
2  HURTS LITTLE BIT
4  HURTS LITTLE MORE
6  HURTS EVEN MORE
8  HURTS WHOLE LOT
10 HURTS WORST

Point to each face using the words to describe the pain intensity. Ask the child to choose a face that best describes their own pain and record the appropriate number below.

NUMERIC RATING SCALE

Assess pain  Using one of the above tools
Plan       Is an intervention required if so what?
Implement  Implement intervention(s)
Evaluate   Re-score at an appropriate interval to evaluate the effectiveness of intervention(s)

0  =  no pain
1 - 3 =  mild pain
4 - 7 =  moderate pain
8 - 10 =  severe pain

© Pain Control Service GOSH 2005
# FLACC Behavioural Pain Assessment Tool

**Suggested age group: 2 months to 7 years**

**GENERAL INSTRUCTIONS**
- Routinely assess pain every 4 hours - increase frequency if score is 4 or above
- Post-op: score hourly for 6 hours then routinely as above

<table>
<thead>
<tr>
<th>CATEGORIES</th>
<th>0</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Face</td>
<td>No particular expression or smile</td>
<td>Occasional grimace or frown, withdrawn, disinterested</td>
<td>Frequent to constant quivering chin, clenched jaw</td>
</tr>
<tr>
<td>Legs</td>
<td>Normal position or relaxed</td>
<td>Uneasy, restless, tense</td>
<td>Kicking, or legs drawn up</td>
</tr>
<tr>
<td>Activity</td>
<td>Lying quietly, normal position, moves easily</td>
<td>Squirming, shifting back and forth, tense</td>
<td>Arched, rigid or jerking</td>
</tr>
<tr>
<td>Cry</td>
<td>No cry (awake or asleep)</td>
<td>Moans or whimpers, occasional complaint</td>
<td>Crying steadily, screams or sobs, frequent complaints</td>
</tr>
<tr>
<td>Consolability</td>
<td>Content, relaxed</td>
<td>Reassured by occasional touching, hugging or being talked to, distractible</td>
<td>Difficult to console or comfort</td>
</tr>
</tbody>
</table>

Each of the five categories: (F) Face; (L) Legs; (A) Activity; (C) Cry; (C) Consolability; is scored from 0 - 2 which results in a total score between 0 and 10

(Merkel et al, 1997)

<table>
<thead>
<tr>
<th>Assess pain</th>
<th>Using the above scoring system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plan</td>
<td>Is an intervention required if so what?</td>
</tr>
<tr>
<td>Implement</td>
<td>Implement intervention(s)</td>
</tr>
<tr>
<td>Evaluate</td>
<td>Re-score at an appropriate interval to evaluate the effectiveness of intervention(s)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>no pain</td>
</tr>
<tr>
<td>1 - 3</td>
<td>mild pain</td>
</tr>
<tr>
<td>4 - 7</td>
<td>moderate pain</td>
</tr>
<tr>
<td>8 - 10</td>
<td>severe pain</td>
</tr>
</tbody>
</table>

© Pain Control Service GOSH 2005
APPENDIX: Psycho-Social Support

Qualified psycho-social support for patients and relatives should be an integral part of the treatment strategy. Faced with the diagnosis of cancer implying the risk of death or of permanent disablement, and the need for long-term, aggressive multimodal therapy, patients and relatives need psychological support and crisis management. Moreover, social issues must be dealt with: housing, financial issues, unscheduled leave from work, etc. Patients (and their families) need to continue their normal lives as much as possible, and to allow their minds to turn away from the disease from time to time. Thus, school, structured and spontaneous play, artwork, music therapy, etc. should be available. In the case of paediatric cancer patients, siblings often feel rejected because all of the attention is directed towards the patient, and they can even feel guilty about being healthy. Parents may wonder if they are responsible for their child's disease (wrong food, smoking?). Special attention needs to be paid and support offered to all family members as well as the patient. Close co-operation and regular exchange with the medical staff is of paramount importance in order to optimise both aspects of patient care.

Well trained personnel who can offer these services should be permanently available, and need to be integrated into each patient's treatment strategy. The psycho-social support team should include members of the following professions:

- Clinical psychologist
- Paediatric nurse
- Social worker
- School teacher
- Nursery school / kindergarten teacher
- Art / music teacher / therapist

Psycho-social support should encompass:

- Social / psychological family history at first contact (intra-family relationships, coping styles, etc.)
- Help and guidance with social services, health insurance, social insurance matters, etc.
- Help and support with practical problems during hospital stays, e.g. housing, transport, organising nannies for siblings, etc.
- Offering assistance and aid to patients and relatives in stressful or painful situations, e.g. on the way to the operating theatre, diagnostic interventions, etc.
- Building stable relationships between patient, family, and support team members
- Crisis intervention (e.g. in case of non-coping, non-compliance, etc.)
- Support with emotional aspects: coping with disease and therapy
- Play, artwork, music, ...
- Visits to the patient's home
- Psychological and social follow up (coping with memories of the disease and its therapy, rehabilitation, occupational problems, ...)
- Psychological support in a terminal care situation.

The brief description given above can obviously not cover all issues of importance. In every patient, his or her specific situation must be met by adjusted care and support, which requires the permanent involvement of an experienced psycho-social team.
30  **APPENDIX: International Trial Co-ordination**

The conduct of this study will be according to the following agreed procedures:

**30.1 Status of Study**
This is a collaborative study between numbers of participating national groups. The co-ordinators for each group are listed below.

**30.2 The Protocol**
A common protocol will be used for the International study by all national groups. The finalised master protocol in the English language will be held at the International Main Data Centre. Other national groups will be responsible for producing a literal translation in their own language.
Each national co-ordinator will be responsible for the distribution of protocols to centres within their national group via their national data centre as appropriate.
Appendices may be added independently by any of the national groups to address local needs, provided they have no bearing on the essential aims of the international protocol. However no change will be allowed to the eligibility criteria or the treatment procedures of the main protocol. Subsequent to finalisation, any amendments to the protocol must be agreed by the SIOP Europe Neuroblastoma Board, the study co-ordinator and the national co-ordinators.

**30.3 Study Forms**
One common set of forms will be used. The master version (in English) of the study forms will be held at the International Data Centre.
Ideally all data shall be entered through the RDE system and no paper forms shall be used.
Each national co-ordinator will be responsible for distribution of forms to centres within his/her country (using their national data centre as appropriate).
Additional forms may be produced independently by any national group for the collection of data additional to that required for the International study.
Subsequent to finalisation, amendments to the forms must be agreed by all national groups. The International Data Centre will be responsible for the issue of amended forms.

**30.4 Inclusion Procedure for New Patients**
New patients will be enrolled online via the internet by each treating physician or through the national co-ordinator. Pre-registration is only possible if the eligibility criteria are met. Registration will be final once the disease description at diagnosis including MYCN results is complete.

**30.5 Data Collection**
Data entry ideally will be done online by each treating physician via internet. If for any reason the local hospital physician prefers to fill in paper forms, the data entry still should be nationally based and done in the data centre of the responsible national co-ordinator via internet through the SIOPEN-R-NET platform https://www.siopen-r-net.org

**30.6 Randomisation Procedure**
Online per internet; Internet address to be added: https://www.siopen-r-net.org
30.7 Confidentiality of Patient Data
The use of names as patient identifiers on paper forms and on national databases will be according to national practice. An abbreviated patient identifier will be used for data transfer and for the master database.

30.8 Data Quality Control
On receipt of forms at the national data centre, common range and logical checks, agreed by the Study Co-ordinators, will be carried out on data prior to transfer to the master database. Any amendment to the checking programme will require mutual agreement of the Study Co-ordinators.
Data entry verification shall be carried out according to current national practice. Cross checks of data entry will be carried out occasionally, between national centres, on a sample of forms.
Data amendments shall only be carried out at the national data centre on the national database. Errors noted on the master database, after receipt of the group database, shall be reported back to the national centre.
Data audit of study forms against the patient record forms at the treating institution shall be performed to satisfy national requirements.

30.9 Data Analysis and Monitoring
Data will be released from the International Database to the Trial statistician responsible for interim analysis at given time intervals.

Results of the interim analysis on outcome and toxicity shall be reported to an independent international DMSC as scheduled in the protocol. The DMSC may recommend early stopping, continuation or extension of the study to the TMC.

The Study co-ordinators (and national co-ordinators) shall meet as appropriate to consider patient treatment, eligibility and outcome to ensure the smooth running of the study.

30.10 Serious Adverse Events
Any serious adverse event (see chapter 27 for specific details) shall be reported immediately by the treating institution to the national centre and relayed to the Study co-ordinators and the International Data Centre for further reporting according to local practice.
The toxicity criteria used in completion of the data forms will be the same for all participating groups and appear in the appendix (chapter 28).

30.11 Chemotherapy Review
Chemotherapy forms shall be reviewed by the national group co-ordinator(s) and protocol deviations noted on the database. The review information shall be reported to the Trial co-ordinators.

30.12 Pathology Review
Pathology review affecting eligibility shall be rapidly reviewed by national group reviewer(s). National group pathology data shall be discussed at the meeting of the international study committee.

30.13 Biological Review
Biology will be established within the reference laboratories only. National group reference laboratory biology data will be discussed at the meeting of the international study committee and/or by online reviewing (ENQUA).
30.14 BM /PBSC Harvest Review
Review affecting eligibility shall be rapidly done by the national group reviewer(s). National group BM / PBSC harvests data shall be discussed at the meeting of the international study committee.

30.15 MIBG Review
Review of mIBG scans shall be rapidly reviewed by national group reviewer(s). National group mIBG data shall be discussed at the meeting of the international study committee.

30.16 Radiological Review
The question of the quality of surgery and local radiotherapy on the local relapse rate, the relapse rate and EFS will be addressed. The imaging of the primary tumour site prior to and post surgery, as well as the radiation fields should be centrally reviewed.

30.17 Follow Up Data
All registered patients will be followed up by the national data centres during and after completion of treatment according to the current protocol.

30.18 Study Approval
The study will be opened consecutively in each of the participating countries.

30.19 Institutional/Local Ethical Approval and Patient Consent
Institutional/local ethical approval will follow accepted national practice. National procedures for patient consent will be used

30.20 Data Monitoring and Safety Committee (DMSC)
An independent DMSC composed of 3 international experts will monitor the progress of the trial on ethical and scientific grounds. The role of the DMSC will be:
- To review the accrual rate.
- To examine the interim analysis.

Each interim analysis will be reported to the DMSC.
- These interim analyses will remain confidential.
- On the basis of these analyses, the DMSC will recommend whether the study can continue, or whether it should be changed or terminated prematurely.

To monitor toxicity.
- Every 12 months the statistician for the trial will circulate a report to the members of the DMSC about toxicity. The DMSC will review these interim toxicity data and any relevant information will be forwarded to each Trial co-ordinator.
- This annual procedure should prevent problems of major toxicity persisting.

To examine other trials.
- The DMSC will review reports of related studies performed by other groups or organisations to determine whether such information materially affects the aims or preliminary findings of the trial.
The DMSC will be asked to review any major modification to the study proposed by the TMC prior to its implementation.

**30.21 Toxicity Monitoring**
Evaluation of non-lethal toxicity is based on CTC-scored reported toxicity of all patients. The evaluation includes myelosuppression, rate of infections, capillary leak syndrome, VOD of the liver, mucosal damage, cardiac, pulmonary, renal, hepatic, central and peripheral nervous toxicity as described in the toxicity grading appendix (chapter 28).

**30.22 Treatment Stopping Rules for Individual Patients**
If any (non-lethal) severe adverse event occurs in a patient, his or her further treatment according to the study protocol must be discussed with the national study co-ordinator immediately. Consideration will be given to how dangerous continuation of protocol therapy might be, and if there is an alternative treatment option available in the particular situation, or if treatment should be discontinued. As the consequences of either decision may be deleterious to the patient, immediate discussion of the event is mandatory.
## 31 APPENDIX: Address List

### 31.1 SIOP Europe Neuroblastoma Executive Committee

<table>
<thead>
<tr>
<th>Name</th>
<th>Contact Information</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BROCK, PENELlope, DR. PRESIDENT</strong></td>
<td>Dept. of Paediatric Oncology Great Ormond Street Hospital GB – London WC1N 3JN Phone: +44 207 829 8838/7924; Switchboard:+44-207 405 9200 Fax: +44-207-813-8588 Email: <a href="mailto:BrockP@gosh.nhs.uk">BrockP@gosh.nhs.uk</a></td>
<td>UK</td>
</tr>
<tr>
<td><strong>BECK-POPOVIC, MAJA, DR. SECRETARY</strong></td>
<td>Dept. of Paediatrics University Hospital (CHUV) Rue du Bugnon CH-1011 Lausanne Phone: +41-21-3143567 Fax: +41-21-3143332 Email: <a href="mailto:Maja.Beck-Popovic@chuv.ch">Maja.Beck-Popovic@chuv.ch</a></td>
<td>Switzerland</td>
</tr>
<tr>
<td><strong>LAUREYS, GENEViÈVE, DR MEMBER</strong></td>
<td>Kliniek voor Kinderziekten (Paediatric Haematology and Oncology) Universitair Ziekenhuis (University Hospital Gent) De Pintelaan 185 B - 9000 Gent Phone: +32-9-2402111 Fax: +32 9 240 3875 Email: <a href="mailto:genevieve.laureys@ugent.be">genevieve.laureys@ugent.be</a></td>
<td>Belgium</td>
</tr>
<tr>
<td><strong>VALTEAU-COUANET, DOMINIQUE, DR MEMBER</strong></td>
<td>Dept Paediatric Oncology Institut Gustave Roussy 39, Rue Camille Desmoulins F - Villejuif Cedex 94805 Phone: +33-1-42114170 Fax: +33 1 4211 5275 Email: <a href="mailto:dominique.valteau-couanet@igr.fr">dominique.valteau-couanet@igr.fr</a></td>
<td>France</td>
</tr>
<tr>
<td><strong>YANIV, ISSAC, DR MEMBER</strong></td>
<td>Dept. Paediatric Haematology/Oncology Schneider Children’s Medical Center of Israel Kaplan Street 14 IL - Petah Tikva 49202 Phone: +972-3-9253669 Fax: +972-3-9253042 Email: <a href="mailto:iyaniv@clalit.org.il">iyaniv@clalit.org.il</a></td>
<td>Israel</td>
</tr>
<tr>
<td>Name</td>
<td>Institution</td>
<td>Country</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Garaventa, Alberto, Dr.</td>
<td>Department of Pediatric Hematology/Oncology Giannina Gaslini Children's Hospital Largo Gerolamo Gaslini 5 16148 Genova Tel: +39-010-5636411 or 450 Fax: +39-010-3762322 Email: <a href="mailto:albertogaraventa@ospedale-gaslini.ge.it">albertogaraventa@ospedale-gaslini.ge.it</a></td>
<td>Italy</td>
</tr>
<tr>
<td>Beiske, Klaus, Dr., Assoc. Prof.</td>
<td>Dept. of Pathology Rikshospitalet Pilestredet 32 N - 0027 Oslo Phone:+47 23 07 14 00, +47 23 07 40 76 (office) Fax: +47 228 68 596 Email: <a href="mailto:klaus.beiske@labmed.uio.no">klaus.beiske@labmed.uio.no</a></td>
<td>Norway</td>
</tr>
<tr>
<td>Cañete, Adela, Dr.</td>
<td>Unidad De Oncologia Pediatrica Bloque G-2 Hospital Universitari I Politecnic La Fe Bulevar Sur S/N Valencia 46026 Phone: +34-96-12412314 or +34-96-1244904 Fax: +34-96-1246232 Email: <a href="mailto:canyete_ade@gva.es">canyete_ade@gva.es</a></td>
<td>Spain</td>
</tr>
<tr>
<td>Wheeler, Kate, Dr.</td>
<td>Consultant in Paediatric Oncology Oxford Children's Hospital Headley Way Oxford OX3 9DU Tel: +44 1 865 234 199 Fax: +44 1 865 234 211 Email: <a href="mailto:Kate.Wheeler@paediatrics.ox.ac.uk">Kate.Wheeler@paediatrics.ox.ac.uk</a></td>
<td>UK</td>
</tr>
<tr>
<td>Ladenstein, Ruth, Ass.Prof. Dr. Advisory Member</td>
<td>St. Anna Kinderkrebsforschung Zimmermannplatz 10 A - 1090 Vienna Tel: +43 1 40470 4750 Fax: +43 1 40470 7430 Email: <a href="mailto:ruth.ladenstein@ccri.at">ruth.ladenstein@ccri.at</a></td>
<td>Austria</td>
</tr>
<tr>
<td>Michon, Jean, Dr Advisory Member</td>
<td>Institute Curie Pediatric Oncology Department 26 rue d’Ulm 75248 Paris Cedex 05 Tel.: + 33-1-44-32-4550 Fax: + 33-1-53-10-4005 Email: <a href="mailto:jean.michon@curie.net">jean.michon@curie.net</a></td>
<td>France</td>
</tr>
<tr>
<td>Name</td>
<td>Position</td>
<td>Organization</td>
</tr>
<tr>
<td>------</td>
<td>----------</td>
<td>--------------</td>
</tr>
<tr>
<td>De Bernardi, Bruno, Dr.</td>
<td>Advisory Member – Former Chair</td>
<td>Dept. Haematology/ Oncology Giannina Gaslini Children’s Hospital Largo Gerolamo Gaslini 5 I - 16148 Genova Tel: +39-335-8248264 or 0039-010-5636464 Fax: +39 010 3762322 Email: <a href="mailto:brunodebernardi@ospedale-gaslini.ge.it">brunodebernardi@ospedale-gaslini.ge.it</a></td>
</tr>
<tr>
<td>Pearson, Andrew, Prof. Dr.</td>
<td>Advisory Member – Former Chair</td>
<td>Paediatric Department Institute of Cancer Research/ Royal Marsden Hospital 15 Cotswold Road UK - SUTTON SM5 2NG Phone: 0044-(0)20 8661 3453 Fax: 0044 (0)20 8661 3617 Email: <a href="mailto:andrew.pearson@icr.ac.uk">andrew.pearson@icr.ac.uk</a></td>
</tr>
<tr>
<td>Papadakis, Vassilios, Dr.</td>
<td>New Country Coordinator</td>
<td>Department of Paediatric Haematology-Oncology Athens General Paediatric Hospital AGHIA SOPHIA Thivon &amp; Livadias Ave. GR - Athens 11527 Tel: 0030-210 746 7158 (-7000 hospital) Fax: 00301-07797-649 Email: <a href="mailto:vpapadak@otenet.gr">vpapadak@otenet.gr</a></td>
</tr>
</tbody>
</table>

#### 31.2 Data Monitoring

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Organization</th>
<th>Country</th>
<th>Contact Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baruchel, Sylvain, Prof.</td>
<td></td>
<td>The Hospital for Sick Children Division of Haematology/Oncology 555 University Avenue Toronto, Ontario M5G 1X8 Tel: +1-416-813-7795 Email: <a href="mailto:sylvain.baruchel@sickkids.ca">sylvain.baruchel@sickkids.ca</a></td>
<td>Canada</td>
<td></td>
</tr>
<tr>
<td>Matthay, Kate K., Dr.</td>
<td></td>
<td>Dept. of Paediatrics University of California 505 Parnassus Avenue, M – 647 USA - San Francisco, CA 94143-0106 Tel: +1-415-476-0603 Email: <a href="mailto:matthayk@peds.ucsf.edu">matthayk@peds.ucsf.edu</a></td>
<td>USA</td>
<td></td>
</tr>
<tr>
<td>Valsecchi, Maria Grazia, Dr.</td>
<td></td>
<td>Section of Medical Statistics University of Milano-Bicocca I – 20052 Monza Tel: +39 039 233 3074 Email: <a href="mailto:grazia.valsecchi@unimib.it">grazia.valsecchi@unimib.it</a></td>
<td>Italy</td>
<td></td>
</tr>
</tbody>
</table>
## 31.3 National Co-Ordinators

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TRAHAIR, TOBY, DR.</strong></td>
<td>Centre for Children’s Cancer &amp; Blood Disorders</td>
<td>Australia</td>
</tr>
<tr>
<td></td>
<td>Sydney Children’s Hospital</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Randwick NSW 2031, High Street</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone: +61 2 9382 2970</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fax: + 61 2 9382 1789</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:Toby.Trahair@sesihs.health.nsw.gov.au">Toby.Trahair@sesihs.health.nsw.gov.au</a></td>
<td></td>
</tr>
<tr>
<td><strong>LADENSTEIN, RUTH, ASS.PROF. DR.</strong></td>
<td>St. Anna Kinderkrebsforschung</td>
<td>Austria</td>
</tr>
<tr>
<td></td>
<td>A - 1090 Vienna, Zimmermannplatz 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tel: +43 1 40470 4750</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fax: +43 1 40470 7430</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:ruth.ladenstein@ccri.at">ruth.ladenstein@ccri.at</a></td>
<td></td>
</tr>
<tr>
<td><strong>LAUREYS, GENEVIEVE, DR.</strong></td>
<td>Paediatric Haematology and Oncology</td>
<td>Belgium</td>
</tr>
<tr>
<td></td>
<td>University Hospital Gent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>De Pintelaan 185</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B - 9000 Gent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone: +32-9-2402111</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fax: +32 9 240 3875</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:genevieve.laureys@ugent.be">genevieve.laureys@ugent.be</a></td>
<td></td>
</tr>
<tr>
<td><strong>MALIS, JOSEF, DR.</strong></td>
<td>Dept. of Paediatric Oncology</td>
<td>Czech Republic</td>
</tr>
<tr>
<td></td>
<td>2nd Department of Paediatrics</td>
<td></td>
</tr>
<tr>
<td></td>
<td>University Hospital Motol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V Uvalu 84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CZ - Prague 5 150 06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone: +420-2244 36456</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fax: +420 224436420</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:josef.malis@lfmotol.cuni.cz">josef.malis@lfmotol.cuni.cz</a></td>
<td></td>
</tr>
<tr>
<td><strong>SCHRØDER, HENRIK, DR.</strong></td>
<td>Department of Paediatrics</td>
<td>Denmark</td>
</tr>
<tr>
<td></td>
<td>University Hospital of Aarhus, Skejby</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brendstrupgaardsvej 100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DK - 8200 Aarhus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone: +45-8-9496700</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fax: +45-8-9496023</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:hsa@sks.aaa.dk">hsa@sks.aaa.dk</a></td>
<td></td>
</tr>
<tr>
<td><strong>VETTENRANTA, KIM, DR.</strong></td>
<td>Chief, Division of Hematology-Oncology</td>
<td>Finland</td>
</tr>
<tr>
<td></td>
<td>Dept of Pediatrics</td>
<td></td>
</tr>
<tr>
<td></td>
<td>University of Tampere</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PO Box 2000, FIN-33521, Tampere Finland</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+358331164933</td>
<td></td>
</tr>
<tr>
<td></td>
<td><a href="mailto:kim.vettenranta@pshp.fi">kim.vettenranta@pshp.fi</a></td>
<td></td>
</tr>
<tr>
<td><strong>VALTEAU-COUANET, DOMINIQUE, DR.</strong></td>
<td>Dept Paediatric Oncology</td>
<td>France</td>
</tr>
<tr>
<td></td>
<td>Institut Gustave Roussy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>39, Rue Camille Desmoulins</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F - Villejuif Cedex 94805</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone: +33-1-42114170</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fax: +33 1 4211 5275</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:dominique.valteau-couanet@igr.fr">dominique.valteau-couanet@igr.fr</a></td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Institution</td>
<td>Country</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>------------------------------------------------------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td><strong>PAPADAKIS, VASSILIOS</strong></td>
<td>Athens General Paediatric Hospital AGHIA SOPHIA Department of Paediatric Haematology / Oncology Thivon &amp; Livadias Ave. GR- 11527 Athens Phone: +30 6938472600 Phone : +30 8041054 Fax: +30 8047639 Email: <a href="mailto:vpapadak@otenet.gr">vpapadak@otenet.gr</a></td>
<td>GREECE</td>
</tr>
<tr>
<td><strong>GARAMI, MIKLOS, ASS.PROF., DR.</strong></td>
<td>Division of Paediatric Oncology Semmelweis University Tüzoltó u. 7-9 H - Budapest 1094 Phone: +36 1 215 1380 Fax: +36 1 218 1381 Email: <a href="mailto:miklos.garami@gyer2.sote.hu">miklos.garami@gyer2.sote.hu</a></td>
<td>HUNGARY</td>
</tr>
<tr>
<td><strong>O’MEARA, ANNE, DR</strong></td>
<td>Our Lady’s Children’s Hospital, Crumlin Dublin 12 Phone: +353 1 409 6100/6654 Fax: +353 1 456 3041 Email: Anne.O’<a href="mailto:Meara@olchc.ie">Meara@olchc.ie</a></td>
<td>IRELAND</td>
</tr>
<tr>
<td><strong>YANIV, ISAAC, DR.</strong></td>
<td>Dept. Paediatric Haematology/Oncology Schneider Children’s Medical Center of Israel Kaplan Street 14 IL - Petah Tikva 49202 Phone: +972-3-9253669 Fax: +972-3-9253042 Email: <a href="mailto:iyaniv@clalit.org.il">iyaniv@clalit.org.il</a></td>
<td>ISRAEL</td>
</tr>
<tr>
<td><strong>LUKSCH, ROBERTO, DR</strong></td>
<td>Istituto Nazionale Tumori di Milano Unitá Operativa Pediatria Via Venezian 1 I - 20133 Milano Phone:+39-02-266462; 0039-02-23902 592/or 694 Email: <a href="mailto:roberto.luksch@istitutotumori.mi.it">roberto.luksch@istitutotumori.mi.it</a></td>
<td>ITALY</td>
</tr>
<tr>
<td><strong>RUUD, ELLEN, DR.</strong></td>
<td>Department of Paediatrics Rikshospitalet Pilestredet 32 N - Oslo 0027 Phone: +47 23 07 45 60 Email: <a href="mailto:ellen.ruud@rikshospitalet.no">ellen.ruud@rikshospitalet.no</a></td>
<td>NORWAY</td>
</tr>
<tr>
<td>Name</td>
<td>Department</td>
<td>Address</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Balwierz, Walentyna, Prof.</strong></td>
<td>Department of Paediatric Oncology and Haematology Polish-American Institute of Paediatrics, Jagiellonian University Medical College</td>
<td>Wielicka 265 PL - 30-663 Kraków Phone: +48 12 658 02 61 Fax: +48 12 658 02 61 Email: <a href="mailto:balwierz@mp.pl">balwierz@mp.pl</a></td>
</tr>
<tr>
<td><strong>Forjaz de Lacerda, Ana Maria, Dr.</strong></td>
<td>Dept. of Paediatrics Instituto Portugues De Oncologia De Francisco Gentil (Portuguese Institute of Oncology) Rua Professor Lima Basto P - Lisboa Codex 1099-023</td>
<td>Phone: +351-21-722-9800 Fax: +351-21-720-0417 Email: <a href="mailto:alacerda@ipolisboa.min-saude.pt">alacerda@ipolisboa.min-saude.pt</a></td>
</tr>
<tr>
<td><strong>Vujic, Dragana, Dr</strong></td>
<td>Mother and Child Healthcare Institute of Serbia ”Dr. Vukan Čupić“ Radoja Dakica 8 11070 Belgrade</td>
<td>Phone: +381-11-310-8227 Fax: +381-11-269-7232 Email: <a href="mailto:vujicd@yubc.net">vujicd@yubc.net</a></td>
</tr>
<tr>
<td><strong>Bician, Pavel, Dr</strong></td>
<td>Department of Paediatric Oncology F.D. Roosevelt University Hospital Nam.L.Svobodu 1 Banska Bystrica Slovakia</td>
<td>Phone: +421 48 4413361 or +421 907 808363 Fax: +421 48 4134813 Email: <a href="mailto:pbician@nspbb.sk">pbician@nspbb.sk</a></td>
</tr>
<tr>
<td><strong>Castel, Victoria, Dr</strong></td>
<td>Unidad De Oncologia Pediatrica Hospital Universitario Infantil La Fe Avenida Campanar 21 E - Valencia 46006</td>
<td>Phone:: +34-96-197.33.04 or +34-96.197.32.03 Fax: +34-96-3987727 or +34 96 3868 700 Email: <a href="mailto:castel_vic@gva.es">castel_vic@gva.es</a></td>
</tr>
<tr>
<td>Name</td>
<td>Affiliation</td>
<td>Country</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------------------------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Kogner, Per, Dr.</td>
<td>Childhood Cancer Research Unit, Barncancerforskningsenheten, Astrid Lindgren Children's Hospital, Q6:05 Karolinska Hospital SE - Stockholm 17176</td>
<td>Sweden</td>
</tr>
<tr>
<td>Beck-Popovic, Maja, Dr.</td>
<td>Dept. of Paediatrics, University Hospital (CHUV), Rue du Bugnon CH-1011 Lausanne</td>
<td>Switzerland</td>
</tr>
<tr>
<td>Brock, Penelope, Dr.</td>
<td>Dept. of Paediatric Oncology, Great Ormond Street Hospital GB – London WC1N 3JN</td>
<td>UK</td>
</tr>
</tbody>
</table>
## SIOOPEN COMMITTEES

### 31.4 BIOLOGY COMMITTEE

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AMBROS, INGE, DR.</strong></td>
<td>St. Anna Children’s Cancer Research Institute (CCRI)</td>
<td>AUSTRIA</td>
</tr>
<tr>
<td></td>
<td>Zimmermannplatz 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A - 1090 Vienna</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone: +43 1 40470 4210</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:inge.ambros@ccri.at">inge.ambros@ccri.at</a></td>
<td></td>
</tr>
<tr>
<td><strong>AMBROS, PETER, DR., ASS. PROF.</strong></td>
<td>St. Anna Children’s Cancer Research Institute (CCRI)</td>
<td>AUSTRIA</td>
</tr>
<tr>
<td></td>
<td>Zimmermannplatz 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A - 1090 Vienna</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone: +43 1 40170-4110</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:peter.ambros@ccri.at">peter.ambros@ccri.at</a></td>
<td></td>
</tr>
<tr>
<td><strong>SPELEMAN, FRANK, DR., PROF.</strong></td>
<td>Centre for Medical Genetics-OK5</td>
<td>BELGIUM</td>
</tr>
<tr>
<td></td>
<td>University of Ghent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>De Pintelaan 185</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B - 9000 Ghent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone: +32-9-240 2451</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fax: +32-9-240 49 70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:franki.speleman@ugent.be">franki.speleman@ugent.be</a></td>
<td></td>
</tr>
<tr>
<td><strong>VAN ROY, NADINE, DR., PROF.</strong></td>
<td>Centre for Medical Genetics-OK5</td>
<td>BELGIUM</td>
</tr>
<tr>
<td></td>
<td>University of Ghent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>De Pintelaan 185</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B - 9000 – Ghent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone: +32-9-240 55 18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fax: +32-9-240 49 70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:nadine.vanroy@ugent.be">nadine.vanroy@ugent.be</a></td>
<td></td>
</tr>
<tr>
<td><strong>VERMEULEN, JELLE, DR</strong></td>
<td>Centre for Medical Genetics-OK5</td>
<td>BELGIUM</td>
</tr>
<tr>
<td></td>
<td>University of Ghent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>De Pintelaan 185</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B - 9000 – Ghent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone: +32-9-240 55 18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fax: +32-9-240 49 70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:joelle.vermeulen@ugent.be">joelle.vermeulen@ugent.be</a></td>
<td></td>
</tr>
<tr>
<td><strong>VICHIA, ALES, DR</strong></td>
<td>Department of Paediatric Oncology University Hospital Motol</td>
<td>CZECH REPUBLIC</td>
</tr>
<tr>
<td></td>
<td>V Uvalu 84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CZ - 150 18 Praha 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone: +420 2 2443 6420</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:avicha@yahoo.com">avicha@yahoo.com</a></td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Institution</td>
<td>Phone</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>BENARD, JEAN, DR.</td>
<td>Laboratoire de Pharmacologie Clinique et Moleculaire</td>
<td>+33-142114818</td>
</tr>
<tr>
<td></td>
<td>Institut Gustave Roussy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>39, rue Camille Desmoulins</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F - Villejuif-Cedex</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone: +33-1 42115280</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fax: +33-1 42115280</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:benard@igr.fr">benard@igr.fr</a></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FRANCE</td>
<td></td>
</tr>
<tr>
<td>COMBARET, VALERIE, DR.</td>
<td>Immunologie-Centre Leon Berard</td>
<td>+33-4-78782828</td>
</tr>
<tr>
<td></td>
<td>28 rue Laennec</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F - 69373 Lyon Cedex 08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone: +33-4-78782828</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fax: +33-4-78782717</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:combaret@lyon.fnclcc.fr">combaret@lyon.fnclcc.fr</a></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FRANCE</td>
<td></td>
</tr>
<tr>
<td>COUTURIER, JEROME, DR.</td>
<td>Laboratoire de Genetique des Tumeurs</td>
<td>+33-1-4432-4216</td>
</tr>
<tr>
<td></td>
<td>Pavillon Trouilhet Rossignol, Institut Curie</td>
<td></td>
</tr>
<tr>
<td></td>
<td>26 rue d’Ulm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F - 75231 Paris Cedex 05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone: +33-1-4432-4216</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:jerome.couturier@curie.net">jerome.couturier@curie.net</a></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FRANCE</td>
<td></td>
</tr>
<tr>
<td>SCHLEIERMACHER, GUDRUN, DR</td>
<td>Institut Curie</td>
<td>+33 (0)1 56 24 45 50</td>
</tr>
<tr>
<td></td>
<td>26 rue d’Ulm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F - +33 (0)1 56 24 66 30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone: +33 (0)1 56 24 66 30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:gudrun.schleiermacher@curie.net">gudrun.schleiermacher@curie.net</a></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FRANCE</td>
<td></td>
</tr>
<tr>
<td>VALENT, ALEXANDER, DR.</td>
<td>Pathologie Moléculaire</td>
<td>+33 1 42 11 54 21</td>
</tr>
<tr>
<td></td>
<td>Institut Gustave Roussy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>39, rue C.Desmoulins</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F - 94805 Villejuif Cedex</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone: +33 1 42 11 54 21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:avalent@igr.fr">avalent@igr.fr</a></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FRANCE</td>
<td></td>
</tr>
<tr>
<td>JEISON, MARTA, DR.</td>
<td>Paediatric Haematology Oncology</td>
<td>+972-3-9253185</td>
</tr>
<tr>
<td></td>
<td>Schneider Children’s Medical Center of Israel</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL - 49202 Petah-Tikva</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone: +972-3-9253042</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fax: +972 3 9253042</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:martaj@clalit.org.il">martaj@clalit.org.il</a></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ISRAEL</td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Address</td>
<td>Country</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-------------------------------------------------------------------------</td>
<td>---------</td>
</tr>
</tbody>
</table>
| Defferrari, Raffaela  | U.O. Anatomia Patologica  
Istituto G.Gaslini  
Largo G. Gaslini, 5  
16147 Genova  
Italia  
Tel +39 010 5636210  
Fax +39 010 3075010  
RaffaellaDefferrari@ospedale-gaslini.ge.it | Italy   |
| Mazzocco, Katia       | U.O. Anatomia Patologica  
Istituto G.Gaslini  
Largo G. Gaslini, 5  
16147 Genova  
Italia  
Tel +39 010 5636210  
Fax +39 010 3075010  
Email: KatiaMazzocco@ospedale-gaslini.ge.it | Italy   |
| Tonini, Gian Paolo, Dr| Laboratory of Population Genetics  
National Institute for Cancer Research/CBA (IST)  
Advance Biotechnology Center  
I - Largo Benzi, 10  
16132 Genova  
Phone: +39-010-5737-463/ or-490/ or-430  
Fax: +39-010-5737-463  
Email: gianpaolo.tonini@istge.it | Italy   |
| Beiske, Klaus, Dr., Assoc. Prof. | Dept. of Pathology  
Rikshospitalet  
Pilestredet 32  
N - 0027 Oslo  
Phone:+47 23 07 14 00, +47 23 07 40 76  
(office)  
Fax: +47 228 68 596  
Email: klaus.beiske@labmed.uio.no | Norway  |
| Marques, Barbara, Dr. | Instituto Nacional de Saúde Dr. Ricardo Jorge, PI  
Genetics Department  
Avenida Padre Cruz  
1649-016 Lisboa  
Phone: +351-21-7526411  
Fax: +351-21-7526410  
Email: Barbara.Marques@insa.min-saude.pt | Portugal|
<table>
<thead>
<tr>
<th>Name</th>
<th>Address</th>
<th>Country</th>
</tr>
</thead>
</table>
| NOGUERA, ROSA, DR, PROF. | Departamento de patologia  
Facultat de medicina  
Avda Blasco Ibañez 17  
E - 46010 Valencia  
Phone: +34-96-386 41 46  
Fax: +34-96-386 41 73  
Email: Rosa.noguera@uv.es | SPAIN   |
| MARTINSSON, TOMMY, DR.   | Department of Clinical Genetics  
Gahlgrenska University Hospital/East  
Dept. of Clinical Genetics  
S-41685 Gothenburg  
Phone: +46-31-434-803  
Fax: +46-31-842 160  
Email: Tommy.Martinsson@clingen.gu.se | SWEDEN  |
| GROSS, NICOLE, DR        | Onco-Hematology Laboratory  
Pediatrics Department  
University Hospital CHUV  
CH - 1011 Lausanne  
Phone: +41-21-314-3622  
Fax: +41-21-314-3558  
Email: nicole.gross@chuv.hospvd.ch | SWITZERLAND |
| BOWN, NICK, DR.          | Cytogenetics Unit  
Institute for Human Genetics  
Central Parkway  
GB - Newcastle upon Tyne NE1 3BZ  
Phone: +44-191 241 8703  
Fax: +44 191 241 8713  
Email: nick.bown@ncl.ac.uk | UK |
| TWEEDLE, DEBBIE, DR      | Cytogenetics Unit  
Institute for Human Genetics  
Central Parkway  
GB - Newcastle upon Tyne NE1 3BZ  
Phone: +44-191 241 8703  
Fax: +44 191 241 8713  
Email: D.A.Tweedle@newcastle.ac.at | UK |
| LUNEC, JOHN, DR.         | Cancer Research Unit  
Medical School  
University of Newcastle upon Tyne  
Framlington Place  
UK - Newcastle upon Tyne NE2 4HH  
Phone: +44-191-222-8057 (Dept.office),  
+44 191 222 8532 (direct)  
Fax: +44-191-222-7556  
Email: john.lunec@newcastle.ac.at | UK |
<table>
<thead>
<tr>
<th>Name</th>
<th>Institution and Contact Information</th>
<th>Country</th>
</tr>
</thead>
</table>
| Ambros, Peter, Dr., Assoc. Prof. | St. Anna Children’s Cancer Research Institute (CCRI)  
Zimmermannplatz 10  
A - 1090 Vienna  
Phone: +43 1 40170-4110  
Email: peter.ambros@ccri.at | Austria     |
| Vicha, Ales, Dr.      | Department of Pediatric Oncology  
2nd Medical Faculty Charles University  
V Uvula 84  
CZ - 150 18 Prague 5  
Phone: +42 02 2443 6494  
Fax: +4202 2443 6420  
Email: avicha@yahoo.com | Czech Republic |
| Klijanienko, Jerzy, Dr. | Institut Curie  
26, Rue D’Ulm  
F - 75005 Paris Cedex 5  
Email: jerzy.klijanienko@curie.net | France      |
| Jeison, Marta, Dr.    | Pediatric Hematology/Oncology Department  
Cancer Cytogenetics Laboratory  
Schneider Children's Medical Center of Israel  
Kaplan 14  
IL – 49202 Petah-Tikva  
Phone: +972-3-9253185  
Fax: +972-3-9253042  
Email: martaj@clalit.org.il | Israel      |
| Sementa, Angela Rita, Dr. | Dept. of Pathology  
Giannina Gaslini Children's Hospital  
Largo Gaslini 5  
I – 16148 Genova  
Phone: +39-010-5636210  
Fax: +39-010-3776590  
Email: angelaritasementa@ospedale-gaslini.ge.it | Italy       |
| Beiske, Klaus, Dr.    | Dept. of Pathology  
Rikshospitalet  
Pilestredet 32  
N - 0027 Oslo  
Phone:+47 23 07 14 00, +47 23 07 40 76 (office)  
Fax: +47 228 68 596  
Email: klaus.beiske@labmed.uio.no | Norway      |
<table>
<thead>
<tr>
<th>Name</th>
<th>Address</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Balwierz, Walentyna, Ass. Prof. Dr</strong></td>
<td>Department of Paediatric Oncology and Haematology Polish-American Institute of Paediatrics, Jagiellonian University Medical College Wielicka 265 PL - 30-663 Kraków Phone: + 48 12 658 02 61 Fax: + 48 12 658 02 61 Email: <a href="mailto:balwierz@mp.pl">balwierz@mp.pl</a></td>
<td><strong>Poland</strong></td>
</tr>
<tr>
<td><strong>Gross, Nicole, Dr.</strong></td>
<td>Onco-Haematology Laboratory Paediatrics Department University Hospital (CHUV) rue du Bugnon CH – 1011 Lausanne Phone: +41 21 314 3622 Fax: +41 21 314 3664 Email: <a href="mailto:Nicole.gross@chuv.hospvd.ch">Nicole.gross@chuv.hospvd.ch</a></td>
<td><strong>Switzerland</strong></td>
</tr>
<tr>
<td><strong>Lammens Tim Dr</strong></td>
<td>Department of Pediatrics and Genetics Laboratorium of Haematology, 1P8, West Ghent University Hospital De Pintelaan 185 B - 9000-Ghent Email: <a href="mailto:tim.lammens@ugent.be">tim.lammens@ugent.be</a></td>
<td><strong>Belgium</strong></td>
</tr>
<tr>
<td><strong>Vicha, Ales, Dr.</strong></td>
<td>Department of Pediatric Oncology 2nd Medical Faculty Charles University V Uvula 84 CZ - 150 18 Prague 5 Phone: +42 02 2443 6494 Fax: +4202 2443 6420 Email: <a href="mailto:avicha@yahoo.com">avicha@yahoo.com</a></td>
<td><strong>Czech Republic</strong></td>
</tr>
<tr>
<td><strong>Tchirkov, Andrei, Dr.</strong></td>
<td>Département de Radiothérapie Centre Jean Perrin 58, rue Montalembert - B.P. 392 F - 63011 Clermont-Ferrand Cedex 1 Phone:+33 (0)4 73 27 81 42 Fax +33 (0)4 73 27 81 25 Email: <a href="mailto:Andrei.TCHIRKOV@cjp.fr">Andrei.TCHIRKOV@cjp.fr</a></td>
<td><strong>France</strong></td>
</tr>
<tr>
<td><strong>Corrias, Maria Valeria, Dr.</strong></td>
<td>Laboratorio di Oncologia Istituto G. Gaslini largo Gaslini 5 I - 16147 Genova Phone: +39 010 5636/342 or 524 Fax: +39-010-3779820 Email:<a href="mailto:mariavalerialcorrias@ospedale-gaslini.ge.it">mariavalerialcorrias@ospedale-gaslini.ge.it</a></td>
<td><strong>Italy</strong></td>
</tr>
</tbody>
</table>

**31.6 Molecular Monitoring Studies Committee**

<table>
<thead>
<tr>
<th>Name</th>
<th>Address</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Balwierz, Walentyna, Ass. Prof. Dr</strong></td>
<td>Department of Paediatric Oncology and Haematology Polish-American Institute of Paediatrics, Jagiellonian University Medical College Wielicka 265 PL - 30-663 Kraków Phone: + 48 12 658 02 61 Fax: + 48 12 658 02 61 Email: <a href="mailto:balwierz@mp.pl">balwierz@mp.pl</a></td>
<td><strong>Poland</strong></td>
</tr>
<tr>
<td><strong>Gross, Nicole, Dr.</strong></td>
<td>Onco-Haematology Laboratory Paediatrics Department University Hospital (CHUV) rue du Bugnon CH – 1011 Lausanne Phone: +41 21 314 3622 Fax: +41 21 314 3664 Email: <a href="mailto:Nicole.gross@chuv.hospvd.ch">Nicole.gross@chuv.hospvd.ch</a></td>
<td><strong>Switzerland</strong></td>
</tr>
<tr>
<td><strong>Lammens Tim Dr</strong></td>
<td>Department of Pediatrics and Genetics Laboratorium of Haematology, 1P8, West Ghent University Hospital De Pintelaan 185 B - 9000-Ghent Email: <a href="mailto:tim.lammens@ugent.be">tim.lammens@ugent.be</a></td>
<td><strong>Belgium</strong></td>
</tr>
<tr>
<td><strong>Vicha, Ales, Dr.</strong></td>
<td>Department of Pediatric Oncology 2nd Medical Faculty Charles University V Uvula 84 CZ - 150 18 Prague 5 Phone: +42 02 2443 6494 Fax: +4202 2443 6420 Email: <a href="mailto:avicha@yahoo.com">avicha@yahoo.com</a></td>
<td><strong>Czech Republic</strong></td>
</tr>
<tr>
<td><strong>Tchirkov, Andrei, Dr.</strong></td>
<td>Département de Radiothérapie Centre Jean Perrin 58, rue Montalembert - B.P. 392 F - 63011 Clermont-Ferrand Cedex 1 Phone:+33 (0)4 73 27 81 42 Fax +33 (0)4 73 27 81 25 Email: <a href="mailto:Andrei.TCHIRKOV@cjp.fr">Andrei.TCHIRKOV@cjp.fr</a></td>
<td><strong>France</strong></td>
</tr>
<tr>
<td><strong>Corrias, Maria Valeria, Dr.</strong></td>
<td>Laboratorio di Oncologia Istituto G. Gaslini largo Gaslini 5 I - 16147 Genova Phone: +39 010 5636/342 or 524 Fax: +39-010-3779820 Email:<a href="mailto:mariavalerialcorrias@ospedale-gaslini.ge.it">mariavalerialcorrias@ospedale-gaslini.ge.it</a></td>
<td><strong>Italy</strong></td>
</tr>
<tr>
<td>Name</td>
<td>Institution and Details</td>
<td>Country</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>----------------------------------------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td><strong>Viprey Virginie Dr</strong></td>
<td>Cancer Medicine Research Unit</td>
<td>UK</td>
</tr>
<tr>
<td></td>
<td>St. James University Hospital</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Beckett Street</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leeds- LS9 7TF UK</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone:+44-113-206 4922</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fax:+44-113-242 9886</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email:<a href="mailto:V.F.Viprey@leeds.ac.uk">V.F.Viprey@leeds.ac.uk</a></td>
<td></td>
</tr>
<tr>
<td><strong>Dallorso Sandro Dr</strong></td>
<td>Istituto G. Gaslini</td>
<td>ITALY</td>
</tr>
<tr>
<td></td>
<td>largo Gaslini 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I - 16147 Genova</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email:<a href="mailto:sandrodallorso@ospedale-gaslini.ge.it">sandrodallorso@ospedale-gaslini.ge.it</a></td>
<td></td>
</tr>
<tr>
<td><strong>Burchill, Sue, Professor.</strong></td>
<td>Cancer Medicine Research Unit</td>
<td>UK</td>
</tr>
<tr>
<td></td>
<td>St. James’s University Hospital</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Beckett Street</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leeds- LS9 7TF UK</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone:+44-113-206 5873</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fax:+44-113-242 9886</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email:<a href="mailto:S.A.Burchill@leeds.ac.uk">S.A.Burchill@leeds.ac.uk</a></td>
<td></td>
</tr>
</tbody>
</table>

### 31.7 IMMUNOTHERAPY COMMITTEE

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution and Details</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ladenstein, Ruth, Ass.-Prof., Dr.</strong></td>
<td>St. Anna Children’s Cancer Research Institute</td>
<td>AUSTRIA</td>
</tr>
<tr>
<td></td>
<td>Zimmermannplatz 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A - 1090 Vienna</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tel: +43 1 40470 4750</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fax: +43 1 40470 7430</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:ruth.ladenstein@ccri.at">ruth.ladenstein@ccri.at</a></td>
<td></td>
</tr>
<tr>
<td><strong>Verhaselt, Bruno, Dr.</strong></td>
<td>CMD UZ Gent, 2P8</td>
<td>BELGIUM</td>
</tr>
<tr>
<td></td>
<td>Ghent University Hospital</td>
<td></td>
</tr>
<tr>
<td></td>
<td>De Pintelaan 185</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B - 9000 Ghent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone: +32 9 240 37 00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fax: +32 9 240</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:bruno.verhasselt@ugent.be">bruno.verhasselt@ugent.be</a></td>
<td></td>
</tr>
<tr>
<td><strong>Valteau-Couanet, Dominique, Dr.</strong></td>
<td>Department of Paediatrics</td>
<td>FRANCE</td>
</tr>
<tr>
<td></td>
<td>Institut Gustave Roussy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>39, rue Camille Desmoulins</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F - 94805 Villejuif</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone: +33-1-42114170</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fax: +33 1 4211 5275</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:valteau@igr.fr">valteau@igr.fr</a></td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Address</td>
<td>Country</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td><strong>LODE, HOLGER, DR., PROF.</strong></td>
<td>Klinik für Allgemeine Pädiatrie und Pädiatrische Hämatologie und Onkologie Soldmannstr. 15 17475 Greifswald T: +49 3834 866301 / 6300 F: +49 3834 866410 Email: <a href="mailto:lode@greifswald.de">lode@greifswald.de</a> Mobil: +49 176 48 32 62 48</td>
<td>GERMANY</td>
</tr>
<tr>
<td><strong>NAGY, KALMAN, DR</strong></td>
<td>Child Health Centre Department of Pediatric Hematology and BMT Unit Szentpéteri kapu 76 H - 3501 Miskole Phone: +36 46 515252, +36 46 411 344 Fax: +36 46 411344 Email: <a href="mailto:igyek.kalman.nagy@axelero.hu">igyek.kalman.nagy@axelero.hu</a></td>
<td>HUNGARY</td>
</tr>
<tr>
<td><strong>GOLAN, CHANA, DR.</strong></td>
<td>Sheba Medical Center IL - Tel Hashomer Phone: +972-3-5303037 Fax: +972-3-5303031 Email:<a href="mailto:golan_il@netvision.net.il">golan_il@netvision.net.il</a></td>
<td>ISRAEL</td>
</tr>
<tr>
<td><strong>PISTOIA, VITO, DR.</strong></td>
<td>Giannina Gaslini Children's Hospital Laboratory of Oncology Largo Gaslini 5 I - 16145 Genova Phone: +39 010 56 36 342 Email: <a href="mailto:vitopiostaia@ospedale-gaslini.ge.it">vitopiostaia@ospedale-gaslini.ge.it</a></td>
<td>ITALY</td>
</tr>
<tr>
<td><strong>DŁUŻNIEWSKA AGNIESZKA, DR</strong></td>
<td>Department of Paediatric Oncologyand Haematology Polish-American Institute of Paediatrics, Jagiellonian University Medical College Wielicka 265 PL - 30-663 Kraków Tel: + 48 12 658 02 61 Fax: + 48 12 658 02 61 Email: <a href="mailto:balwierz@mp.pl">balwierz@mp.pl</a></td>
<td>POLAND</td>
</tr>
<tr>
<td><strong>DEMBOWSKA, BOŻENA, DR</strong></td>
<td>Department of Oncology The Children’s Memorial Health Institute Al. Dzieci Polskich 20 PL - 04-730 Warszawa Phone: + 48 22 815 38 53 Fax: + 48 22 815 75 75 Email: <a href="mailto:b-demb@czd.waw.pl">b-demb@czd.waw.pl</a></td>
<td>POLAND</td>
</tr>
<tr>
<td><strong>Cañete, Adela, Dr.</strong></td>
<td>Spain</td>
<td></td>
</tr>
<tr>
<td>------------------------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td><strong>University de Oncologia Pediatrica</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bloque G-2 Hospital Universitari I Politecnic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>La Fe Bulevar Sur S/N</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>46026 Valencia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Phone:</strong> +34 963 12412314, +34 96 1244904</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fax:</strong> +34 96-1246232</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Email:</strong> <a href="mailto:canyete_ade@gva.es">canyete_ade@gva.es</a></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Anderson, John, Dr.</strong></th>
<th>UK</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>University College London</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Senior Lecturer and Consultant Oncologist</strong></td>
<td></td>
</tr>
<tr>
<td><strong>University College London</strong></td>
<td></td>
</tr>
<tr>
<td><strong><a href="mailto:j.anderson@ich.ucl.ac">j.anderson@ich.ucl.ac</a>.</strong></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Abrahamsson, Jonas, Dr.</strong></th>
<th>Sweden</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Queen Silvia's Children's Hospital</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Sv/ostra</strong></td>
<td></td>
</tr>
<tr>
<td><strong>SE – 41685 Goteborg</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Phone:</strong> +31 3434100</td>
<td></td>
</tr>
<tr>
<td><strong>Fax:</strong> +31 215486</td>
<td></td>
</tr>
<tr>
<td><strong>Email:</strong> <a href="mailto:jonas.abrahamsson@vgregion.se">jonas.abrahamsson@vgregion.se</a></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>31.8 Nuclear Medicine and Physics Committee</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Boubaker, Ariane, Dr.</strong></td>
</tr>
<tr>
<td><strong>Sevice de Médecine Nucléaire BH-07</strong></td>
</tr>
<tr>
<td><strong>Centre Hospitalier Universitaire Vaudois</strong></td>
</tr>
<tr>
<td><strong>rue du Bougnon</strong></td>
</tr>
<tr>
<td><strong>CH - 1011 Lausanne</strong></td>
</tr>
<tr>
<td><strong>Phone:</strong> +41 21 131 44353</td>
</tr>
<tr>
<td><strong>Fax:</strong> +41 21 314 43 43</td>
</tr>
<tr>
<td><strong>Email:</strong> <a href="mailto:Ariane.Boubaker@chuv.hospvd.ch">Ariane.Boubaker@chuv.hospvd.ch</a></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Staudenherz, Anton, Dr</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>University Clinic for Nuclear Medicine</strong></td>
</tr>
<tr>
<td><strong>Währinger Gürtel 18-20</strong></td>
</tr>
<tr>
<td><strong>A-1090 Vienna</strong></td>
</tr>
<tr>
<td><strong>Phone:</strong> +43 1 40400 5550</td>
</tr>
<tr>
<td><strong>Fax:</strong> +43 1 40400 5552</td>
</tr>
<tr>
<td><strong>Email:</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Lambert, Bieke, Dr.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Department of Nuclear Medicine (P7)</strong></td>
</tr>
<tr>
<td><strong>University Hospital Ghent</strong></td>
</tr>
<tr>
<td><strong>De Pintelaan 185</strong></td>
</tr>
<tr>
<td><strong>B - 9000 Ghent</strong></td>
</tr>
<tr>
<td><strong>Phone:</strong> +32 9 240 38 07</td>
</tr>
<tr>
<td><strong>Fax:</strong> +32-9-240-3807</td>
</tr>
<tr>
<td><strong>Email:</strong> <a href="mailto:Bieke.Lambert@ugent.be">Bieke.Lambert@ugent.be</a></td>
</tr>
<tr>
<td>Name</td>
</tr>
<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td>KRIZOVA, HANA, DR</td>
</tr>
<tr>
<td>ESKILD-JENSEN, ANNI, DR.</td>
</tr>
<tr>
<td>GIAMARILLE, FRANCESCO, DR.</td>
</tr>
<tr>
<td>BAR-SEVER, ZVI, DR.</td>
</tr>
<tr>
<td>CASTELLANI, MARIA RITA, DR.</td>
</tr>
<tr>
<td>FJELD, JAN GUNNAR, DR.</td>
</tr>
<tr>
<td>Name</td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>Kaminska, Anna, Dr</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Colarinha, Paula, Dr</td>
</tr>
<tr>
<td>Mitjavila, Mercedes, Dra.</td>
</tr>
<tr>
<td>Jacobsson, Hans, Prof. Dr.</td>
</tr>
<tr>
<td>Cook, Gary, Dr.</td>
</tr>
<tr>
<td>Lorenzo Biassoni, Dr</td>
</tr>
<tr>
<td><strong>31.9 PATHOLOGY COMMITTEE</strong></td>
</tr>
<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td><strong>AMANN, GABRIELE, DR.</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>HEIMANN, PIERRE, DR.</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>SCIOT, RAF, MD</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>PETersen, Bodil LAUB, DR.</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>PEUCHMAUR, MICHEL, PROF.</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>PAJOR, LÁSZLO, PROF.</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
|**Tornoczky Tamás, Dr.**  | Institute of Pathology  
Faculty of Medicine  
University of Pécs  
Szigeti út 12  
H - 7601 Pécs  
Phone: +36 72 536621  
Fax: +36 72 536621  
Email: yst@pathology.pote.hu | **Hungary** |
|------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
|**Feinmesser, Meora, Dr.**  | Rabin Medical Center  
Beilinson Campus  
IL - Petah-Tikva 49100  
Phone: +972-3-9376098  
Fax: +972-3-9376186  
Email: raphael5@netvision.net.il | **Israel** |
|**D'Amore, Emanuele S.G., Dr.**  | Institute of Pathology  
Via Gabelli 61  
I – 35100 Padova  
Phone: +39-0444 753274  
Fax: +39 049 827 753273  
Email: emanuele.damore@unipd.it | **Italy** |
|**Gambini, Claudio, Dr.**  | Giannina Gaslini Children's Hospital  
Largo Gaslini 5  
I - 16148 Genova  
Phone: +39-010-5636210  
Fax: +39-010-3776590  
Email: claudio.gambini@ospedale-gaslini.ge.it | **Italy** |
|**Beiske, Klaus, Dr.**  | Dept. of Pathology  
Rikshospitalet  
Sognsvannsveien 20  
N - 0027 Oslo  
Phone: +47-23-071400 or office: +47-23-074076  
Fax: +47-23-071410  
Email: klaus.beiske@labmed.uio.no | **Norway** |
|**Klepacka, Teresa, Ass, Prof., Dr.**  | Pathomorphology Department  
National Research, Institute of Mother and Child  
Kasprzaka 17a  
PL - 01-211 Warszawa  
Phone: +48 22 632 12 81  
+ 48 22 632 81 68  
Email: pathology@imid.med.pl | **Poland** |
<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mieżyński, Witold Dr</strong></td>
<td>Department of Pathology&lt;br&gt;Polish-American Institute of Pediatrics,&lt;br&gt;Jagiellonian University Medical College&lt;br&gt;Wielicka 265&lt;br&gt;PL - 30-663 Kraków&lt;br&gt;Phone: +48 12 658 24 86&lt;br&gt;Fax: +48 12 658 17 56&lt;br&gt;Email: <a href="mailto:8849735@pharmanet.com.pl">8849735@pharmanet.com.pl</a></td>
<td>Poland</td>
</tr>
<tr>
<td><strong>Kluge, Przemysław Dr</strong></td>
<td>Department of Pathology&lt;br&gt;The Children’s Memorial Health Institute&lt;br&gt;Al. Dzieci Polskich 20&lt;br&gt;PL - 04-730 Warszawa&lt;br&gt;Phone: +48 22 815 19 60&lt;br&gt;Fax: +48 22 815 27 32&lt;br&gt;Email: <a href="mailto:przeklug@czd.waw.pl">przeklug@czd.waw.pl</a></td>
<td>Poland</td>
</tr>
<tr>
<td><strong>Lemos, Maria Manuel, Dr.</strong></td>
<td>Dept. of Pathology&lt;br&gt;Instituto Portugues De Oncologia&lt;br&gt;De Francisco Gentil (Portuguese Institute of Oncology)&lt;br&gt;Rua Professor Lima Basto&lt;br&gt;PT - 1099-023 Lisboa Codex&lt;br&gt;Email: <a href="mailto:mlemos@ipolisboa.min-saude.pt">mlemos@ipolisboa.min-saude.pt</a></td>
<td>Portugal</td>
</tr>
<tr>
<td><strong>Mendonca, Eveline, Dr.</strong></td>
<td>Dept. of Pathology&lt;br&gt;Instituto Portugues De Oncologia&lt;br&gt;De Francisco Gentil (Portuguese Institute of Oncology)&lt;br&gt;Rua Professor Lima Basto&lt;br&gt;PT - 1099-023 Lisboa Codex&lt;br&gt;Email: <a href="mailto:emendonca@ipolisboa.min-saude.pt">emendonca@ipolisboa.min-saude.pt</a></td>
<td>Portugal</td>
</tr>
<tr>
<td><strong>Navarro, Samuel, Dr.</strong></td>
<td>Departamento de patologia&lt;br&gt;Facultad de medicina&lt;br&gt;Avda. Blasco Ibañez 17&lt;br&gt;E - 46010 Valencia&lt;br&gt;Phone: +34 96 3864 146&lt;br&gt;Fax: +34 96 3864 173&lt;br&gt;Email: <a href="mailto:Samuel.navarro@uv.es">Samuel.navarro@uv.es</a></td>
<td>Spain</td>
</tr>
<tr>
<td><strong>Sandstedt, Bengt, Dr.</strong></td>
<td>Dept. of Paediatric Pathology&lt;br&gt;Karolinska Hospital&lt;br&gt;PO Box 100&lt;br&gt;SE - 17176 Stockholm&lt;br&gt;Phone: +46-87296162&lt;br&gt;Fax: + 46 8 729 6165&lt;br&gt;Email: <a href="mailto:Bengt.Sandstedt@pat.ds.sll.se">Bengt.Sandstedt@pat.ds.sll.se</a></td>
<td>Sweden</td>
</tr>
</tbody>
</table>
| MEAGHER-VILLEMURE, KATHLEEN, DR. | University Hospital (CHUV)  
rue du Bugnon  
CH - 1011 Lausanne  
Phone: +41 21 314 7162  
Fax: +41 21 314 7115  
Email: Kathleen.Meagher-Villemure@chuv.hospvd.ch | SWITZERLAND |
|-------------------------------|---------------------------------|---------------|
| CULLINANE, CATHERINE, DR, FCRPATH | Pathology Dpt.  
St. James's University Hospital  
Beckett Street  
UK - Leeds LS9 7TF  
Phone: +44 113 2065432, +44 113 2065900  
Email: ccullinane@doctors.org.uk | UK |
| VERSTRAETE, ALAIN, DR | Laboratorium voor klinische biologie  
De Pintelaan 185  
BE - 9000 Gent  
Phone: +32 9 240 34 07  
Email: alain.verstraete@ugent.be | BELGIUM |
| BOULIGAND, JEROME, DR | UPRES EA3535  
Pharmacologie et nouveaux traitements des cancers  
Institut Gustave Roussy  
39 Rue Camille Desmoulins  
F - 94805 Villejuif  
Phone : +33-1-42 11 49 80  
Fax : +33 1 42 11 53 08  
Email : jboulig@igr.fr | FRANCE |
| CHATELUT, ETIENNE, DR | Unité de Pharmacocinétique  
Institut Claudius Regaud  
F – 31052 Toulouse  
Phone: +33-5 61 42 42 71  
Fax: +33-5-61 42 46 31  
Email: Chatelut@jcr.fnclcc.fr | FRANCE |
| DOZ, FRANCOIS, DR | Department of Pharmacology  
Institut Curie  
26, Rue D'Ulm  
F – 75005 Paris Cedex 5  
Phone: +33-1 44324550  
Fax: +33-1-44324005  
Email: francois.doz@curie.net | FRANCE |
| **PACI, ANGELO, DR** | UPRES EA3535  
Pharmacologie et nouveaux traitements des cancers  
Institut Gustave Roussy  
39 Rue Camille Desmoulins  
F - 94805 Villejuif  
Phone: +33-1-42 11 47 30  
Email: apaci@igr.fr | **FRANCE** |
|----------------------|---------------------------------------------------|---------|
| **VASSAL, GILLES, PROF. DR.** | Department of Pharmacology  
Institut Gustave Roussy  
39 Rue Camille Desmoulins  
F – 94805 Villejuif  
Phone: +33-1-4211-4947 (Lab), +33-1-4211-4622 (Paediatric Dpt.)  
Fax: +33-1-4211-5308 (Lab), +33-1-4211-5275 (Paed. Dpt.)  
Email: gvassal@igr.fr | **FRANCE** |
| **KITRA, VASSILIKI, DR.** | BMT Unit  
St. Sophia Children's Hospital  
Thivon and Levadias  
GR - 11527 Athens  
Phone: 0031210 7467303/4  
Fax: 0031 210 7778822  
Email : paedbmt@hellasnet.gr | **GREECE** |
| **BEN ARUSH, MYRIAM, DR** | Paediatric Haematology-Oncology Department  
Meyer Children’s Hospital of Haifa  
Rambam Medical Center  
P.O.Box 9602  
IL - Haifa 31096  
Phone: +972-4-8542502  
Fax: +972-3-8542007  
Email: m_benarush@rambam.health.gov.il | **ISRAEL** |
| **KRIVOV, NORBERTO, DR** | Medicine B and Clinical Pharmacology Unit  
Faculty of Medicine  
Rambam Medical Center  
P.O.Box 9602  
IL - 31096 Haifa  
Phone: +972 4 8542676  
Fax: +972 4 543252  
Email: n_krivoy@rambam.health.gov.il | **ISRAEL** |
<table>
<thead>
<tr>
<th>Name</th>
<th>Institution and Contact Information</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BERGAN, STEIN, DR</strong></td>
<td>Department of Medical Biochemistry Rikshospitalet University Hospital NO - 0027 Oslo Phone: +47 23071082 Fax: +47 23071080 Email: <a href="mailto:stein.bergan@rikshospitalet.no">stein.bergan@rikshospitalet.no</a></td>
<td>NORWAY</td>
</tr>
<tr>
<td><strong>PERIS, JOSÉ-ESTEBAN, DR</strong></td>
<td>Department of Pharmacy and Pharmaceutical Technology Faculty of Pharmacy University of Valencia Avda. Andres Estelles, s/n. E – 46100 Burjassot Phone: +34 96 386 49 14 Fax: +34 96 386 49 11 Email: <a href="mailto:jose.e.peris@uv.es">jose.e.peris@uv.es</a></td>
<td>SPAIN</td>
</tr>
<tr>
<td><strong>HASSAN, MOUSTAPHA, DR.</strong></td>
<td>Laboratory of Haematology Huddinge University Hospital KFC, Novum, 5th Floor S - 141 86 Stockholm Phone: +46-8-585 838 62 (Office), +46-8-585-82676 (Laboratory) Fax: +46-8-58583810 Email: <a href="mailto:Moustapha.Hassan@medhs.ki.se">Moustapha.Hassan@medhs.ki.se</a></td>
<td>SWEDEN</td>
</tr>
<tr>
<td><strong>BODDY, ALAN, DR.</strong></td>
<td>Northern Institute for Cancer Research Paul O’Gorman Building Medical School Framlington Place GB - NE2 4HH Newcastle upon Tyne Phone: +44-191 246-4412 Fax: +44 191 246 4301 Email: <a href="mailto:alan.boddy@newcastle.ac.uk">alan.boddy@newcastle.ac.uk</a></td>
<td>UK</td>
</tr>
<tr>
<td><strong>VEAL, GARETH, J. DR.</strong></td>
<td>Northern Institute for Cancer Research Paul O’Gorman Building Medical School Framlington Place GB - NE2 4HH Newcastle upon Tyne Phone: +44-191 246-4332 Fax: +44 191 246 4301 Email: <a href="mailto:G.J.Veal@newcastle.ac.uk">G.J.Veal@newcastle.ac.uk</a></td>
<td>UK</td>
</tr>
<tr>
<td>Name</td>
<td>Institution and Details</td>
<td>Country</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-----------------------------------------------------------------------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Hörmann, Marcus, Dr.</td>
<td>Dept. of Radiology&lt;br&gt;University of Vienna&lt;br&gt;Währinger Gürtel 18-20&lt;br&gt;A – 1090 Vienna&lt;br&gt;Phone: +43 1 40400-7620/ or-4891&lt;br&gt;Email: <a href="mailto:marcus.hoermann@univie.ac.at">marcus.hoermann@univie.ac.at</a></td>
<td>Austria</td>
</tr>
<tr>
<td>Clapuyt, Philippe, Dr.</td>
<td>Imagerie médicale&lt;br&gt;Cliniques universitaires St. Luc&lt;br&gt;Avenue Hippocrate 10/2972&lt;br&gt;B – 1200 Bruxelles&lt;br&gt;Phone: +32-2-764 2974&lt;br&gt;Fax: +32-2-7705574&lt;br&gt;Email: <a href="mailto:clapuyt@rdgn.ucl.ac.be">clapuyt@rdgn.ucl.ac.be</a></td>
<td>Belgium</td>
</tr>
<tr>
<td>Avni, Fred, Dr.</td>
<td>Hôpital Erasme&lt;br&gt;Route de Lennik, 808&lt;br&gt;BE - 1070 Brussels&lt;br&gt;Email: <a href="mailto:favni@ulb.ac.be">favni@ulb.ac.be</a></td>
<td>Belgium</td>
</tr>
<tr>
<td>Meerschaut, Valérie, Dr.</td>
<td>University of Gent&lt;br&gt;De Pintelaan 185&lt;br&gt;B – 9000 Gent&lt;br&gt;Belgium&lt;br&gt;Phone: +32 9 240 43 72&lt;br&gt;Fax: +32 9 240 49 77&lt;br&gt;Email: <a href="mailto:Valerie.meerschaut@ugent.be">Valerie.meerschaut@ugent.be</a></td>
<td>Belgium</td>
</tr>
<tr>
<td>Cumlivska, Eliska, Dr.</td>
<td>University Hospital Motol&lt;br&gt;150 18 Praha 5&lt;br&gt;Email: <a href="mailto:eliska.cumlivska@lfmotol.cuni.cz">eliska.cumlivska@lfmotol.cuni.cz</a></td>
<td>Czech Republic</td>
</tr>
<tr>
<td>Brisse, Hervé, Dr.</td>
<td>Institut Curie&lt;br&gt;26 Rue D’Ulm&lt;br&gt;F – 7548 Paris&lt;br&gt;Email: <a href="mailto:herve.brisse@curie.net">herve.brisse@curie.net</a></td>
<td>France</td>
</tr>
<tr>
<td>Benz-Bohm, Gabriele, Prof.Dr.</td>
<td>Paediatric Radiology&lt;br&gt;University Clinic for Paediatrics&lt;br&gt;Joseph-Stelzmann.Str. 9&lt;br&gt;D – 50933 Köln&lt;br&gt;Phone: +49 221 478 4228&lt;br&gt;Fax: +49 221 478 3347&lt;br&gt;Email: <a href="mailto:gabriele.benz-bohm@medizin.uni-koeln.de">gabriele.benz-bohm@medizin.uni-koeln.de</a></td>
<td>Germany</td>
</tr>
</tbody>
</table>
| **LOMBAY, BÉLA, DR** | Department of Paediatric Radiology  
Child Health Centre  
Szentpéteri kapu 76  
H - 3501 Miskolc  
Phone: +36 46 515230  
Email: dr.lombaysr@axelero.hu | **HUNGARY** |
| **WEISENBACH, JÁNOS, DR** | University of Pécs  
Department of Paediatrics  
Division of Radiology  
Attila u. 7  
H – 7623 Pécs  
Phone: +36 72 535900  
Fax: +36 72 535971  
Email: weisenbachjanos@hotmail.com | **HUNGARY** |
| **DONOGHUE, VERONICA, DR.** | Radiology Department  
Paediatric Radiology  
Children's University Hospital  
Temple Street  
Dublin 1  
Phone: +353-1-8784264/8784264  
Fax: +353-1-8748355  
Email: veronica.donoghue@tsch.ie | **IRELAND** |
| **KORNREICH, LEORA, DR.** | Imaging Department  
Schneider Children's Medical Center of Israel  
IL - 49202 Petah Tikva  
Phone: +972 3 9253036  
Fax:972 3 972 3 9253618  
Email: korn@netvision.net.il | **ISRAEL** |
| **GIAN MICHELE MAGNANO, DR.** | Department of Radiology  
Giannina Gaslini Children’s Hospital  
Largo Gaslini 5  
I - 16148 Genova - Quarto  
Phone: +39-010-5636 531  
Fax: +39-010-385599  
Email: magnano@panet.it | **ITALY** |
| **TOMA, PAOLO, DR.** | Service of Radiology  
Giannina Gaslini Children’s Hospital  
Largo Gaslini 5  
16148 Genova  
Email: paolotoma@ospedale-gaslini.ge.it | **ITALY** |
<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
<th>Country</th>
</tr>
</thead>
</table>
| SMEVIK, BJARNE, DR          | Rikshospitalet  
Sognsvannsveien 20  
NO - 0027 Oslo  
Phone : +47 23073383  
Fax : + 47 23073754  
Email : bjarne.smevik@rikshospitalet.no | NORWAY      |
| WYROBEK, ŁUKASZ, DR         | Department of Clinical Radiology  
University Children’s Hospital of Krakow  
Wielicka 265  
PL - 30-663 Kraków  
Phone: + 48 12 658 20 11  
Email: lukaszwyrobek@interia.pl | POLAND      |
| JURKIEWICZ, ELŻBIETA, DR    | Department of Radiology  
The Children’s Memorial Health Institute  
Al. Dzieci Polskich 20  
PL - 04-730 Warszawa  
Phone: + 48 22 815 42 40  
Fax: + 48 22 815 42 40  
Email: elajur@hotmail.com | POLAND      |
| SILVA, JOÃO PAULO CONCEIÇAO E, DR | Instituto Portugues De Oncologia De Francisco Gentil (Portuguese Institute of Oncology)  
Rua Professor Lima Basto  
P - 1099-023 Lisboa Codex  
Email: csilvaj@sapo.pt | PORTUGAL    |
| MUDO, M. DOLORES, DR        | Servicio de Radiologia Infantil  
Hospital Infantil La Fe  
Avda de Campanar 21  
E - 46009 Valencia  
Phone: +34 963868772  
Fax: +34 963868773  
Email: mpal@servitel.es | SPAIN       |
| JACOBSSON, HANS, PROF. DR   | Department of Radiology  
Karolinska Hospital  
SE - 17176 Stockholm  
Phone: +46 8 517 73581  
Fax: +46-8517-74939 | SWEDEN      |
<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Address</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vergesslich, Klara, Prof. Dr.</td>
<td>Dept. of Paediatric Radiology</td>
<td>University Children's Hospital Römergasse 8 CH - 4058 Basel</td>
<td>Switzerland</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phone: +41 61 685 6282 Fax: +41 61 685 6565 Email: <a href="mailto:klara.vergesslich@unibas.ch">klara.vergesslich@unibas.ch</a></td>
<td></td>
</tr>
<tr>
<td>McHugh, Kieran, Dr.</td>
<td>Great Ormond Street Hospital</td>
<td>Great Ormond Street UK - London WC1N 3JN</td>
<td>UK</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Email: <a href="mailto:mchugk@gosh.nhs.uk">mchugk@gosh.nhs.uk</a></td>
<td></td>
</tr>
<tr>
<td>Dickinson, Fiona, Dr.</td>
<td>Department of Radiology</td>
<td>Leicester Royal Infirmary Infirmary Close UK – Leicester LE1 5WW</td>
<td>UK</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phone: +44-116-258-6461 Email: <a href="mailto:fiona.dickinson@uhl-tr.nhs.uk">fiona.dickinson@uhl-tr.nhs.uk</a></td>
<td></td>
</tr>
<tr>
<td>31.12 Radiotherapy Committee</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dieckmann, Karin, Dr.</td>
<td>Univ.Klinik f. Strahlentherapie und Strahlenbiologie</td>
<td>Währinger Gürtel 18-20 A - 1180 Vienna Phone: +43-1-40400 2665 Fax: +43-1-40400 2693 Email: <a href="mailto:karin.dieckmann@meduniwien.ac.at">karin.dieckmann@meduniwien.ac.at</a></td>
<td>Austria</td>
</tr>
<tr>
<td>Potter, Richard, Prof. Dr.</td>
<td>Univ.Klinik f. Strahlentherapie und Strahlenbiologie</td>
<td>Währinger Gürtel 18-20 A - 1180 Vienna Phone: +43-1-40400 2694 Fax: +43-1-40400 2693 Email: <a href="mailto:Richard.Poetter@meduniwien.ac.at">Richard.Poetter@meduniwien.ac.at</a></td>
<td>Austria</td>
</tr>
<tr>
<td>Boterberg, Tom, Dr</td>
<td>Department of Radiotherapy-Oncology</td>
<td>University Hospital De Pintelaan 185 B - 9000 Ghent Phone: +32 9 240 30 15 Fax: +32 9 240 30 40 Email: <a href="mailto:tom@krtkg1.ugent.be">tom@krtkg1.ugent.be</a></td>
<td>Belgium</td>
</tr>
<tr>
<td>Name</td>
<td>Institution and Contact Information</td>
<td>Country</td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>--------------------------------------------------------------------------------------------------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>MALINOVA, BELA, DR.</td>
<td>Department of Radiotherapy and Oncology University Hospital Motol V. Uvalu 84 150 18 Praha 5 Phone: +42 0 224434710 Fax: +42 0 224434764 Email: <a href="mailto:bela.malinova@fnmotol.cz">bela.malinova@fnmotol.cz</a>; <a href="mailto:bela.malinova@lfmotol.cuni.cz">bela.malinova@lfmotol.cuni.cz</a></td>
<td>CZECH REPUBLIC</td>
<td></td>
</tr>
<tr>
<td>HABRAND, JEAN LOUIS, PROF.</td>
<td>Service de Radiothérapie Institut Gustave Roussy 39, rue C. Desmoulins F - 94800 Villejuif Phone: +33 1 42 11 49 95 Email: <a href="mailto:habrand@igr.fr">habrand@igr.fr</a></td>
<td>FRANCE</td>
<td></td>
</tr>
<tr>
<td>HELFRE, SYLVIE, DR</td>
<td>Service de Radiothérapie B Institut Curie 26 rue d’Ulm F – 7548 Paris Phone: +33-1 44324636 Fax: +33-144-324616 Email: <a href="mailto:sylvie.helfre@curie.net">sylvie.helfre@curie.net</a></td>
<td>FRANCE</td>
<td></td>
</tr>
<tr>
<td>KOCSIS, BÉLA, DR</td>
<td>National Institute of Oncology XII. Ráth György u. 7-9 Pf 21 H - 1525 Budapest Phone: +36 1 2248600 Fax: +36 1 2248620</td>
<td>HUNGARY</td>
<td></td>
</tr>
<tr>
<td>PEYLAN-RAMU, NILI, DR.</td>
<td>Department of Paediatric Haematology Oncology Hadassah Hospital IL - Jerusalem Phone: +972-2-6776724 Fax: +972-2-6431259 Email:<a href="mailto:ramu@hadassah.org.il">ramu@hadassah.org.il</a></td>
<td>ISRAEL</td>
<td></td>
</tr>
<tr>
<td>GANDOLA LORENZA, DR.SSA</td>
<td>Istituto Nazionale Tumori Dip.di Radioterapia Via Venezian 1 I - 20133 Milano Tel: +39-02-23902471 Fax: +39-02-2665605, +39-010-3367874 Email: <a href="mailto:gandola@istitutotumori.mi.it">gandola@istitutotumori.mi.it</a></td>
<td>ITALY</td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Position</td>
<td>Institution</td>
<td>Address</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-----------------------------------</td>
<td>--------------------------------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td><strong>TOMA, PAOLO, DR.</strong></td>
<td>Service of Radiology</td>
<td>Giannina Gaslini Children’s Hospital</td>
<td>Largo Gaslini 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16148</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Email: <a href="mailto:paolotoma@ospedale-gaslini.ge.it">paolotoma@ospedale-gaslini.ge.it</a></td>
</tr>
<tr>
<td><strong>SALVINA, BARRA, DR.</strong></td>
<td>Service of Radiotherapy</td>
<td>National Cancer Research Institute</td>
<td>Viale Benedetto XV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 - 16132</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phone: +39 010 5600014</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fax: +39 010 5600014</td>
</tr>
<tr>
<td><strong>OLDENBURGER, FOPPE, DR.</strong></td>
<td>Academic Medical Center</td>
<td>Dept. of Radiotherapy</td>
<td>Meibergdreef 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NL - 1105</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phone: +31 20 5664231</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fax: +31 20 6091278</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Email: <a href="mailto:F.Oldenburger@amc.uva.nl">F.Oldenburger@amc.uva.nl</a></td>
</tr>
<tr>
<td><strong>LOTE, KNUT, DR.</strong></td>
<td>Radiumhospital</td>
<td>Sognsvannsveien 20</td>
<td>0027</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phone: +47 2293 4000'</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fax: +47 2252 5559</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Email: <a href="mailto:knut.lote@radiumhospitalet.no">knut.lote@radiumhospitalet.no</a></td>
</tr>
<tr>
<td><strong>SKOWROŃSKA-GARDAS, ANNA ASS. PROF., DR</strong></td>
<td>Radiotherapy Department</td>
<td>Center of Oncology</td>
<td>Wawelska 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PL - 00-973</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phone: +48 22 822 54 51</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fax: +48 22 822 24 29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Email: <a href="mailto:gardas@cmkp.edu.pl">gardas@cmkp.edu.pl</a></td>
</tr>
<tr>
<td><strong>KORAB-CHRZANOWSKA, ELŻBIETA, DR</strong></td>
<td>Department of Radiotherapy</td>
<td>University Children’s Hospital of Krakow</td>
<td>Wielicka 265</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PL - 30-663</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phone: +48 12 657 37 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Email: <a href="mailto:elkorchrzanowska@poczta.onet.pl">elkorchrzanowska@poczta.onet.pl</a></td>
</tr>
</tbody>
</table>
| BADAL, DOLORES, DR | Servicio de Radioterapia  
Hospital Universitario La Fe  
Avda Campanar 21  
E - 46009 Valencia  
Phone:+34 963862700 Ext.:50445  
Fax: +34-963-868-789  
Email: lbadal@ctv.es | SPAIN |
|-------------------|--------------------------------------------------------------------------------|-------|
| BJÖRK-ERIKSSON, THOMAS, DR. | Department of Oncology  
Sahlgrenska University Hospital  
SE -413 45 Gothenberg  
Phone: +46 313 421000  
Fax: +46 313 820114  
Email: thomas.bjork-eriksson@oncology.gu.se | SWEDEN |
| HJELM-SKOG, ANNA LENA, DR. | Karolinska University Hospital Solna  
SE – 17176 Stockholm  
Phone: +46 966 1935  
Fax: +46 51772501  
Email : anna-lena.hjelm-skog@karolinska.se | SWEDEN |
| GAZE, MARK, DR. | University College Hospital  
First Floor Central  
250 Euston Road  
London  
NW1 2PQ  
Phone: +44-20-7380-9090  
Email: mark.gaze@uclh.nhs.uk | UK |

<table>
<thead>
<tr>
<th>31.13 STATISTICAL COMMITTEE</th>
</tr>
</thead>
</table>

| PÖTSCHGER, ULRIKE, M.SC. | St. Anna Children’s Cancer Research Institute  
Zimmermannplatz 10  
A - 1090 Vienna  
Phone: +43-1-40470-4770  
Fax: +43-1-40470-7430  
Email: ulrike.poetschger@ccri.at | AUSTRIA |
|--------------------------|-------------------------------------------------------------------------------------------------|-------|
| WHEATLEY, KEITH | Cancer Research UK Clinical Trials Unit (CRCTU)  
School of Cancer Sciences  
University of Birmingham  
Edgbaston  
Birmingham B15 2TT  
Tel: +44 (0) 121 415 1049  
Fax: +44 (0) 121 414 3700  
Email: k.wheatley@bham.ac.uk | UK |
| MOSSERI, VÉRONIQUE, DR. | Institut Curie  
26 rue D’Ulm  
F - 75248 PARIS CEDEX 5  
Phone: +33 1 4432-4665  
Fax: +33-1-44-32-40-78  
Email: veronique.mosseri@curie.net | FRANCE |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>31.14 STEM CELL COMMITTEE</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| WITT, VOLKER, DR. | St. Anna Children’s Hospital  
Zimmermannplatz 10  
A - 1090 Vienna  
Phone: +43 1 40170-325  
Fax: +43-1-40170-752  
Email: witt@stanna.at | AUSTRIA |
| VAN HAUTE, INGE, DR. | BTC Oost-Vlaanderen  
Ottergemsesteenweg 413  
B -9000 Ghent  
Phone: +32-92445656  
Fax: +32-92445664  
Email: Inge.Vanhaute@rodekruis.be | BELGIUM |
| BOCCACCIO, CATHERINE, DR. | Cellular Therapy Unit  
Institut Gustave Roussy  
11 rue Camille Desmoulins  
F - 94805 Villejuif Cedex  
Phone: 33142115148  
Fax: 33142115303  
Email: boccacio@igr.fr | FRANCE |
| MICHON, JEAN, DR. | Dept. of Paediatric Oncology  
Institut Curie  
26 Rue D’Ulm  
75248 Paris Cedex 5  
Phone: +33-1-44324558  
Fax: +33-1-44324005  
Email: jean.michon@curie.net | FRANCE |
| NAGY, KALMAN, DR | Child Health Centre  
Department of Pediatric Hematology and BMT Unit  
Szentpéteri kapu 76  
H - 3501 Miskolc  
Phone: +36 46 515252, +36 46 411 344  
Fax: +36 46 411344  
Email: igyek.kalman.nagy@axelero.hu | HUNGARY |
<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Institution</th>
<th>Phone</th>
<th>Fax</th>
<th>Email</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kriván, Gergely, DR</td>
<td>BMT Unit</td>
<td>St. László Hospital</td>
<td>+36 1-455 8100</td>
<td>+36 1-455 8200</td>
<td><a href="mailto:krivang@hu.inter.net">krivang@hu.inter.net</a></td>
<td>Hungary</td>
</tr>
<tr>
<td>Yaniv, Isaac, Dr.</td>
<td>Dept. Paediatric Haematology/Oncology</td>
<td>Schneider Children’s Medical Center of Israel</td>
<td>+972-3-9253669</td>
<td>+972-3-9253042</td>
<td><a href="mailto:iyaniv@clalit.org.il">iyaniv@clalit.org.il</a></td>
<td>Israel</td>
</tr>
<tr>
<td>Kapelushnik, Yosi, Dr.</td>
<td>Paediatric Haematology Oncology</td>
<td>Soroka Medical Center</td>
<td>+972-8-6405931</td>
<td>+972-8-6400528</td>
<td><a href="mailto:kapelush@bgumail.bgu.ac.il">kapelush@bgumail.bgu.ac.il</a></td>
<td>Israel</td>
</tr>
<tr>
<td>Dallorso, Sandro, Dr.</td>
<td>Unita' Trapianto di Midollo Osseo</td>
<td>Institute Gaslini</td>
<td>+39 010 5636-405 (or-507, or-508)</td>
<td>+39 010 3777 133</td>
<td><a href="mailto:sandrodallorso@ospedale-gaslini.ge.it">sandrodallorso@ospedale-gaslini.ge.it</a></td>
<td>Italy</td>
</tr>
<tr>
<td>Kowalczyk, Jerzy, Professor, Dr.</td>
<td>Haematology and Oncology Department, Children’s Hospital University</td>
<td>Chodżki 2</td>
<td>+48 81 718 55 20</td>
<td>+48 81 747 72 20</td>
<td><a href="mailto:jkowalcz@dsk.lublin.pl">jkowalcz@dsk.lublin.pl</a></td>
<td>Poland</td>
</tr>
<tr>
<td>Chybicka, Alicja, Professor, Dr.</td>
<td>Department of Children Oncology and Haematology</td>
<td>Wroclaw Medical University</td>
<td>+48 71 328 20 40</td>
<td>+48 71 328 20 40</td>
<td><a href="mailto:klin@pedhemat.am.wroc.pl">klin@pedhemat.am.wroc.pl</a></td>
<td>Poland</td>
</tr>
<tr>
<td>Name</td>
<td>Institution/Unit</td>
<td>Country</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td>----------------------------------------------------------------------------------</td>
<td>---------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Nuno, Miranda, Dr.</strong></td>
<td>BMT Unit</td>
<td>Portugal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Instituto Portugues De Oncologia De Francisco Gentil (Portuguese Institute of Oncology)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rua Professor Lima Basto 1099</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PT - 1099-023 Lisboa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fax:+351 21 722985</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:nmiranda@ipolisboa.min-saude.pt">nmiranda@ipolisboa.min-saude.pt</a></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>De La Rubia, Javier, Dr.</strong></td>
<td>Servicio de Hematologia</td>
<td>Spain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hospital Universitario La Fe</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Avda. Campanar No. 21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>E - 46009 VALENCIA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone: +34-963862721</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fax: +34 963868757</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:delarubia_jav@gva.es">delarubia_jav@gva.es</a></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Abrahamsson, Jonas, Dr.</strong></td>
<td>Queen Silvia's Children's Hospital</td>
<td>Sweden</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sv/ostra</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SE – 41685 Goteborg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone: +31 3434100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fax: +31 215486</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:jonas.abrahamsson@pediat.gu.se">jonas.abrahamsson@pediat.gu.se</a></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Picton, Sue, Dr.</strong></td>
<td>Consultant Paediatric Oncologist</td>
<td>UK</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Regional Paediatric Oncology Unit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>St James's University Hospital</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GB - Leeds LS9 7TF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone: +44 - 113 206 4984</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fax: +44- 113 2470248</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:susan.picton@leedsth.nhs.uk">susan.picton@leedsth.nhs.uk</a></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 31.15 Surgery Committee

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Horcher, Ernst, Prof.</strong></td>
<td>Dept. For Paediatric Surgery</td>
<td>Austria</td>
</tr>
<tr>
<td></td>
<td>Univ. Clinic for Surgery</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Währinger Gürtel 18-20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A - 1090 Vienna</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone: +43 1-40400 6838</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fax:+43 1-40400 6898</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:Ernst.Horcher@meduniwien.ac.at">Ernst.Horcher@meduniwien.ac.at</a></td>
<td></td>
</tr>
<tr>
<td><strong>De Wever, Ivo, Prof. Dr.</strong></td>
<td>Head of Department Surgical Oncology</td>
<td>Belgium</td>
</tr>
<tr>
<td></td>
<td>University Hospital Gasthuisberg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Herestraat 49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B - 3000 Leuven</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phone: +32 16 34 6831</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fax: +32 16 34 68 34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:Ivo.Dewever@uz.kuleuven.ac.be">Ivo.Dewever@uz.kuleuven.ac.be</a></td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Department</td>
<td>Address</td>
</tr>
<tr>
<td>-----------------------</td>
<td>--------------------------------------------------</td>
<td>----------------------------------------------</td>
</tr>
<tr>
<td>RASMUSSEN, LARS, DR</td>
<td>Department of Surgical Gastroenterology A</td>
<td>Odense University Hospital DK- 5000 Odense C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SARNAKCI, SABINE, DR</td>
<td>Service de Chirurgie Pédiatrique et INSERM E-0212</td>
<td>Hopital et Faculté Necker Enfants-Malades 149 rue de Sèvres 75743 Paris Cedex 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PINTER, ANDRÁS, DR</td>
<td>Department of Paediatrics</td>
<td>University of Pécs József Attila u. 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H - 7623 Pécs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FREUD, ENRIQUE, DR.</td>
<td>Department of Paediatric Surgery</td>
<td>Schneider Children’s Medical Center of Israel</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kaplan Street 14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL - Petah-Tikva 49292</td>
</tr>
<tr>
<td>CECCHETTO, GIOVANNI, PROF.</td>
<td>Paediatric Surgery</td>
<td>Divisione Chirurgia Pediatrica</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Istituto di Chirurgia Pediatrica dell'università di Padova</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Via Giustiniani 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I - 35128 Padova</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MONCLAIR, TOM, DR.</td>
<td>Paediatric Surgical Service</td>
<td>Department of Surgery</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rikshospitalet</td>
</tr>
<tr>
<td></td>
<td></td>
<td>The National Hospital University of Oslo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N - 0027 Oslo</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**HRNBL1.5/SIOPEN valid per 01.06.2011**
<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BYSIEK, ADAM,</strong> <strong>ASS. PROF., DR</strong></td>
<td>Department of Peadiatric Surgery Polish-American Institute of Peadiatrics, Jagiellonian University Medical College Wielicka 265 PL - 30-663 Kraków Phone: + 48 12 658 02 32 Fax: + 48 12 658 13 25 Email : <a href="mailto:Adam.bysiek@wp.pl">Adam.bysiek@wp.pl</a></td>
<td><strong>POLAND</strong></td>
</tr>
<tr>
<td><strong>GODZINSKI JAN, DR.</strong></td>
<td>Department of Paediatric Surgery T. Marciniak Hospital in Wroclaw Phone: +48 71 32 32 409 Fax: +48 71 78 90 156</td>
<td><strong>POLAND</strong></td>
</tr>
<tr>
<td><strong>MARTINS, ANÍTONO GENTIL, DR.</strong></td>
<td>Instituto Portugues De Oncologia De Francisco Gentil (Portuguese Institute of Oncology) Rua D. Francisco Manuel De Melo P - 1000 Lisboa Email: <a href="mailto:agentilmartins@netc.pt">agentilmartins@netc.pt</a></td>
<td><strong>PORTUGAL</strong></td>
</tr>
<tr>
<td><strong>VIDISCAK, MARIAN, ASSOC.PROF.DR.</strong></td>
<td>Department of Paediatric Surgery University Children's Hospital SK-Limbova 1833 40 Bratislava Phone: +421 593 71174 Fax: +421 2 547 88 419 Email: <a href="mailto:vidiscakm@yahoo.com">vidiscakm@yahoo.com</a></td>
<td><strong>SLOVAKIA</strong></td>
</tr>
<tr>
<td><strong>MARTINEZ, LEOPOLDO, DR.</strong></td>
<td>Servicio de Cirugia Infantil Hospital La Paz Paseo de la castellana no.261 E – 28046 Madrid Phone: +34 917277223 Fax: +34-917277042 Email: <a href="mailto:lmartinezm.hulp@salud.madrid.org">lmartinezm.hulp@salud.madrid.org</a></td>
<td><strong>SPAIN</strong></td>
</tr>
<tr>
<td><strong>KULLENDORFF, CARL,</strong> <strong>MAGNUS, DR.</strong></td>
<td>Carl-Magnus Kullendorff Section of Pediatric Surgery Deaprtments of Pediatrics University Hospital SE - 221 85 Lund Phone : 0046 46 178298 Fax : 0046 46 178120 Email : <a href="mailto:Carl-Magnus.Kullendorff@skane.se">Carl-Magnus.Kullendorff@skane.se</a></td>
<td><strong>SWEDEN</strong></td>
</tr>
</tbody>
</table>
### 31.16 Summary of National Contact Details for Material Transfer
Section to be completed by each National Coordinator according to identified reference and coordinating national sites

<table>
<thead>
<tr>
<th>Primary Tumour Material and Bone Marrow Biopsies</th>
<th>Pathology Contact Details</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Biology Contact Details</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bone Marrow and Peripheral Blood Samples (QRT-PCR, cytospins)</th>
<th>Reference Lab Contact Details</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Nuclear Medicine Department – mIBG scans                     | Contact Details               |
|                                                             |                               |

| Radiology - images                                           | Contact Details               |
|                                                             |                               |

| Radiotherapy - images                                        | Contact Details               |
|                                                             |                               |

| Pharmaco-Toxicology Serum samples                            | Contact Details               |
|                                                             |                               |

| Immunotherapy Serum samples                                  | Contact Details               |
|                                                             |                               |
32 APPENDIX: Declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly Helsinki, Finland, June 1964 and amended by the
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008

32.1 Introduction

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data. The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.

2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.

3. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

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

5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.

6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.

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

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

9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special
protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.

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

32.2 Principles for All Medical Research

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.

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

13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.

14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.

15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.

16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.

17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or
community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.

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

19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.

20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.

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

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

23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.

24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject’s freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.

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

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

28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject’s dissent should be respected.

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

30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

32.3 Additional Principles for Medical Research Combined with Medical Care
31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

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

- The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
- Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.

33. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.

34. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient’s decision to withdraw from the study must never interfere with the patient-physician relationship.

35. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized
representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.
33 **APPENDIX: Sample Information Sheet/Consent Forms**

As there are a number of different countries participating in this study, and therefore a number of different languages involved, it is left to each National co-ordinator to write the information sheets for parents and informed consent form as appropriate. The following parent information sheet and informed consent document has been prepared for use within this protocol, and may be adapted by other participants.

33.1 **Sample Information Sheet for study enrolment and induction treatment**

Dear Parent

You have been told that your child has been diagnosed as having a disease called neuroblastoma. Neuroblastoma is a rare malignant disease (cancer) and in children over the age of one year is often very difficult to treat. However, some children are cured, but others despite very intensive treatment do not do well. Children with high-risk neuroblastoma often respond to standard treatment at first, but there is a high risk that the cancer will come back. We invite you to agree to your child’s participation in the research study named the High-Risk Neuroblastoma Study1/SIOPEN which aims to increase the number of children with high-risk neuroblastoma who can be cured. This research study has been reviewed and approved by the Institutional Review Board (IRB) {name of the institution}, which is an independent board composed of {name of institution} physician’s and staff members, and representatives of the community. The IRB has reviewed this study, evaluated the potential benefits and risks and has granted approval for the inclusion of participants. The hospital maintains a Multiple Project Assurance of Compliance, a document that explains how the hospital provides for protection of human subjects. You/your child will receive a copy of this assurance if so requested.

This study is a clinical trial (a research study involving human patients). Clinical trials include only patients who choose to take part. You are being asked to allow your child to take part in this study because he/she has been diagnosed with high-risk neuroblastoma. Please take your time to make your decision. Before you can decide whether or not to volunteer/allow your child to volunteer for this study, you must understand the purpose, how it may help you/your child, any risks to you/your child, and what is expected of you/your child. This process is called informed consent. You are asked to read this information sheet and discuss your decision with friends and family.

This information sheet gives you information about the study which will be discussed with you/your child. Once you understand (and possibly your child understands) the study, and if you agree to participate/to allow your child to participate, you will be asked to sign the Informed Consent Form, assuming that your child will not be able to sign. (However, your child’s assent will be required if the child is seven years of age or older.) Before you learn about the study, it is important to know the following:

- You/your child’s participation in the study is entirely voluntary.
- You may decide not to participate/allow your child to participate or to withdraw/withdraw your child from the study at any time without penalty.

If the study is changed in any way which could affect your/your child’s participation, you will be told about the changes and may be asked to sign a new informed consent form.
WHAT IS THIS STUDY ABOUT?

Neuroblastoma is a rare disease and doctors will not see enough cases to be able to plan treatments based just on their own experience, or even that seen in all the centres in their own country. For this reason doctors in a number of European countries have worked together to plan this Clinical Trial in order to study and treat in a planned and systematic way children with high-risk Neuroblastoma and to collect information on a large group of patients.

Doctors specialised in treating this disease have combined their knowledge and experience to create a treatment protocol based on what they all believe to be the best available treatment. Experts from many centres in Europe have contributed to the design of this treatment program which is believed to be as effective (or more effective) than treatment used in other major centres throughout the world. These specialists of the field are working together within the International Society of Pediatric Oncology (SIOP) Europe Neuroblastoma Group (SIOPEN). The SIOP Europe Neuroblastoma Group represents more than 22 participating countries including more than 200 hospitals that treat children with cancer. It is common medical practice world-wide to treat children with neuroblastoma on research studies like this one.

What is high-risk neuroblastoma?

Neuroblastoma is a cancerous tumour that grows as a lump or mass in the abdomen or around the spinal cord in the chest, neck, or pelvis. This treatment protocol is called High-Risk Neuroblastoma Study and aims to improve survival in children recognised to have particularly high-risk neuroblastoma. This may be due to disease that has spread throughout the body, in particular bone marrow, bones, lymph nodes, liver or more rarely brain or lungs. In addition over recent years it has become apparent that certain biological features (that it is now possible to test for) may provide additional information about the anticipated prognosis. To date the most important biological feature is something called \textit{MYCN}. \textit{MYCN} amplification has been recognised to be related to a poor survival chance even if the disease is still localised. All these patient’s groups mentioned above are assumed to need very intensive treatment to have a chance of surviving this disease.

How can a malignant disease (cancer) be treated?

There are different methods of attacking and ultimately destroying or overcoming tumour cells: one is \textbf{CHEMOTHERAPY}, using a group of drugs which interact with tumour cells and destroy them. Since tumour cells usually show rapid growth these drugs mainly affect the tumour cells, some however interact with normal tissues and cause what is known as side effects of chemotherapy, i.e. loss of hair, poor blood counts, pronounced immunosuppression, elevated risk of infection and mucositis. All treatments of high-risk tumours are associated with side effects which may be very severe and even life-threatening. These risks are justified in view of the life-threatening nature of the cancer which without treatment could cause the child’s death within a short period of time. Drugs may be used at different dose intensity which affects the type of supportive care associated with their administration. At an appropriate time surgeons will try to remove (excise) the main tumour bulk aiming at a \textbf{COMPLETE SURGICAL EXCISION} of the primary tumour. \textbf{RADIOTHERAPY} also has the capacity to kill residual tumour cells, causing damage in cells with a high turn over rate (i.e. tumour cells). Newer approaches to treatment include induction of differentiation of the tumour cells, in other words to turn aggressive immature tumour cells into non-aggressive mature cells. This effect on cells is seen after treatment with certain vitamins, in particular Vitamin A (Retinoic acid) and is called \textbf{DIFFERENTIATION TREATMENT}. A recent study demonstrated the beneficial effect on patient survival when this was added. \textbf{IMMUNOTHERAPY} means treatment to boost the immune response against the tumour cells and is another approach that has been shown to improve survival in patients who have responded well to the previous components of the treatment.

What is a randomisation?
For some treatment options, developed and accumulated over the years in the various countries world-wide, it is not known today which one is superior in its capacity to improve survival on the one hand and which is characterised by a better toxicity profile on the other hand. In order to compare two types of treatment patients need to be randomised.

Random assignment means that the treatment to which your child is assigned is based on chance. It is like flipping a coin, except that this assignment is done by a computer. Neither you nor the researcher chooses which group your child will be in. Your child will have an equal chance of being placed in either group.

Parents need to understand and agree to this philosophy prior to their child entering the study and have to allow the randomisation process by signing a consent form. Parents should remember that if doctors knew which of the treatment was better, they would offer it to your child. Parents are not therefore being asked to choose the treatment offered. Doctors will however inform you of the different toxicities associated with both approaches. The parts of the study which include a randomisation should not be regarded as experimental since proposed treatments have been explored and used previously in national treatment protocols. Doctors hope that by studying treatments in a randomised way they will understand the disease better, and will then be able to provide the best treatment available, based on sound evidence of which is best able to cure the disease.

Which treatment options are randomised within this study?

Comparison of two chemotherapy regimens (R3)
This randomisation compares two different chemotherapy regimens that are given at induction (i.e. at the start of treatment):
1) Rapid COJEC which is the chemotherapy schedule that has been used in the UK for 20 years and widely in Europe for the past 10 years. This includes chemotherapy given every 10 days for 80 days
2) Modified N7 regimen, which has been used for more than 20 years but widely in the US for the past decade, and also by a number of national groups in Europe. This regimen comprises 5 courses of chemotherapy given every three weeks.
Both regimens have shown good results in previous studies in the US and Europe, and so the aim of this randomisation is to compare the efficacy and toxicity of the two regimens to determine if one regimen is more effective or less toxic than the other.

Explores the potential benefit of using immunotherapy in addition to differentiation treatment with cis-retinoic acid (R2)
This randomisation will investigate whether the addition of immunotherapy in the form of a neuroblastoma specific, humanised mouse antibody (ch14.18/CHO) with or without aldesleukin in addition to differentiation treatment could help to further improve survival. Both will be given in a treatment phase where usually only minimal residual disease, which is currently very difficult to detect, is suspected in your child’s body. This immunotherapy consists of a monoclonal antibody directed against neuroblastoma cells and is an investigational drug not available for use outside of a clinical trial. Aldesleukin is a man-made protein that stimulates the immune system; it is known to be efficacious in stimulating immune cells, but is has never been shown clearly if this additional stimulation results in improved outcome (unclear effectiveness).

Parents may withdraw their agreement to randomisation at any given time point. Your doctors will accept such a decision and continue to treat your child with the treatment arm that is referred to as “standard” in this study. Also in the case that parents do not agree to the randomisation process at all, the child will receive what is called the “standard” treatment. Refusal to participate in the randomisation or a decision to withdraw will not affect your child’s treatment or relationship with the doctor or team.
WHAT IS STANDARD TREATMENT?

Standard treatment is the treatment that is considered the best proven treatment in Europe at the moment. This will be the treatment that your child will receive if you decline to consent to the randomisations. Standard treatment for children with high-risk neuroblastoma comprises of:

- Induction treatment with Rapid COJEC chemotherapy
- Surgery (non-randomised treatment element)
- **MAT** treatment with busulfan and melphalan (BUMEL) followed by stem cell reinfusion
- Local radiotherapy (non-randomised treatment element)
- Differentiation treatment with 13 cis-retinoic acid without additional immunotherapy

SPECIFIC TREATMENT PLAN OF THE HR-NBL1/SIOPEN STUDY

The current protocol consists of 5 distinct treatment periods. These treatment phases are outlined in the attached, “Outline of Treatment Plan for High Risk Neuroblastoma.”

1) **Induction treatment**: tries to reduce the tumour burden in the body, both disease which has spread and at the tumour site. As described above, your child will be randomly assigned to one of the chemotherapy regimens: Rapid-COJEC where 8 courses of chemotherapy are given every 10 days or Modified N7 where 5 courses of chemotherapy are given every 3 weeks. Both the chemotherapy and side effects of chemotherapy mean your child will spend significant periods of time in hospital during this induction chemotherapy treatment. Depending on the response of your child’s disease to chemotherapy, your doctor may decide to give additional courses of chemotherapy. Initially this will be 2 cycles of Topotecan, Vincristine and Doxorubicin (called TVD). Depending on the disease response to TVD further induction treatment may be offered. The aim of the induction treatment is to clear the body of disease as much as possible particularly from distant sites, often bone and bone marrow.

2) **Interim phase without chemotherapy**: Time to evaluate disease response carefully with a variety of diagnostic measures and to aim for complete surgical excision (removal) of the main tumour at the so-called primary tumour site. During this period doctors will also try to harvest stem cells (Stem cells are the cells that create new blood cells, such as red blood cells, white blood cells, and platelets). These very young cells are usually found in the bone marrow and have the capacity to develop into all the necessary ‘blood’ cells and replace those which have been destroyed by very high doses of chemotherapy (myeloablative therapy: MAT). These stem cells are harvested from your child’s peripheral blood and frozen for use after high-dose therapy.

3) **Myeloablative treatment (MAT)** is a very intensive way of administering chemotherapy. High doses of two chemotherapy drugs, busulfan and melphalan are given to attack any residual tumour cells, in a more intensive manner than the induction chemotherapy; this treatment is given over about a week followed by a recovery period in hospital of about 3 additional weeks. Once your child has received MAT his/her own ‘stem cells’ are thawed and reinfused via a central venous line. Those cells have the capacity to re-establish the whole bone marrow population and to allow for normal peripheral blood values within a time frame of 10 to 20 days. During this period your child will be cared for in the hospital.
4) **Radiotherapy** (lasts 2 to 3 weeks), is to be initiated in all children once he/she has recovered from the MAT procedure and will involve the primary tumour site (where the tumour initially arose). This should improve the control of the disease, since the primary tumour site is an area where previously re-growth of the tumour has been observed with a fairly high frequency (reported in up to 40% of affected children) even after complete surgical excision.

5) **Differentiation Treatment** with 13 cis-retinoic acid (Vitamin A) This is 6 months of oral drug treatment and the drug encourages any surviving neuroblastoma cells to behave in a less aggressive (or more benign) manner.

6) **Immunotherapy** is given to all patients participating in the R2 randomisation process. They all receive a “neuroblastoma antibody” (ch14.18/CHO), and then patients are randomly allocated to receive this antibody with or without aldesleukin. Immunotherapy is given over the same period as differentiation therapy.

**WHY IS THIS STUDY BEING DONE?**

The primary goals of this study are to find out
- Whether there is any difference in the number of children who’s disease responds (whose tumours shrink and where disease that has spread to bone or bone marrow is cleared) with one of the two induction chemotherapy regimens (Rapid-COJEC or Modified N7) and more importantly in the number of children that are ultimately cured.
- Whether immunotherapy with ch14.18/CHO with or without aldesleukin, following MAT, in addition to differentiation therapy with isotretinoin (13-cis-RA), adds to the cure rate observed in patients with high-risk neuroblastoma.

The secondary goals of this study are
- To find out if the biological features of neuroblastoma tumours can be used to predict survival.
- To improve the rate of local control with the help of more aggressive local surgery aiming at complete primary tumour resection and the addition of local radiotherapy in all patients.
- To determine the efficacy of and to compare the acute and long term toxicities of I.V. Busulfan (Busilvex®) and melphalan (BUMEL).
- To study the long-term side effects of treatment for neuroblastoma on health and quality of life.

*A side effect is an unintended result of a treatment unrelated to the reason the treatment is being given.*

**HOW MANY PEOPLE WILL TAKE PART IN THIS current HR-NBL-1.5/SIPEN STUDY?**

It is expected that about 600 patients from more than 22 European countries will be treated on this study over the study period.

**WHAT WILL HAPPEN TO MY CHILD ON THIS STUDY?**

If your child is eligible for the induction chemotherapy randomisation your doctors will explain to you the details of both regimens and ask you again for your consent. You will be given a separate information sheet and informed consent document to read and sign (R3).

After successful completion of MAT and planning of local radiotherapy the second randomisation will be due. Your child will be eligible for this randomisation providing that certain response timelines are met. Your child will randomly be assigned to receive immunotherapy with or without Aldesleukin in addition to differentiation treatment. If your child is eligible, your doctors will inform you again in detail about the treatment options and ask your consent again to participate in the second
randomisation. You will be given a separate information sheet and informed consent document to read and sign (R2).

It may be that your child is ineligible for either or both of the randomisations (R3 and R2) for different reasons which will be explained to you. In this case your child will receive the standard treatment or alternative treatment. Your child’s doctor will discuss this with you.

**Methods for Giving Drugs**

Various methods will be used to give drugs to your child. Some drugs will be given in tablet form by mouth. Other drugs will be given using a needle subcutaneously (inserted under the skin) or directly into a central venous line.

**Central Line**

For drugs to be given intra-venously, your child’s doctor will be likely to recommend that your child has a central venous line placed. A central line is a special type of tubing inserted into a large vein in the chest by a surgeon during a short operation. Your child will be anaesthetised for this procedure and receive pain medication afterwards to keep him/her comfortable. The central line is used to administer chemotherapy drugs and to withdraw small amounts of blood for testing during treatment. The risks associated with central lines will be explained to you. If your child is to have a central line inserted, you will be given a separate informed consent document to read and sign. Schedules of how and when the drugs will be given during each phase of therapy can be found in the attached “Flowsheet of Therapy for High-Risk Neuroblastoma”.

**Induction Phase**

All patients will begin treatment with the induction phase of chemotherapy and will be randomly assigned to one of the following arms (R3):

1) Rapid COJEC, which is divided into 8 chemotherapy cycles given every 10 days (regardless of blood count recovery). There are three different drug combinations in use during the eight cycles. Cycle A uses the anti-cancer drugs vincristine, etoposide and carboplatin, cycle B uses vincristine and cisplatin and cycle C cyclophosphamide, etoposide and vincristine.

2) Modified N7, which is divided into 5 chemotherapy cycles given every 21 days (or when the blood count has recovered adequately). There are two different drug combinations in use during the five cycles. Cycle CAV consists of cyclophosphamide, doxorubicin and vincristine and cycle PE consists of cisplatin and etoposide.

If you decide to allow your child to be randomised, you will be asked to sign a separate informed consent document.

Additional drugs given during these cycles are MESNA, G-CSF and drugs reducing nausea and vomiting (antiemetics). MESNA is given to help protect the bladder from the potentially damaging effects of cyclophosphamide. G-CSF (granulocyte colony stimulating factor) which stimulates the production of white blood cells (infection fighting cells) will be given following chemotherapy to reduce the number of days in hospital, those with fever and those requiring antibiotics. It is likely that your child will still need blood transfusions (either red packed blood cells or platelets), admissions for i.v. antibiotics and feeding through a naso-gastric tube during this induction period.

Depending on the response of your child’s tumour to chemotherapy, your doctor may decide to give two additional courses of chemotherapy, including topotecan, vincristine and doxorubicin (called TVD).

**Stem Cell Harvest**

After induction is completed and the disease has responded sufficiently, according to given criteria, your child’s stem cells will be collected using a procedure called leukapheresis. Either a special catheter will be placed into a large vein or the pre-existing central line (if in place and appropriate)
will be used. One side of the catheter will collect circulating blood into a machine that filters out the stem cells. The filtered blood will be returned to your child’s body through the other side of the catheter or a different venous line. Each leukapheresis procedure takes up to 6 hours. The procedure may need to be done several times to collect a sufficient number of stem cells. The physician may decide that the collection of stem cells should come from the bone marrow instead of the peripheral blood, if the collection from the peripheral blood is not satisfactory. This alternative will be discussed in more detail if it affects your child.

Your child’s collected stem cells (either from the peripheral blood or from the bone marrow) will be frozen and stored at your child’s treating institution.

**Surgery**

After the end of induction chemotherapy, your child will have surgery to remove as much of the primary tumour as possible.

**Myeloablative Chemotherapy and Stem Cell Transplant**

If your child is ready to undergo stem cell transplant, he/she will begin consolidation chemotherapy after the end of induction therapy. Most of the children will have already undergone surgery, but this is not a prerequisite prior to MAT. The consolidation phase of therapy is one course of treatment with high doses of the anti-cancer drugs BUMEL consisting of I.V. busulfan (Busilvex®) and melphalan. This regimen is now used for all children within this study. A randomisation within this study compared two regimens for MAT: BUMEL (widely used in Europe before) versus CEM (widely used in the US). This randomisation has recently been closed as we now know that BUMEL is more effective than CEM (more children were alive and fewer children had relapsed at three years after treatment with BUMEL) and BUMEL is less toxic than CEM.

After these anti-cancer drugs are given, your child will have a rest period during which no drugs are given. Your child’s stored stem cells will then be given to him/her through the central line, like a transfusion. Five days after the stem cell transplant, your child will be given daily G-CSF injections. This is because G-CSF is beneficial after transplant. Your child will continue to receive G-CSF for 2-3 weeks, or until blood tests show that the cell count in your child’s blood is adequate. During this phase of therapy, your child will be hospitalised. Your child will also require blood transfusions and probably feeding through a naso-gastric tube. It may take only 10 days, but may extend to two or more weeks for the transplanted stem cells to grow and begin to make enough white blood cells so that your child can go home. When your child is medically stable, he/she will be discharged from the hospital. Your child may still need blood transfusions and feeding through a naso-gastric tube for another one or two months.

**Radiation Therapy**

Approximately two months after transplant, your child will need to have radiation treatment. Radiation will be given once a day on 5 week days (rest at week-ends) until a total dose of 21 Gy has been administered. Thus radiation will be given on 14 working days and will not exceed a total period of 21 days.

**Isotretinoin (13-cis-RA) Therapy**

Approximately 3 months following stem cell transplant or immediately after radiation therapy, your child will receive treatment for six months with isotretinoin (13-cis-RA). Isotretinoin (13-cis-RA) is a drug closely related to vitamin A and has been shown to help stop the multiplication of any remaining neuroblastoma cells in your child’s body. Your child will take this drug twice a day by mouth for 14 days and then not take it for the following 14 days. This 28-day cycle will be repeated 6 times.

**Entry into the last treatment phase or immunotherapy**
If your child was eligible for stem cell transplant, your child's physician will approach you about the option of entering your child into the last randomisation (R2). If you allow your child to be entered for randomisation into the last treatment phase, he/she will receive the neuroblastoma specific monoclonal antibody,(ch14.18/CHO) for 5 courses added to the 6 courses of isotretinoin (13-cis-RA) described in the previous paragraph. Your child will be randomised to receive or not 5 additional courses of immunotherapy with IL-2 each given over two weeks during the rest periods of the isotretinoin (13-cis-RA).

The randomisation will be explained to you in more detail after the MAT procedure before radiotherapy. If you decide to allow your child to be randomised, you will be asked to sign a separate informed consent document.

**HOW LONG WILL MY CHILD BE ON THIS STUDY?**

The treatment portion of the study will last about 12 months. However, the researchers would like to continue to observe your child periodically for many years following the study. The researchers may decide to remove your child from the treatment part of the study if your child’s cancer gets worse, or your child experiences effects from the treatment that are considered too severe. You can remove your child from the treatment part of the study at any time and your rights and your child’s medical care will not be affected. However, if you consider removing your child from the study, we encourage you to talk to your child’s regular physician and to the research physician before making a final decision.

**WHAT ARE THE RISKS OF THE STUDY?**

While on the study, your child is at risk for the side effects listed below. Your child will not necessarily suffer all of the listed side-effects. None of these side-effects would be accepted in a healthy child, but the risk to encounter some of them during treatment is only accepted in view of your child’s life-threatening disease. Your child will be closely monitored for the risk of these side effects, and most of the side-effects can be managed effectively. Your child should be aware of an increased risk of infections. It might be asked to stay at home for periods and not to eat certain foods to decrease the chance of infection. There may also be other side effects that cannot be predicted. Some of the increased side effects may occur during therapy (such as low blood counts, allergic reaction, increased risk of infection, reduced kidney function, impaired hearing or poor nutritional status). Other side effects can occur later for example those relating to fertility. Some side effects can become life threatening, or even fatal. It is also rare, but possible that your child could develop another cancer as a result of treatment on this study. You should discuss these potential risks with the researcher and/or your regular doctor. Other drugs will be given to make certain side effects less serious and/or less uncomfortable. Patients are watched carefully and where possible treatment is stopped if serious side effects develop.

**Reproductive risks:**

It is unknown what effect(s) these treatments may have on an unborn child. For this reason, if your child is of child-bearing age, your child will be asked to practice an effective method of birth control while participating in this study.

Females of childbearing potential must have a negative pregnancy test and must agree to use an effective birth control method. Female patients who are lactating must agree to stop breast-feeding. The use of chemotherapy may cause a fertility impairment, in particular high doses as used during the MAT phase. In particular busulfan is known to cause sterility in the majority of patients; melphalan is thought to reduce fertility particularly in boys. There are measures which can be taken to try and enable your child to have children in the future either by banking sperm or eggs. You are encouraged to discuss these options with your child’s doctor who will be able to advise you.

**Side effects of chemotherapy drugs:**
As well as killing cancer, chemotherapy can damage normal parts of the body and produce side effects (things that will make your child feel unwell). Side effects are often mild but maybe more serious. The doctors and nurses looking after your child may give them medicines to help lessen the side effects. Many side effects go away when the chemotherapy finishes. Occasionally side effects do not go away and this can cause serious complications. A person can die from these and other complications.

Common side effects include:
- Hair loss – in most cases the hair grows back
- Not wanting to eat, feeling and being sick
- Diarrhoea
- Sore mouth
- Tiredness – sometimes blood transfusions are needed
- Infections

Some side effects may be permanent and can occur in the long-term, after years of completing treatment, and these can include hearing loss, deterioration of kidney function, toxicity to the heart and there is also the possibility that a second cancer may develop as a result of the chemotherapy.

If you would like more information about the side effects of your child’s treatment then please speak to your child’s doctor.

**Side effects of radiation therapy:**
Side effects will vary depending on the site being irradiated. Some side effects may only become apparent months or years after treatment. Please ask your child’s doctor about the specific side effects associated with your child’s particular radiation treatment. General side effects include: nausea and vomiting, sleepiness, temporary weakness, sore mouth or tummy if this is included in the treatment area. Temporary redness or soreness of the skin usually develops in the area of treatment. A more permanent decrease in growth of muscles and bone in the treatment area as well as an increased risk of developing a second cancer are also possible.

**Side effects of peripheral blood stem cell collection include:**
Nausea and vomiting, dizziness and/or fainting, seizures (rare), blood loss, infection, muscle cramps, tingling of the lips, changes in heart rhythm (rare and easily treated), skin rash, hives, flushing (redness and warmthness of the skin), increased bleeding (caused by a drug given to prevent clotting of the blood during collection).

**Side effects of bone marrow stem cell collection include:**
Pain and swelling at the collection site and risk of infection.

**Side effects of consolidation chemotherapy and stem cell transplant:**
The high doses of chemotherapy used during preparation for the stem cell transplant cause severe lowering of the number of blood cells, damage to normal tissues and severe impairment of your child’s ability to fight infection. Busulfan and Melphalan when combined cause permanent severely reduced fertility or infertility in both boys and girls.

Due to the severe lowering of peripheral blood cells, transfusions are required and your child will be at risk of potentially life-threatening infections. The general side effects related to the stem cell transplant are severe mouth sores, nausea and vomiting, diarrhoea, abdominal pain, skin damage with redness and peeling, irritation of the liver. If there are tumour cells in the harvested stem cells, it is possible that they may cause tumour re-growth in your child when the stem cells are given back to
your child. This risk if reduced if peripheral blood stem cells are used rather than a bone marrow harvest.

Side effects of minimal residual disease therapy:
This includes 13 cis retinoic acid and immunotherapy.
The side effects of 13 cis retinoic acid are mainly chapped lips and dry skin and can be helped by using ointment containing vitamin E. Liver function and urine analysis can become temporarily abnormal and then doses may be adjusted.
The side effects of the immunotherapy include temporary but severe pain (mainly in the tummy) which will be managed and controlled with strong pain medication including intravenous morphine. Other side effects also occur during the administration which include, fever, cough and severe allergic reactions.

Please note that not all of these side effects will be experienced by all children; they will be closely monitored and managed long term.

WILL MY CHILD BENEFIT FROM THIS STUDY?
While the hope is that more children with neuroblastoma are cured on this study than on previous studies, there may not be any direct medical benefit to your child if he/she participates in the study. It is hoped that the information learned from this study may however help future patients with high-risk neuroblastoma.

ARE THERE OTHER TREATMENT OPTIONS?
There are other options, which will vary from country to country, and are mainly used in non-European countries. Your doctor will be able to provide advice on other treatment options.

WILL MY CHILD’S RECORDS BE CONFIDENTIAL?
You may read your child’s medical record. Efforts will be made to keep your child’s personal information confidential. Unfortunately, absolute confidentiality cannot be guaranteed. Information about your child may be disclosed if required by law. Your child’s name will not be used when the research results are published.
Organisations that may inspect/or copy your child’s research records for quality assurance and data analysis include:
- The SIOPEN Group
- The Data Monitoring Committee
- The Institutional Review Board of your child’s hospital or the regulatory authorities from your country

WILL I HAVE TO PAY FOR THIS TREATMENT?
Taking part in this study may lead to added costs for your insurance company. Please ask about any expected added costs or insurance problems. In the case of injury or illness resulting from this study, emergency medical treatment will always be provided. You or your insurance company or your social security will be charged for continuing medical care and/or hospitalisation. However, additional insurance is provided for the investigational drug “ch14.18/CHO” within immunotherapy and for I.V. Busulfan (Busilvex®) administered during high dose chemotherapy. No-one will receive payment for taking part in this study.

WHAT ARE MY CHILD’S RIGHTS AS A STUDY PARTICIPANT?
Taking part in this study is voluntary. You may choose not to allow your child to take part in this study. If you allow your child to participate, you may remove your child from the study at any time.
If you remove your child from the study, physicians and hospital personnel will still take care of your child. You also have the right to know about new information that may affect your child’s health, welfare, or your willingness to let him/her participate in the study. You will be provided with this information as soon as it becomes available. Whether you participate or not, your child will continue to get the best medical care your hospital can provide.

**WHAT IF I HAVE QUESTIONS OR PROBLEMS?**
For questions about the study or an injury related to the research, please call

____________________ ___________________
NAME at TELEPHONE NUMBER

For questions about your rights as a study participant, please call

______________________________________________________
NAME OF INSTITUTIONAL REVIEW BOARD REPRESENTATIVE*

___________________
at TELEPHONE NUMBER

*The Institutional Review Board is a group of people who review the research study to protect your rights.

**WHAT HAPPENS TO THE INFORMATION COLLECTED ON MY CHILD?**
If you are willing to allow your child to participate in this study, information about your child's tumour and his/her response to the treatment given will be collected, and stored on a computerised database (held in your own country and also in the main data centre). The data will be analysed along with the information from other similar children from all over Europe, so that we can answer some of the questions we have about this disease. We hope in this way that we will be able to more accurately plan treatment for children in the future. All information relating to your child will be treated in the strictest confidence. Any publication or presentation relating to the results of the study will not include any information that enables your child to be identified.
If you do decide to allow your child to be entered into the study you may decide at any time to change this decision and this will not affect your child’s treatment in any way.
Please ask the doctors caring for your child for any further information. You will be given a copy of this consent form.
33.2 Consent form for study enrolment and induction treatment

<table>
<thead>
<tr>
<th>High Risk Neuroblastoma 1.5 study /SIOPEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRIAL CONSENT FORM</td>
</tr>
</tbody>
</table>

The parent(s) should complete the whole of this sheet himself/herself, (themselves)  
Please delete as necessary

| I have been given a copy of and read the information sheet dated……version……. | YES / NO |
| I have had an opportunity to ask questions and discuss this study. | YES / NO |
| I have received satisfactory answers to all of my questions. | YES / NO |

I understand that sections of any of my child’s medical notes, imaging, blood, bone marrow or tissue specimens may be looked at by responsible individuals from my child’s hospital, the National and International study centres, or from regulatory authorities where it is relevant to my child’s taking part in research. I understand that extra samples will be taken for research purposes and may be sent to another country for analysis.

| YES / NO |

I have understood that if I agree to my child taking part in the study he/she will be treated following the protocol guidelines. For each randomisation a separate consent will be sought at the different time points.

| YES / NO |

Who have you spoken to Dr/Mr/Ms .............................
I have understood that I can withdraw my child from the study:
• at any time
• without having to give a reason
• and without affecting the future medical care of my child

| YES / NO |

I agree to allow my child to take part in this study.

| YES / NO |

| Name of Patient ................................................. |
| Name of Parent/Guardian ........................................ |
| Signed ........................................................... |
| Date .............................................................. |
| Name of Physician ................................................. |
| Signature ........................................................ |
| Date .............................................................. |
33.3 Randomisation R3 concerning the induction chemotherapy regimen

You have been told that your child has high-risk neuroblastoma which is a life threatening disease. Intensive treatments as proposed below are intended to offer a realistic chance of your child overcoming this disease. Potential toxicity from the treatment has been carefully weighed up against the severity of your child’s disease. The reason for randomisation has been explained to you. We would now like you to confirm your willingness to participate in the R3 randomisation after which your child will be assigned one of the two proposed induction chemotherapy regimens by chance.

WHAT IS INDUCTION CHEMOTHERAPY FOR?

The aim of induction chemotherapy is to reduce the burden of tumour, both where the primary tumour is (where it initially arose) and in all sites where it might have spread. We know that this is best achieved with intensive chemotherapy. Several studies across Europe and the US have shown that the use of intensive induction chemotherapy improves the survival of children with high-risk neuroblastoma. The use of intensive chemotherapy avoids the neuroblastoma cells becoming resistant to treatment. Improvement in supportive care has also made further intensification of the treatment possible. This means that thanks to better use of antibiotics and G-CSF, which helps the blood count to recover quicker, we can deliver induction chemotherapy more safely.

WHAT TYPE OF CHEMOTHERAPY IS PROPOSED? HAS IT BEEN USED BEFORE?

Two different chemotherapy induction regimens are proposed in this trial, namely Rapid-COJEC and Modified N7. For both regimens investigators have reported improvements in survival in comparison with other regimens given in the past that were less intensive. However, these two regimens have never been compared head-to-head in the same trial demonstrating that one is better than the other in reducing the burden of tumour, improving the number of children that are cured or in reducing toxicity.

1. The Rapid COJEC regimen was developed in the United Kingdom. This regimen was shown in a previous randomised study to be better than less intensive chemotherapy. About 1500 patients have received it throughout Europe in the past decade. There are three different drug combinations used over eight cycles. Cycle A uses the anti-cancer drugs vincristine, etoposide and carboplatin, cycle B uses vincristine and cisplatin and cycle C cyclophosphamide, etoposide and vincristine. They are given every 10 days over 80 days regardless of blood count recovery.

2. The Modified N7 regimen was initially developed in the Memorial Sloan Kettering Cancer Centre in the US and has been extensively used since, with some modifications, across the US, France and Austria in more than 500 patients. The regimen that will be used in this trial includes five courses of chemotherapy. It has been shown that giving more than 5 courses does not improve outcome and adds more toxicity. There are two different drug combinations used during the 5 cycles. Cycle CAV consists of Cyclophosphamide, doxorubicin (Adriamycin) and Vincristine and cycle PE consists of cisPlatin and Etoposide.

WHAT ARE THE SCIENTIFIC AIMS OF THE R3 RANDOMISATION?
The scientific aims are to test whether Rapid COJEC or Modified N7 induction chemotherapy results in improved tumour response and ultimately better survival.

**HOW WILL MY CHILD BENEFIT?**

The potential benefit to be gained from participation in this research study is improved control of your child’s disease. Information will also be gained that will be useful to researchers studying this disease that will benefit other children with neuroblastoma in the future.

**WHO IS ALLOWED ON STUDY?**

All children with widespread metastatic disease (stage 4) that have not started treatment yet and are enrolled in the HR-NBL1 trial are eligible for this R3 randomisation.

**ARE THERE SIDE EFFECTS TO BE EXPECTED?**

**The side effects of induction chemotherapy**

The chemotherapy used during induction may cause:

- Severe lowering of the number of blood cells
- Severe impairment of your child’s ability to fight infection.
- Mouth ulcers or other ulcers along the gastrointestinal tract (gut) this is called mucositis, diarrhoea and/or abdominal pain or conversely constipation.
- Nausea and vomiting (feeling and being sick)

Due to the severe lowering of blood cells, transfusions are required and your child is at risk of potentially life-threatening infections.

Some permanent side effects may occur in some children in the long-term, after months or even years of completing the treatment, and those may include hearing loss, deterioration of the kidney function or toxicity to the heart and there is also the possibility that a second cancer may develop as a result of the chemotherapy.

Please note that not all of these side effects will be experienced by all children; they will be closely monitored and managed where necessary long term.

**WHAT WILL HAPPEN TO THE INFORMATION COLLECTED ON MY CHILD?**

If you are willing to allow your child to participate in this randomisation, information about your child's tumour and his/her response to the treatment given will be collected and stored on a computerised web-based database. The data will be analysed along with the information from other similar children from all over Europe, so that we can answer some of the questions we have about this disease. We hope in this way that we will be able to more accurately plan treatment for children in the future. All information relating to your child will be treated in the strictest confidence. Any publication or presentation relating to the results of the study will not include any information that enables your child to be identified.

If you do decide to allow your child to be entered into the study you may decide at any time to change this decision, your child will then be treated with the European standard treatment.

Please do not hesitate to ask the doctors caring for your child for any further information.
# 33.4 Consent Form for Induction Randomisation R3

## High Risk Neuroblastoma 1.5 Study /SIOPEN

**CONSENT FORM**

**For the Induction Randomisation R3**

<table>
<thead>
<tr>
<th>The parent(s) should complete the whole of this sheet by themselves Please delete as necessary</th>
<th>YES / NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>I have been given a copy of and read the information sheet dated…. and version…..</td>
<td>YES / NO</td>
</tr>
<tr>
<td>I have had an opportunity to ask questions and discuss this study.</td>
<td>YES / NO</td>
</tr>
<tr>
<td>I have received satisfactory answers to all of my questions.</td>
<td>YES / NO</td>
</tr>
<tr>
<td>I have understood that if I agree my child will be randomised for this induction chemotherapy question and will receive chemotherapy as indicated by the randomisation result.</td>
<td>YES / NO</td>
</tr>
</tbody>
</table>
| I have understood that I can withdraw my child from the study:  
  • at any time  
  • without having to give a reason  
  • and without affecting the future medical care of my child | YES / NO |
| I agree to allow my child to take part in this study. | YES / NO |

| Name of Patient | .............................................. |
| Name of Parent/Guardian | .............................................. |
| Signed | .............................................. |
| Date | .............................................. |

| Name of Physician | .............................................. |
| Signature | .............................................. |
| Date | .............................................. |
33.5 Immunotherapy Randomisation R2

INFORMATION SHEET

This randomisation will investigate if the addition of immunotherapy with a man-made protein (aldesleukin (IL-2)) to the neuroblastoma specific, humanised antibody (ch14.18/CHO) could help to further improve the survival chances of your child. During the same period your child will also receive oral differentiation treatment with cis-retinoic acid (13cis-RA). This treatment will be given even if only minimal residual disease is suspected in your child’s body. This means disease which is no longer detectable with methods available to date (scans or biopsies) but small amounts of tumour cells may still be present which could be responsible for producing recurrent disease later. Immunotherapy consists of an antibody (ch14.18/CHO), which adheres to a specific neuroblastoma cell surface marker, like a lock and key mechanism, and is directed against neuroblastoma cells and a man-made protein (aldesleukin (IL-2)) that stimulates the immune system and which is known to support the function of the antibody mentioned above.

HOW IS THE IMMUNOTHERAPY EXPECTED TO WORK?

Ch14.18/CHO is a monoclonal antibody. Monoclonal antibodies are proteins made in the lab, designed to bind to specific cancer cells. Ch14.18/CHO was designed to bind to neuroblastoma cells and other cancer cells that express the GD-2 antigen. When ch14.18/CHO binds to the neuroblastoma cells, the body’s immune system is stimulated to attack and kill the neuroblastoma cells. Ch14.18/CHO represents a new kind of cancer therapy that, unlike chemotherapy and radiation, targets the destruction of cancer cells without destroying nearby healthy cells. There is laboratory evidence to suggest that ch14.18/CHO can target the body’s own immune cells to destroy cancer cells. These immune cells armed by ch14.18/CHO include cells that are activated by aldesleukin (IL-2).

Aldesleukin (IL-2) is a substance that is similar to a substance made by the body in all individuals. Under normal circumstances, the body makes small amounts of aldesleukin (IL-2) which helps white blood cells fight infection. It is now possible to make aldesleukin (IL-2) outside of the body and give humans much higher doses than their own body makes. There is some evidence that, in the lab and in animals, aldesleukin (IL-2) increases the anticancer effect of monoclonal antibodies like ch14.18/CHO. We wish to study whether aldesleukin (IL-2) actually improves the efficacy and effectiveness of ch14.18/CHO in humans.

WHAT ARE THE TREATMENT OPTIONS?

This randomisation has two treatment options. The two treatment options are:

- Arm A: Treatment with isotretinoin (13-cis-RA) and the antibody ch14.18/CHO. Isotretinoin (13-cis-RA) is a drug closely related to vitamin A and has been shown to help stop the multiplication of remaining neuroblastoma cells.
- Arm B: Treatment with isotretinoin (13-cis-RA), the antibody ch14.18/CHO and aldesleukin (IL-2). This is the experimental arm of the study.
WHAT IF I DON’T WANT MY CHILD TO PARTICIPATE IN THIS PART OF THE STUDY?

If you do not want your child to participate in this randomisation then your child will receive maintenance treatment with 6 months of isotretinoin (13-cis-RA) alone which will start approximately three months after the stem cells are given back to your child. This drug will be given twice a day by mouth for 14 days and then not given for the next 14 days. This 28 day course will be repeated six times.

HAS IMMUNOTHERAPY ALREADY BEEN USED?

Yes. A recent study done by the Children’s Oncology Group, in the US, showed that survival rates (proportion of children that are alive and free of disease) at 2 years were better for patients who received immunotherapy, in addition to isotretinoin (13-cis-RA).

WHAT IS THE SCIENTIFIC AIM OF THE R2 RANDOMISATION?

Today it is not clear which part of the immunotherapy combination increases survival. The addition of immune modulators to the antibody may increase the efficacy of the antibody however they are known to increase the toxicity of the immunotherapy. It may or may not be necessary to give a combination therapy to improve survival. The current study will recruit enough patients to address this question. The antibody with or without aldesleukin (IL-2) will be given in addition to isotretinoin (13-cis-RA) after high-dose therapy and local irradiation to patients who are randomised. Two independent mechanisms of anti-neuroblastoma activity will be used to target the tumour from two different angles. One is indirect via activation of the immune system (the antibody and aldesleukin both work in this way), the other (isotretinoin (13-cis-RA)) is direct by induction of neuroblastoma cell death. The aim of this part of the study is to see if adding aldesleukin to treatment with isotretinoin (13-cis-RA) and the antibody ch14.18/CHO increases survival.

WHO IS ALLOWED ONTO THIS PART OF THE STUDY?

In this trial the monoclonal antibody ch14.18/CHO will be given to all patients fulfilling the R2 entry criteria and who are randomised. Prior to the start of the antibody treatment, patients have to be in a stable condition without clinical, radiological or laboratory signs of infection. Aldesleukin will only be given to those patients randomised in R2 to receive it. (i.e. Arm B).

IS MY CHILD ALLOWED TO RECEIVE IMMUNOTHERAPY OUTSIDE THE RANDOMISATION?

Neuroblastoma antibody is not readily available. This is because the antibody is produced specifically for children with neuroblastoma and is not yet commercially available.

WHEN WILL IMMUNOTHERAPY BE GIVEN?

In this trial patients will be randomised (R2) to either Arm A (isotretinoin (13-cis-RA) and the antibody) or Arm B (isotretinoin (13-cis-RA), the antibody and aldesleukin) after completion of the disease evaluation following high-dose therapy. Patients will usually get radiotherapy to the primary tumour prior to starting immunotherapy. All randomised patients will receive the antibody. Patients will start the antibody treatment three weeks after the start of treatment with isotretinoin (13-cis-RA). The antibody is given every day for five days every four weeks for five courses.
Patients randomised to Arm B will also receive aldesleukin; this will begin directly after each treatment with isotretinoin (13-cis-RA). Aldesleukin is given subcutaneously every day for 5 days over two weeks for five courses.

**HOW IS IMMUNOTHERAPY GIVEN?**

The antibody is given by slow intravenous infusion over about eight hours every day for 5 days. Your child will be admitted to hospital the day or the evening prior to the antibody infusion. Before the infusion starts your child will be given pre-medications. This will consist of anti-allergic medication, and a continuous pain killing morphine infusion. The morphine co-medication is necessary to control the pain which would otherwise occur during the antibody infusion.

Aldesleukin is given subcutaneously (an injection under the skin). During the days that the antibody is also given, Aldesleukin will be given two hours after the stop of the antibody infusion.

**BLOOD SAMPLING DURING IMMUNOTHERAPY**

For each of the five courses of immunotherapy a total of 10 blood samples (1-2 ml) will be taken from a central line of your child. The total amount of blood taken over each 28 day cycle will be 10-20 mls (2-4 tea-spoons). Patients will also be asked for additional samples to be taken on immunotherapy treatment days 0 and 4 for each cycle. The total amount of blood taken over each 28 day cycle for pharmacokinetics with these additional samples will be 24-48 mls (around 3 tablespoons).

**ARE THERE ANY SIDE EFFECTS?**

Yes. While receiving treatment in this part of the study your child may experience the side effects mentioned in the following tables. There may also be side effects that cannot be predicted. Some of the increased side effects may occur during therapy (such as low blood counts and increased risk of infection). Other side effects that could occur later might also be life threatening, or even fatal. It is rare, but possible that your child could develop another cancer as a result of treatment on this study. You should discuss these potential risks with your child’s doctor. Other drugs will be given to make side effects less serious and less uncomfortable. Patients are watched carefully and treatment is stopped if serious side effects develop.

**Reproductive risks:** It is unknown what effect(s) these treatments may have on an unborn child. For this reason, if your child is of child-bearing age, your child will be asked to practice an effective method of birth control while participating on this study. The use of isotretinoin (13-cis-RA) can cause serious birth defects to unborn children if taken during pregnancy.

**Side effects of drugs given in the R2 randomisation**

Risks and side effects related to isotretinoin (13-cis-RA) include those which are:

<table>
<thead>
<tr>
<th>Likely</th>
<th>Less likely</th>
<th>Rare but serious</th>
</tr>
</thead>
</table>
| • Dryness of the skin and mucous membranes  
  • Dry, cracked and bleeding lips  
  • An increased tendency to sun burn  
  • Bloody nose from dry membranes of the nose  
  • Aches and pains in the | • Rash and itching  
  • Headache  
  • Increase in cholesterol and a decrease in the good fat in the blood  
  • Red eyes  
  • Elevation in the blood of certain enzymes found in the liver which may mean liver irritation or damage.  
  • Fewer red blood cells and white blood | • Severe allergic reaction which can be life threatening with shortness of breath, low blood pressure, rapid heart rate, chills and fever  
  • Irritation of the small airways in the lungs that can cause coughing and wheezing  
  • An allergic reaction in the |
<table>
<thead>
<tr>
<th>Joints</th>
<th>Blood vessels of the skin which turn the skin red, inflamed and bumpy and which may lead to skin breakdown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Back pain</td>
<td>A severe lowering of the white blood count which can cause susceptibility to infections which could be life threatening</td>
</tr>
<tr>
<td>Elevation of the fats in the blood</td>
<td>Convulsions</td>
</tr>
<tr>
<td>Increase in calcium in the blood which may require decreasing the dose</td>
<td>Brain swelling that can cause symptoms of severe headache, nausea and vomiting, and changes to vision including blurriness and pressure behind the eyes</td>
</tr>
<tr>
<td>An increase in a laboratory test on the blood that may measure some non specified inflammation which may or may not be of any importance</td>
<td>Life threatening or fatal changes in moods have occurred including severe depression or feelings of suicide and feelings of aggressiveness and violent behaviour</td>
</tr>
<tr>
<td>Too many platelets in the blood</td>
<td>Thinning of the bone (osteoporosis) which could lead to weakness of the bone, bone fractures or delay in healing of fractures</td>
</tr>
<tr>
<td>Loss or thinning of hair</td>
<td>Inflammation of the pancreas which can lead to severe abdominal pain and in some very rare cases can be fatal</td>
</tr>
<tr>
<td>Appetite disturbances causing lack of appetite or unusually hungriness</td>
<td>Damage to the muscle which can release a protein that can cause severe damage to the kidneys</td>
</tr>
<tr>
<td>Weight loss</td>
<td>Inflammation of the intestinal tract which can result in diarrhoea and bleeding</td>
</tr>
<tr>
<td>Increase in blood sugar levels</td>
<td>&quot;cells and platelets in the blood&quot;</td>
</tr>
<tr>
<td>A darkening or lightening of the skin</td>
<td>1. a low number of red blood cells can cause tiredness and weakness</td>
</tr>
<tr>
<td>Finger and toe nail changes including breaking or splitting more easily</td>
<td>2. a low number of white blood cells can make it easier to get infections</td>
</tr>
<tr>
<td>The sudden appearance of little yellow raised bumps on the skin usually because the cholesterol in the blood is too high (xanthomas)</td>
<td>3. a low number of platelets causes bruising and bleeding to happen more easily</td>
</tr>
<tr>
<td>Dizziness</td>
<td>Too many platelets in the blood</td>
</tr>
<tr>
<td>Difficulty falling asleep or staying asleep and strange dreams</td>
<td>Loss or thinning of hair</td>
</tr>
<tr>
<td>A feeling of tiredness or not feeling well</td>
<td>Appetite disturbances causing lack of appetite or unusually hungriness</td>
</tr>
<tr>
<td>Nervousness</td>
<td>Weight loss</td>
</tr>
<tr>
<td>Numbness and tingling in the fingers and toes</td>
<td>Increase in blood sugar levels</td>
</tr>
<tr>
<td>Difficulty hearing clearly or a ringing in the ears</td>
<td>A darkening or lightening of the skin</td>
</tr>
<tr>
<td>Changes in vision including more difficulty seeing at night, blurred vision, changes in colour vision, pain or squinting in bright light, and cataract formation</td>
<td>Finger and toe nail changes including breaking or splitting more easily</td>
</tr>
<tr>
<td>Fluid retention</td>
<td>The sudden appearance of little yellow raised bumps on the skin usually because the cholesterol in the blood is too high (xanthomas)</td>
</tr>
<tr>
<td>Chest pain</td>
<td>Too many platelets in the blood</td>
</tr>
<tr>
<td>Inflammation of the gums</td>
<td>Loss or thinning of hair</td>
</tr>
<tr>
<td>A dry throat which could lead to a change in your child’s voice and more throat infections</td>
<td>Appetite disturbances causing lack of appetite or unusually hungriness</td>
</tr>
<tr>
<td>Slowed growth</td>
<td>Weight loss</td>
</tr>
<tr>
<td>Irregular periods</td>
<td>Increase in blood sugar levels</td>
</tr>
<tr>
<td>Mild kidney damage which could lead to blood or protein in the urine or renal stones</td>
<td>A darkening or lightening of the skin</td>
</tr>
<tr>
<td>Extra bone growth along the spine and a tendency for calcium deposits in the tendons and ligaments where they attach to the bone which can lead to pain or stiffness and arthritis of the back and tendonitis</td>
<td>Finger and toe nail changes including breaking or splitting more easily</td>
</tr>
</tbody>
</table>

- Too many platelets in the blood
- Loss or thinning of hair
- Appetite disturbances causing lack of appetite or unusually hungriness
- Weight loss
- Increase in blood sugar levels
- A darkening or lightening of the skin
- Finger and toe nail changes including breaking or splitting more easily
- The sudden appearance of little yellow raised bumps on the skin usually because the cholesterol in the blood is too high (xanthomas)
- Dizziness
- Difficulty falling asleep or staying asleep and strange dreams
- A feeling of tiredness or not feeling well
- Nervousness
- Numbness and tingling in the fingers and toes
- Difficulty hearing clearly or a ringing in the ears
- Changes in vision including more difficulty seeing at night, blurred vision, changes in colour vision, pain or squinting in bright light, and cataract formation
- Fluid retention
- Chest pain
- Inflammation of the gums
- A dry throat which could lead to a change in your child’s voice and more throat infections
- Slowed growth
- Irregular periods
- Mild kidney damage which could lead to blood or protein in the urine or renal stones
- Extra bone growth along the spine and a tendency for calcium deposits in the tendons and ligaments where they attach to the bone which can lead to pain or stiffness and arthritis of the back and tendonitis
- Too many platelets in the blood
Risks and side effects related to the antibody include those which are:

<table>
<thead>
<tr>
<th>Likely</th>
<th>Less Likely</th>
<th>Rare but serious</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Pain which may be in the back, abdomen (belly) as cramping, joints, arms, legs and other parts of the body and will require administration of pain relieving medications during the infusion</td>
<td>• A moderate drop or rise in blood pressure which may require treatment</td>
<td>• Severe allergic reactions which can be life threatening with shortness of breath or wheezing, low oxygen levels in the blood, low blood pressure and a rapid heart rate</td>
</tr>
<tr>
<td>• Numbness and tingling in the fingers and toes</td>
<td>• Vomiting</td>
<td>• Severe allergic reaction which can be life threatening with rapid build-up of fluid under the skin, in the lining of the intestine and possibly in the throat or swelling of the tongue which could make it difficult to breathe</td>
</tr>
<tr>
<td>• A slight drop or rise in blood pressure</td>
<td>• Diarrhoea</td>
<td>• Irritation of the small airways of the lungs that can cause coughing and wheezing</td>
</tr>
<tr>
<td>• A fast heartbeat which may cause pain in the chest</td>
<td>• Low level of salts in the blood such as sodium or potassium which may require treatment</td>
<td>• Seizures</td>
</tr>
<tr>
<td>• Itching and hives</td>
<td>• A feeling of sleepiness, an inability to stay awake or aware or be aroused by someone else</td>
<td>• A rapid heart beat which could be life threatening</td>
</tr>
<tr>
<td>• Fever</td>
<td>• Weight loss</td>
<td>• Chest pain that may mean heart damage</td>
</tr>
<tr>
<td>• Nausea</td>
<td>• An allergic reaction that causes fever, aches and pains in the joints, skin rash and swollen lymph glands</td>
<td>• A sudden stopping of the heart or breathing</td>
</tr>
<tr>
<td>• Low level of salt in the blood</td>
<td>• Increased levels of a chemical (creatinine) in the blood which could mean kidney damage</td>
<td>• Vascular leak syndrome: A condition in which fluid and proteins leak out of tiny blood vessels and flow into surrounding tissues, resulting in dangerously low blood pressure. Vascular capillary leak syndrome may lead to multiple organ failure such as kidney, heart or liver failure and shock</td>
</tr>
<tr>
<td>• Loss of Appetite</td>
<td>• Elevation in the blood of certain enzymes found in the liver</td>
<td>• Severe rashes which can result in loss of skin and damage to mucous membranes and which may be life-threatening</td>
</tr>
</tbody>
</table>

- Fewer platelets in the blood, a low number of platelets causes bruising and bleeding to happen more easily
- A problem in nerve function that may cause pain, numbness, tingling, and muscle weakness in various parts of the body
- Drooping of the eyelids, blurred vision or inability of the eye to react to bright or dim light, increased sensitivity to bright light. This is usually reversible but vision may be affected for a number of months and during this period glasses may improve vision or existing prescription glasses may need to be altered
- Clotting of the intravenous central line

- Severe allergic reaction which can be life threatening with rapid build-up of fluid under the skin, in the lining of the intestine and possibly in the throat or swelling of the tongue which could make it difficult to breathe
- Irritation of the small airways of the lungs that can cause coughing and wheezing
- Seizures
- A rapid heart beat which could be life threatening
- Chest pain that may mean heart damage
- A sudden stopping of the heart or breathing
- Vascular leak syndrome: A condition in which fluid and proteins leak out of tiny blood vessels and flow into surrounding tissues, resulting in dangerously low blood pressure. Vascular capillary leak syndrome may lead to multiple organ failure such as kidney, heart or liver failure and shock
- Severe rashes which can result in loss of skin and damage to mucous membranes and which may be life-threatening
- Swelling in the back of the eye caused by an increase in pressure in the brain
- Damage to the optic nerve (nerve from the brain to the eye) leading to potentially permanent decreased vision
Risks and side effects related to aldesleukin include those which are:

<table>
<thead>
<tr>
<th>Likely</th>
<th>Less Likely</th>
<th>Rare but Serious</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever and chills including shaking chills</td>
<td>Nausea and Vomiting</td>
<td>A severe allergic reaction that can be life-threatening and may lead to difficulty in breathing, a drop in blood pressure, and an irregular heart beat.</td>
</tr>
<tr>
<td>Flu like symptoms with headache, tiredness, aches and pains</td>
<td>Low levels of certain salts in the body like sodium, calcium, potassium, magnesium and phosphate which may require treatment</td>
<td>Heart attack or severe pain in the chest (angina) that can be life-threatening or fatal.</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>Upset of the normal acid levels in the blood</td>
<td>Inflammation of the heart muscle which could lead to heart failure</td>
</tr>
<tr>
<td>Loss of Appetite</td>
<td>A condition (vascular or capillary leak syndrome) in which fluid and proteins leak out of tiny blood vessels and flow into surrounding tissues, resulting in dangerously low blood pressure which may lead to multiple organ failure such as kidney, heart or liver failure and shock.</td>
<td>A severe drop in blood pressure that will require treatment</td>
</tr>
<tr>
<td>A feeling of weakness and/or tiredness not relieved by sleep or rest</td>
<td>Heart problems including an irregular or rapid heart beat, chest pain</td>
<td>A decrease in the factors in the blood that help the blood to clot normally</td>
</tr>
<tr>
<td>A mild drop in blood pressure</td>
<td>Dizziness</td>
<td>Bleeding which can occur in the head, stools, the nose, urine and other parts of the body</td>
</tr>
<tr>
<td>Rashes with itching</td>
<td>Cough, runny nose</td>
<td>Seizures</td>
</tr>
<tr>
<td>Fluid retention and build-up in the tissues usually of the lower legs leading to an increase in weight</td>
<td>Headache</td>
<td>Prolonged loss of consciousness (coma)</td>
</tr>
<tr>
<td>Increased levels of a chemical (creatinine) in the blood which could mean kidney damage</td>
<td>Enlarged abdomen (belly) and weight gain</td>
<td>Inflammation of the colon (large bowel) which could lead to bloody diarrhoea and may be life threatening or a hole may develop in the intestines which would cause leakage into the abdomen (belly) with pain and infection</td>
</tr>
<tr>
<td>A temporary decrease in the amount of urine which could mean the kidneys are not working as well</td>
<td>Pain in the abdomen (belly) or other parts of the body</td>
<td>Inflammation of the pancreas which could cause pain in the abdomen and may be life-threatening</td>
</tr>
<tr>
<td>Elevation in the blood of certain enzymes or bilirubin found in the liver</td>
<td>High blood sugar which may require treatment</td>
<td>Severe kidney damage (which may be permanent)</td>
</tr>
<tr>
<td>An increase in the blood of a type of white blood cell called an Eosinophil. These are sometimes associated with allergic reactions</td>
<td>Mood changes including depression, inability to sleep or excessive sleepiness, irritability and agitation, anxiety, confusion, and mood swings such as feelings of suicide, feelings of aggressiveness</td>
<td>If your child has ever been told that he/she has a disease such as lupus, rheumatoid arthritis or other disease that is caused by a disturbance in the immune system “autoimmune disease”. Aldesleukin (IL-2) may cause these to be worse.</td>
</tr>
<tr>
<td>An increase in the number of white blood cells in the blood</td>
<td>Aches and Pains in the muscles and joints, sleep difficulties, a feeling of extreme tiredness or not feeling well</td>
<td>Damage to the lungs which could lead to shortness of breath</td>
</tr>
<tr>
<td>Fewer red blood cells and platelets in the blood</td>
<td>Inflammation and/or sores in the mouth that may make swallowing difficult and are painful (painful mouth sores)</td>
<td>Sudden death</td>
</tr>
<tr>
<td>o a low number of red blood cells can cause feel tiredness and weakness</td>
<td>Severe rashes which can result in loss of skin</td>
<td></td>
</tr>
<tr>
<td>o a low number of platelets causes bruising and bleeding to happen more easily</td>
<td>Infections including those caused by bacteria, virus, and fungus which may require treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blurred vision</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A flushing of the skin with redness and a feeling of warmth</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fewer white cells in the blood</td>
<td></td>
</tr>
<tr>
<td></td>
<td>o a low number of white blood cells may make it easier to get infections</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Decrease or increase in the thyroid hormones</td>
<td></td>
</tr>
</tbody>
</table>
WHAT WILL HAPPEN TO INFORMATION COLLECTED ABOUT MY CHILD?

If you are willing to allow your child to participate in this randomisation, information about your child's tumour and his/her response to the treatment given will be collected and stored on a computerised database (accessible in your own country and also in the main data centre in Vienna). The data will be analysed along with the information from other similar children from all over Europe and beyond, so that we can answer some of the questions we have about this disease. We hope in this way that we will be able to more accurately plan treatment for children in the future. All information relating to your child will be treated in the strictest confidence. Any publication or presentation relating to the results of the study will not include any information that enables your child to be identified.

WHAT IF I CHANGE MY MIND?

If you do decide to allow your child to be entered into the study you may decide at any time to change this decision. If you do change your mind the doctors and nurses will still continue to look after your child.

Please do not hesitate to ask the doctors caring for your child for any further information which you might require.
## Consent Form for Randomisation R2

### High Risk Neuroblastoma 1.5 Study / SIOPEN

**CONSENT FORM**

for the Randomisation R2 of the antibody (ch14.18/CHO) with or without aldesleukin (IL-2)

---

<p>| The parent(s) should complete the whole of this sheet himself/herself, (themselves) |</p>
<table>
<thead>
<tr>
<th>Please delete as necessary</th>
</tr>
</thead>
<tbody>
<tr>
<td>I have been given a copy of and read the information sheet dated… and version ….</td>
</tr>
<tr>
<td>I have had an opportunity to ask questions and discuss this study and in particular have discussed the potential side effects of this therapy listed in the information sheet.</td>
</tr>
<tr>
<td>I have received satisfactory answers to all of my questions.</td>
</tr>
<tr>
<td>I have understood that if I agree my child will be randomised and will receive the antibody with the addition of aldesleukin only if indicated by the randomisation result.</td>
</tr>
<tr>
<td>I have had time to ask questions and understood that I can withdraw my child from the study: • at any time • without having to give a reason • and without affecting the future medical care of your child</td>
</tr>
<tr>
<td>I agree to allow my child to take part in this randomisation.</td>
</tr>
</tbody>
</table>

| Name of Patient | ................................................. |
| Name of Parent/Guardian | ................................................. |
| Signed | ................................................. |
| Date | ................................................. |

| Name of Physician | ................................................. |
| Signature | ................................................. |
| Date | ................................................. |
34 Reference List


HRNBL1.5/SIOPEN valid per 01.06.2011
