A multi-centre open label randomised phase III trial of the efficacy of Sodium Thiosulphate in reducing ototoxicity in patients receiving Cisplatin chemotherapy for

**STANDARD RISK HEPATOBLASTOMA**

International Childhood Liver Tumour Strategy Group – SIOPEL

Version 4.0, 01 May 2011

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2 CONFIDENTIALITY STATEMENT AND SIGNATURES

This document describes a multi-centre open label randomised phase III clinical trial of the efficacy of Sodium Thiosulphate in reducing ototoxicity in children receiving Cisplatin chemotherapy for standard risk hepatoblastoma and provides information for entering patients into it. It is not intended for use as an aide-memoire or guide for the treatment of non-registered patients. Although every care was taken in its drafting, amendments may be necessary. These will be circulated to known participants in the trial, but centres entering patients for the first time should contact the Data Centre to confirm the correctness of the protocol in their possession and to obtain the necessary authorisation to enter patients into the trial. Participants are requested to maintain confidentiality regarding the contents of this protocol. Responsibility for the administration of the protocol treatments and compliance with national legal requirements lies with the participants.

Protocol approved by:

Signatures:

__________________________________
International Chairman              Date

__________________________________
International Vice-Chairman           Date

__________________________________
Responsible Statistician               Date
<table>
<thead>
<tr>
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<th>Past SIOPEL Chairman</th>
<th>SIOPEL 6 International Chairman</th>
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<th>Statistician</th>
<th>Transplant Co-ordinator</th>
<th>Audiology Co-ordinator</th>
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## 4. LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ABR</td>
<td>Auditory Brainstem Response</td>
</tr>
<tr>
<td>AC</td>
<td>Air Conduction</td>
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<tr>
<td>AE</td>
<td>Adverse Event</td>
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<tr>
<td>AFP</td>
<td>Alpha-Fetoprotein</td>
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<tr>
<td>ALT</td>
<td>Alanine Aminotransferase</td>
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<tr>
<td>ANC</td>
<td>Absolute Neutrophil count</td>
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<td>ARP</td>
<td>Advisory Radiological Panel</td>
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<td>ASHA</td>
<td>American Speech-Language-Hearing Association</td>
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<tr>
<td>AST</td>
<td>Aspartate Aminotransferase</td>
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<td>ß-HCG</td>
<td>Beta-Human Chorionic Gonadotropin</td>
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<td>BC</td>
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<td>BP</td>
<td>Blood Pressure</td>
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<td>BSA</td>
<td>British Society of Audiology</td>
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<tr>
<td>BSA</td>
<td>Body Surface Area</td>
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<tr>
<td>CCLG</td>
<td>Children’s Cancer and Leukaemia Group (formerly UKCCSG)</td>
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<td>CDDP</td>
<td>Cisplatin</td>
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<tr>
<td>CINECA</td>
<td>Consorzio Interuniversitario</td>
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<td>CIOMS</td>
<td>Council for International Organization of Medical Sciences</td>
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<td>CR</td>
<td>Complete Remission</td>
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<td>CRCTU</td>
<td>Cancer Research UK Clinical Trials Unit</td>
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<tr>
<td>CRR</td>
<td>Complete Resection Rate</td>
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<td>CT</td>
<td>Computed Tomography</td>
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<td>CTA</td>
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<tr>
<td>CTCAE</td>
<td>Common Terminology Criteria for Adverse Events</td>
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<tr>
<td>CXR</td>
<td>Chest X-Ray</td>
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<td>dB</td>
<td>decibel</td>
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<tr>
<td>daPa</td>
<td>deca Pascal</td>
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<tr>
<td>DPOAE</td>
<td>Distortion Product Otoacoustic Emission</td>
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<tr>
<td>DNA</td>
<td>Deoxyribo Nucleic Acid</td>
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<td>ECochG</td>
<td>Electrocochleography</td>
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<td>EFS</td>
<td>Event Free Survival</td>
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<tr>
<td>FBC</td>
<td>Full Blood Cell count</td>
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<td>FFPE</td>
<td>Formalin Fixed Paraffin Embedded</td>
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<td>FT</td>
<td>Frozen Tumour</td>
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<td>GCP</td>
<td>Good Clinical Practice</td>
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<tr>
<td>GFR</td>
<td>Glomerular Filtration Rate</td>
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<td>HB</td>
<td>Hepatoblastoma</td>
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<td>HCC</td>
<td>Hepatocellular Carcinoma</td>
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<td>HL</td>
<td>Hearing Level</td>
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<tr>
<td>HR-HB</td>
<td>High Risk Hepatoblastoma</td>
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<td>ICH</td>
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<td>IDMC</td>
<td>Independent Data Monitoring Committee</td>
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<tr>
<td>IHCs</td>
<td>Inner hair cells</td>
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<tr>
<td>IMP</td>
<td>Investigational Medicinal Product</td>
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<td>IRB</td>
<td>Institutional Review Board</td>
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<td>IV</td>
<td>Intravenous</td>
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<td>LLS</td>
<td>Left Lateral Section</td>
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<td>Left Medial Section</td>
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<tr>
<td>LTX</td>
<td>Liver Transplantation</td>
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<tr>
<td>LRLTX</td>
<td>Living Related Donor Liver Transplantation</td>
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<tr>
<td>MHRA</td>
<td>Medicines and Healthcare Products Regulatory Agency</td>
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</table>
MR  Magnetic Resonance Imaging
mmho  milli-mho, equal to millisiemens (mS)
MRA  Magnetic Resonance Angiography
MUAC  Mid-Upper Arm Circumference
NCI  National Cancer Institute
NHL  Normal Hearing Level
OAE  Otoacoustic Emission
OS  Overall Survival
OHCs  Outer hair cells
OLT  Orthoptic Liver Transplantation
PA  Posterior-Anterior
PD  Progressive Disease
PLADO  Cisplatin (=Platinol) and Doxorubicin
POSTEXT  Post-treatment Extent of disease
PR  Partial Response
PRETEXT  Pre-treatment Tumour Extension
RAS  Right Anterior Section
RDE  Remote Data Entry
RPS  Right Posterior Section
RR  Response Rate
RRR  Rapid Radiological Review
SAE  Serious Adverse Event
SAR  Serious Adverse Reaction
SD  Stable Disease (tumour evaluation)
SD  Standard Deviation
SIOP  International Society of Paediatric Oncology
SIOPEL  International Childhood Liver Tumour Strategy Group
SF  Shortening Fraction
SOP  Standard Operating Procedure
SPL  Sound Pressure Level
SR-HB  Standard Risk Hepatoblastoma
STS  Sodium Thiosulphate
SUSAR  Suspected Unexpected Serious Adverse Reaction
TEOAE  Transient Evoked Otoacoustic Emission
UKCCSG  United Kingdom Children's Cancer Study Group (now CCLG)
UNHS  Universal Neonatal (or Newborn) Hearing Screening
US  Ultrasonography
VRA  Visual Reinforcement Audiometry
5 SYNOPSIS

5.1 Title
A multi-centre open label randomised phase III trial of the efficacy of Sodium Thiosulphate (STS) in reducing ototoxicity in patients receiving Cisplatin chemotherapy for standard risk hepatoblastoma.

5.2 Primary objective
- To assess the efficacy of STS to reduce the hearing impairment caused by Cisplatin chemotherapy.

5.3 Secondary objectives
- To carefully monitor any potential impact of STS on response to Cisplatin and survival.
- To assess the short- and long-term tolerability of the combination of STS and Cisplatin.
- To prospectively evaluate and validate biological, radiological and pathological features of standard risk hepatoblastoma for future risk adapted management.
- To investigate the effect of STS on the formation of Cisplatin-DNA adducts.
- To prospectively collect patient DNA specifically for the analysis of possible genetic factors that may contribute to the development of treatment related ototoxicity and nephrotoxicity.

5.4 Trial design
Randomised phase III clinical trial: 1:1 randomisation between Cisplatin alone and Cisplatin + Sodium Thiosulphate.

5.5 Primary end-point
- Rate of Brock grade ≥1 hearing loss determined after end of trial treatment or at an age of at least 3.5 years, whichever is later (see Appendix 5).

5.6 Secondary endpoints
- Response to preoperative chemotherapy
- Complete resection
- Complete remission
- Event free survival (EFS)
- Overall survival (OS)
- Toxicity as graded by CTCAE v 3.0
- Long-term renal clearance
- Feasibility of central audiology review

5.7 Sample size and duration
- Projected accrual: 35 patients/year
- Projected total accrual: 102 evaluable patients (115 randomised patients)
- Expected duration of recruitment period: 3.8 years
5.8 Selection of patients

5.8.1 Inclusion criteria

- Histologically confirmed newly diagnosed hepatoblastoma
- Standard risk hepatoblastoma (defined in section 10.1)
- Age ≤ 18 years and > 1 month
- Written informed consent and national/local ethics committee and regulatory approval
- Centre/country willing and able to organise audiometry at the minimum required quality standard and to provide the contact details of the Consultant Audiologist or Ear Nose and Throat Surgeon who will take the responsibility for seeing that this is done (see Section 15.9)
- Ability to comply with requirements for submission of material for central review
- For females of child-bearing potential, a negative pregnancy test prior to study treatment is required.
- Any patient who is of reproductive age should agree to use adequate contraception for the duration of the trial.

5.8.2 Exclusion criteria

- High risk hepatoblastoma (defined in section 10.2)
- Hepatocellular carcinoma
- Treatment starting more than 15 days from written biopsy report
- Abnormal renal function (defined in section 10.2)
- Any previous chemotherapy
- Recurrent disease
- Previous hypersensitivity to STS
- Patient unable to follow the protocol for any reason

5.9 Trial treatment

The Investigational Medicinal Products (IMPs) to be studied in this trial are Sodium Thiosulphate (STS) and Cisplatin. STS will be supplied free of charge by Adherex Technologies.

Trial treatment consists of the following phases:

- Pre-operative randomised 1:1 chemotherapy (4 courses of Cisplatin, with or without STS, every second week)
- Surgical removal of all remaining tumour lesions. If surgery has to be delayed, 1 or 2 cycles of the post-operative chemotherapy may be given pre-operatively.
- Post-operative chemotherapy (2 courses of Cisplatin, with or without STS, every second week)
- Patients with progressive disease after 2 or more courses of Cisplatin, with or without STS, will stop trial treatment

5.10 Toxicity monitoring

- The trial treatment is potentially toxic and children are at risk of developing myelo-, mucosal- or other reversible toxicity as well as irreversible oto- and renal toxicity.
- Toxicity monitoring will be required for each child throughout trial treatment and at regular follow-up intervals.
- Reporting of specific renal and oto-toxicity will be required as an adverse event. Serious Adverse Events (SAE, see section 17) must be reported immediately on knowledge of the event. The trial committee will evaluate toxicity reports on a regular basis and may stop the trial if an unacceptable rate of severe toxicity is recognized.
Dose and treatment modifications due to toxicity are specified in the protocol.
Long-term follow up of patients in this trial will include any late toxicity.

5.11 Central radiological, pathological and audiological review
Central review of pathology slides and of radiological images is mandatory. All audiologic data will be centrally reviewed, see section 15.9.9.

5.12 Statistical considerations

5.12.1 Sample size
The trial is designed to detect a 25% reduction in the rate of Brock grade ≥1 hearing loss with a chi-square test, from a 60% hearing loss in the Cisplatin alone arm to a 35% hearing loss in the Cisplatin + STS arm.

5.12.2 Early stopping and Interim analysis
Early stopping may be warranted in case of convincing evidence that a reduction in hearing impairment by at least 25% is corroborated.

Two interim analyses are planned for early stopping in case of a greater than expected difference between treatment arms in terms of hearing loss.

Interim evaluations of efficacy of the chemotherapy will also be carried out after 20, 40, 60 and 80 patients are evaluable for response, and the results will be reviewed by an Independent Data Monitoring Committee. Early stopping will be considered in case of concerns on efficacy of chemotherapy in either treatment arm.

5.12.3 Final evaluation
The final evaluation will be done as soon as the primary endpoint can be determined for 102 patients.

5.12.4 Definition of study completion
The primary endpoint will be reached when the last patient has completed his/her audiological testing or reached 3.5 years of age, whichever is later. Patients will then be followed up for the secondary endpoints according to national guidelines.
6 PREAMBLE

The SIOPEL 6 trial is part of a comprehensive research strategy developed by the International Childhood Liver Tumour Strategy Group (SIOPEL) which among others presently includes:

- a prospective single arm clinical trial for high risk hepatoblastoma with intensified pre-operative chemotherapy and radical surgery (SIOPEL 4)
- a phase II trial for relapsing and/or resistant hepatoblastoma (with Irinotecan)
- a clinical trial on hepatocellular carcinoma (HCC) family of tumours in children, adolescents and young adults (SIOPEL 5 (HCC-1))
- an international childhood liver tumour tissue banking program.

The SIOPEL 6 trial is part of the fourth generation of clinical trials run by the SIOPEL group. The present trial is a co-operative international randomised phase III trial of the efficacy of Sodium Thiosulphate (STS) in reducing ototoxicity in children and adolescents receiving Cisplatin chemotherapy for standard risk hepatoblastoma. The standard risk group includes those hepatoblastomas that involve at most three hepatic sections, classified as PRETEXT I, II or III according to the pre-treatment extent of disease system (2005 PRETEXT revision) developed for the SIOPEL trials, and without evidence of extra-hepatic disease and with alpha-fetoprotein (AFP) value at diagnosis >100 µg/L. Patients with low AFP, PRETEXT IV and/or additional PRETEXT criteria are classified as high risk and treated according to SIOPEL 4.

The cure rate for standard risk hepatoblastoma reaches promising levels with Cisplatin monotherapy and surgery but Cisplatin is well known to cause permanent bilateral high frequency hearing loss. The present SIOPEL 6 trial is based on the hypothesis that in a group of patients with high chance of cure it might be possible to prevent ototoxicity and late sequelae without reducing the cure rate.
7 BACKGROUND AND RATIONALE

7.1 Classification of hepatoblastoma

Hepatoblastoma (HB) is an embryonal tumour containing hepatic epithelial parenchyma and/or mesenchymal components (epithelial types and mixed epithelial and mesenchymal types). Based on the epithelial components, four major histological sub-types exist, whereas the two mixed sub-types are distinguished by the presence or absence of teratoid features. Epithelial sub-types are frequently intermixed, but each may exclusively comprise a tumour.

7.1.1 Epithelial morphology: sub-types

A - Fetal - Tumour cells are smaller than hepatocytes in adjacent liver, have a low nucleus-cytoplasm ratio, minimal nuclear pleomorphism and small nucleoli. Mitoses are infrequent. Fetal cells may form either slender cords which often contain canaliculi and definite sinusoids or compact mosaic sheets. Fetal cells may contain abundant lipid or glycogen or have granular eosinophilic or amphophilic cytoplasm. When 100% of the tumour is composed of this epithelial cell type, the term, pure fetal histology, has been proposed. Central vein-like vessels may be present but biliary ducts are not a feature of fetal HB.

B - Embryonal - Tumour cells have a higher nucleus-cytoplasm ratio and sparse basophilic cytoplasm in contrast to those of fetal HB. The nuclei have coarser chromatin and prominent nucleoli. Mitoses are frequent. Embryonal cells occur in sheets or trabeculae of various thicknesses. They sometimes lose cohesiveness and all architectural features of epithelium. They may form acini, tubules or pseudorosettes, resembling the early ducts of the liver of a 6-week gestation embryo.

C - Macrotrabecular - is a term that indicates a repetitive arrangement of fetal and/or embryonal tumour cells in cords or plates. The tumour cell size may exceed that of the normal liver cells and may resemble the malignant cells of hepatocellular carcinoma (HCC).

D - Small cell undifferentiated HB - was previously designated anaplastic HB. It consists of sheets of loosely cohesive, almost monotypic cells with scanty cytoplasm and high mitotic rate. The tumour cells are typically round to oval but in some areas they may be spindle shaped.

In addition to HB exclusively composed of small undifferentiated cells, small cells of poor differentiation may also occur as a minor component in other sub-types of HB (‘focal anaplasia’).

7.1.2 Mixed epithelial and mesenchymal morphology: sub-types

A - Mixed pattern without teratoid features - HB characterised by a combination of fetal or embryonal epithelial patterns intermixed with immature mesenchymal components. Osteoid-like tissue is a common feature of these tumours.

B - Mixed pattern with teratoid features - This pattern refers to HB containing, in addition to epithelial and immature mesenchymal components, various combinations of heterologous tissues such as cartilage, skeletal muscle, intestinal-type and squamous epithelium and melanin-producing cells.
7.1.3 HB NOS (not otherwise specified)

This category comprises rare HB that, owing to their unusual histological presentation, cannot be classified as representing one of the standard sub-types. It is also proposed to classify HB as NOS in situations where, owing to suboptimal sampling, only a diagnosis of HB as such, but no further sub-typing is possible.

7.2 Background to SIOPEL 6

This trial is part of the fourth generation of clinical trials run by the SIOPEL group. Previous studies have led to the decision to design future trials according to the risk adapted treatment approach initiated with the SIOPEL 2 trial. This implies a clear understanding of consolidated risk factors for childhood hepatoblastoma and search for new prognostic factors including pathological and biological markers.

7.2.1 SIOPEL 1

During the late eighties the prognosis of childhood hepatoblastoma was dramatically improved because effective chemotherapy capable of reducing tumour volume and making unresectable tumours resectable became available. The use of Cisplatin in association with other agents, mainly Doxorubicin, seems to have been the key factor.

SIOPEL 1 was the first international clinical trial run by the SIOPEL group and was based on pre-operative chemotherapy using the Cisplatin-Doxorubicin (PLADO) combination regimen for all patients. The results were encouraging with a 79% 3-yr overall survival (OS) (CI 73-85%) and 67% event-free survival (EFS) (CI 60-75%). The pre-treatment extent of disease was defined by the new PRETEXT classification developed by the group. PRETEXT category proved to be a significant prognostic factor regarding event free and overall survival.

![Figure 7.1](image)

Figure 7.1 5 year EFS by PRETEXT category in the SIOPEL 1

These results led to the identification of two prognostic risk groups, standard risk and high-risk hepatoblastoma. The standard risk group consists of patients with tumour completely confined to the liver with up to three sections involved (PRETEXT 1, 2 and 3) and the high-risk group consists of patients with large tumours extending into all four sections (PRETEXT 4) and/or with extension beyond the liver into large blood vessels, the abdomen or with distant metastases (See Appendix 3). This stratification into risk groups made it possible to plan studies with intensified treatment for high risk patients with worse prognosis and on the other hand studies aiming to reduce chemotherapy and risk for short- and long term toxicity for standard risk patients with relatively good prognosis (1-3).
7.2.2 SIOPEL 2

The SIOPEL 2 pilot study took this concept further by introducing Cisplatin monotherapy for standard risk patients and a three drug (“Super-PLADO”) intensification regime for high risk patients. The results for the standard risk group were excellent and are presented in table 7.1 (4).

Table 7.1 Response, resection rates and 3-year survival rate by PRETEXT category for standard risk hepatoblastoma in the SIOPEL 2 trial

<table>
<thead>
<tr>
<th>PRETEXT</th>
<th>No. of patients</th>
<th>No of positive responses (%)</th>
<th>No of patients with a complete tumour resection incl OLT (%)</th>
<th>No patients who died of surgical complications</th>
<th>No. of patients who died of disease</th>
<th>OS at 3 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard risk</td>
<td>77</td>
<td>69 (90%)</td>
<td>75 (97%)</td>
<td>2 (3%)</td>
<td>5 (6%)</td>
<td>91%</td>
</tr>
<tr>
<td>PRETEXT I</td>
<td>6</td>
<td>6 (100%)</td>
<td>39 (100%)</td>
<td>-</td>
<td>-</td>
<td>100%</td>
</tr>
<tr>
<td>PRETEXT II</td>
<td>39</td>
<td>37 (95%)</td>
<td>39 (100%)</td>
<td>1 (3%)</td>
<td>1 (3%)</td>
<td>95%</td>
</tr>
<tr>
<td>PRETEXT III</td>
<td>32</td>
<td>26 (81%)</td>
<td>30 (94%)</td>
<td>1 (3%)</td>
<td>4 (13%)</td>
<td>84%</td>
</tr>
</tbody>
</table>

Legend: Positive response rate = complete and partial responses; Complete resection rate = including patients having microscopical residuals after surgery. OLT = Orthoptic Liver Transplantation. Standard risk patients also include those who were treated with HR regimen (n=11)

7.2.3 SIOPEL 3

The SIOPEL 3 protocol compared Cisplatin monotherapy with Cisplatin-Doxorubicin combination therapy (PLADO) for standard risk patients in a randomized non–inferiority trial. Final results are not yet available, as the trial closed on 31 December 2006. Confidential interim results have been submitted to the Independent Data Monitoring Committee (IDMC), who saw no reason for early stopping and encouraged the SIOPEL group to continue the trial to the final accrual goal of 250 patients.

7.3 Rationale for use of STS

7.3.1 STS Source and Pharmacology

Sodium Thiosulphate is a water-soluble thiol compound with reducing agent properties. Its approved application is for the treatment of cyanide poisoning.

It has been used in oncology to prevent Cisplatin nephrotoxicity, Cisplatin and Carboplatin ototoxicity and as an antidote for extravasation of various chemotherapy agents. The mechanism by which Sodium Thiosulfate reduces the nephrotoxicity caused by Cisplatin and the ototoxicity by Cisplatin and Carboplatin is not fully understood. However, the proposed mechanism of actions are that as a thiol, Sodium Thiosulphate is electrophilic, and as such is thought to act as a free radical scavenger and/or to act by covalent binding inactivating the platinum compound. In ototoxicity protection, it is also hypothesized that there is direct interaction with the hair cells of the cochlea to rescue them from platinum that is bound to the hair cells. It is also believed that Sodium Thiosulphate protects against nephrotoxicity by reducing delivery of Cisplatin to the kidneys and by neutralizing Cisplatin in the kidneys where Sodium Thiosulphate is highly concentrated.
7.3.2 Reducing ototoxicity

Cisplatin is the most active chemotherapy agent for the treatment of hepatoblastoma. It is also widely used for the treatment of other solid tumours in children. At commonly used doses and schedules, Cisplatin is associated with ototoxicity. It is known to cause permanent bilateral high frequency hearing loss, the extent of which is evaluated in different ways (5,6). The incidence, therefore, ranges from 26% to 100% depending on the criteria used to define ototoxicity, dosing and patient related factors (7-16). Even minimal and mild hearing loss in high frequency regions above 2000 Hz increases a child’s risk for academic difficulty, social-emotional problems and increased levels of fatigue in the learning environment (24-28).

Therefore as the cure rate for standard risk hepatoblastoma reaches promising levels with Cisplatin monotherapy and surgery, it seems logical to try and reduce the toxicity of the treatment without reducing the cure rate.

7.3.3 Preclinical hearing studies with Sodium Thiosulphate (STS)

Based on positive reports of STS as a protectant against Cisplatin-induced nephrotoxicity in human cancers, Edward Neuwelt’s team initiated animal studies to investigate whether STS had a protective effect against the hearing loss caused by platinum agents.

Initial studies used a guinea pig model in which Carboplatin and Cisplatin were ototoxic when administered with furosemide. STS (11.6 g/m²) was protective against Carboplatin-induced cochlear damage when administered 2, 4, or even 8 hours after Carboplatin, as determined by physiological measurements and counts of outer hair cells. STS was also protective against Cisplatin in the guinea pig, blocking 40-50 dB threshold shifts at frequencies between 4 kHz and 32 kHz. In another guinea pig study, the maximum tolerated dose of Cisplatin therapy and overall survival were increased due to lower platinum-related toxicity. In a recently developed rat model of platinum ototoxicity, Neuwelt et al delivered Cisplatin (6-8 mg/kg) to the vertebral arteries via a retrograde right external carotid artery infusion to the aorta. Auditory brainstem responses taken 5 days after administration of Cisplatin demonstrated ≥20 dB change in threshold at 4-16 kHz (Figure 7.2). To evaluate delayed STS otoprotection, a dose of STS (8 g/m²) was used that provided equivalent serum thiol concentration as achieved with patients given 20 g/m². When STS was administered 8 hours after Cisplatin, significant hearing protection was achieved at every frequency (Figure 7.2) (25, 29-33).

With respect to potential tumour protection, STS given 8 hours after Carboplatin/ Etoposide in a rat
subcutaneous tumour model had no significant impact on chemotherapy efficacy. The time to
tumour progression (8.1 ± 0.7 days) was not significantly different from chemotherapy alone
(8.9 ± 0.6 days, n = 18, p = 0.188). In another model, nude rats (n = 8 per group) with
intracerebral human lung carcinoma xenografts were treated with a Carboplatin tri-drug regimen
delivered intra-arterially with osmotic blood-brain barrier disruption. There was no difference in
tumour volume between the groups that received chemotherapy, whether or not they also
received STS (Figure 7.3). The authors conclude there is no evidence of chemoprotection of
tumour in animal models (29-30, 34).

7.3.4 Clinical studies with STS to prevent ototoxicity
Carboplatin is effective in the treatment of malignant brain tumours. However, when
administered to patients intra-arterially in conjunction with osmotic opening of the blood-brain
barrier, Carboplatin is ototoxic. On the basis of encouraging results in animal models, Neuwelt et
al began a clinical protocol in 1996. Adult patients with malignant brain tumours underwent
monthly treatment with intra-arterial Carboplatin in conjunction with osmotic opening of the
blood-brain barrier, for up to one year. Dose escalation of STS was conducted. A dose of 20
g/m² caused transient hypernatremia and grade II nausea and vomiting (NCI CTC, version 2.0).
The nausea and vomiting was well controlled with antiemetics. Thereafter, STS was
administered i.v. as one (20 g/m²) or two (20 g/m² and 16 g/m²) 15 minute doses, depending on
baseline hearing status (patients with impaired baseline hearing received two doses of STS). An initial group
received the first STS dose 2 (or 2 and 6 hours) after Carboplatin (STS2), and a subsequent group
received STS 4 (or 4 and 8 hours) after Carboplatin (STS 4). Audiologic data (conducted at
baseline and within 24 hours before each monthly treatment) were
compared with a historical
comparison group (HCG) treated
with Carboplatin without STS. Spearman correlation coefficients
comparing STS 2 (n = 24) and STS 4
(n = 17), and HCG (n = 19) indicated significantly lower rates of ototoxicity with increased
delay in STS (p = 0.0006). There were significant differences between the two STS groups and
the HCG at 8 kHz (p = 0.0010) and at 4 kHz (p = 0.0018) (Figure 7.4). STS delayed to 4 hours
after Carboplatin significantly decreased time to development of ototoxicity and rate of
ototoxicity when compared with the HCG (35-36).

7.3.5 Clinical studies suggesting that STS may permit increased platinum dose
intensity
In a further review by Neuwelt et al of 24 patients who received Carboplatin without STS and 29
patients who received Carboplatin with STS, the proportion of patients requiring dose reduction
for thrombocytopenia decreased from 33% in the former group to 0% in the latter (37-38).
Kumar and Blakely report on a series of 70 patients with head and neck squamous cell
carcinoma (39). STS was given following the first pass of Cisplatin through the tumour bed. While ototoxicity did occur, hearing losses were primarily at frequencies greater than 2000 Hz
and treatment was not altered due to hearing losses. Robbins et al reported on 42 patients with
advanced squamous cell carcinoma of the larynx and/or pharynx who received Cisplatin therapy
(150 mg/m\(^2\) weekly x4) with concurrent IV STS (40). No grade III or IV ototoxicity was observed in this series. Thus STS appears to permit increased doses of platinum that may be given in adults by limiting platinum-related toxicity.

7.3.6 Pilot paediatric trial with Carboplatin +/- STS

In the early 1990’s Neuwelt et al treated three paediatric patients with malignant brain tumours with the intra-arterial Carboplatin regimen, without STS. All patients experienced a significant decrease in hearing sensitivity following treatment. Based on the safety profile of delayed STS in adults undergoing intra-arterial Carboplatin treatment, they administered delayed STS to 10 paediatric patients (1.5 to 12 years) treated with the Carboplatin regimen. The 10 patients underwent 93 Carboplatin treatments. Three patients (30%) had a significant decrease in hearing sensitivity during treatment. The three patients had impaired hearing at baseline; two had prior treatment with Cisplatin and in the third patient the cause of impaired baseline hearing is unknown.

Hypernatremia occurred in some adults treated with high dose delayed STS, so sodium levels were closely monitored in the paediatric patients. One patient had 13 Carboplatin treatments and in each treatment the maximum sodium exceeded the normal range (by 1 to 25 mmol/L). In four patients, after 1 to 3 treatments the sodium value was above the normal range (136–145 mmol/L). Five patients had no sodium values outside the normal range across treatments. The final sodium value following the treatment (usually the following morning) was within the normal range for all treatments except one (which was 1 mmol/L above the normal range). The median difference between the last sodium value and the initial sodium value was –1 mmol/L. No toxicities required significant intervention or modification of subsequent therapy due to transient hypernatremia (41-43).

The difference in this trial is that the average age of patients will be very low, around 18 months, and therefore neonates under the age of 1 month have been excluded. This is because their sodium haemostasis is less well developed. The dose of Sodium Thiosulphate to be administered is 20g/m\(^2\). This equates to a sodium load of 160mmol/m\(^2\), equivalent for a neonate to 10mmol/kg. This is a large dose but given as a one off infusion in an afebrile child with well established diuresis, well controlled blood pressure and normal stable neurology should be well tolerated (44).

Severe hypernatremia in the neonate is usually attributed to low water and dehydration. Intravenous sodium overload is rarely the underlying cause of hypernatremia (45). Oral salt poisoning which is well documented in infants is usually chronic and in a different league in terms of sodium load.

7.3.7 Dose Selection rationale summary

The dose selection rationale is based on three published studies that investigated the effects of delayed administration of STS on ototoxicity associated with Carboplatin and osmotic blood brain barrier disruption (35-36, 41-42).

A brief description of the dose selection rationale follows:

- The standard dose of STS in cyanide poisoning is 12.5 g administered IV as 50 ml of a 250 mg/ml (25%) solution over 10 minutes, which can be repeated at half the original dose if needed. Thus, in cyanide poisoning the total standard dose of STS is up to 18.75 g (or 11 g/m\(^2\) for an individual with a body surface area of 1.7 m\(^2\)).
• STS doses of 4, 8, 12, 16, and 20 g/m² administered IV 2 hours after Carboplatin have been evaluated for safety (35). Mild nausea and/or vomiting were noted in a few subjects receiving 4 or 8 g/m². Higher doses were administered after prophylactic administration of droperidol. Transient hypernatremia (10 to 15% increase over baseline) associated with transient hypertension (10 to 15% increase over baseline) was noted in subjects receiving 16 or 20 g/m².
• STS doses of 4 or 8 g/m² administered IV 2 hours after Carboplatin did not protect against ototoxicity compared to a historical control group (35).
• STS doses of 16 or 20 g/m² administered IV 2 hours after Carboplatin did protect against ototoxicity compared to a historical control group (35).
• In another study, STS was administered as one (20 g/m²) or two (20 and 16 g/m²) 15 minute infusions, depending on the patient’s baseline hearing status. Patients with impaired baseline hearing received two doses of STS. STS given at 4 hours or 4 and 8 hours after Carboplatin significantly delayed the development of ototoxicity and decreased the rate of ototoxicity when compared with the historical control group (36).

In addition, there is clinical experience at a range of STS doses given with platinum agents in over 1000 subjects (see the Investigator’s Brochure).

7.3.8 Renal toxicity
Cisplatin causes both permanent tubular and glomerular toxicity. In infants and young children it is also known to cause transitory electrolyte disturbances. When first introduced in patients a number of methods were used to try and reduce the renal toxicity by adding hydration, mannitol and electrolytes to the pre- and post-Cisplatin infusions, by lengthening the time of the Cisplatin administration or by dividing the dose over a number of days. Permanent toxicity has also been reduced by limiting the total cumulative dose administered. In children Cisplatin is now given in a number of different ways according to disease specific treatment protocols. In previous SIOPEL studies Cisplatin has been administered as a 24 hour infusion with the aim of reducing renal toxicity. In the USA it is standard practice to administer Cisplatin over 6 hours. To date only small studies have been undertaken in children comparing long- and medium-term infusions and renal toxicity and have not shown significant differences. There is however some evidence in adult studies that long term infusions reduce renal toxicity. To date in SIOPEL studies the evaluations of renal toxicity have been poorly complied with but the data which we do have has shown acceptable levels of renal toxicity even in this young age group. By moving to a shorter infusion time we risk seeing an increase in renal toxicity over audiological toxicity. It will be essential that adequate toxicity monitoring is done in all patients so that this effect can be carefully followed and assessed (9,46-47).

7.4 Biological studies
The biological studies in SIOPEL 6 are planned to address two broad biological questions: the first will be the identification and validation of candidate biological markers for future risk adapted management of hepatoblastoma; and the second will be the collection of genomic DNA specifically for the analysis of possible genetic determinants that may contribute to the development of treatment related ototoxicity and renal toxicity.

7.4.1 Biology of hepatoblastoma
Unlike other embryonal tumours of childhood such as neuroblastoma and Wilms tumour, no biological markers for hepatoblastoma have been identified and validated within a clinical trial setting. While some independent small studies have looked at prognostic biological markers,
these studies have been seriously limited by the lack of available tumour tissue, and have not been part of a large statistically significant clinical trial (48-53).

The recent SIOPEL Strategy group Spring meetings in Edinburgh (2004), Bern (2005) and Amsterdam (2006) identified the need to integrate biological studies into all future SIOPEL trials, with the long term goals of improving the understanding of this tumour and identifying prognostic markers for risk stratification. To this end, SIOPEL has established a Biological Studies Committee, and has supported the development of a childhood liver tumour bank based in Zurich.

More recently, SIOPEL has supported a genome wide microarray expression analysis of hepatoblastoma (Dr Beundia, Paris), and a complementary analysis of a large cohort of tumours from patients enrolled in the SIOPEL 2 and 3 trials, with the aim of identifying and validating candidate prognostic biological markers (Dr Sullivan, New Zealand). It is anticipated that any candidate prognostic markers identified by these, and associated studies, will be validated by the analysis of tumours from patients enrolled in the SIOPEL 4 high-risk study and the SIOPEL 6 standard risk study.

However, like most other contemporary clinical trials it will not be possible to address any biological questions in SIOPEL 6 without the submission of biological material to the SIOPEL tumour bank in Zurich (UK patients should bank in the CCLG Tumour Bank) with informed consent for future specified research, which is linked to clinical outcome.

7.4.2 Biological mechanism and genetic determinants of Cisplatin induced hearing loss

Since a primary objective of this study is the reduction of treatment related ototoxicity, it will be important to understand any potential genetic contribution which might predispose a patient to Cisplatin associated hearing loss.

The biological mechanisms underpinning Cisplatin related ototoxicity appear to be the induction of apoptotic cell death within the auditory sensory cells. Liu et al and Cheng et al showed Cisplatin caused auditory cell apoptosis using in vitro models, and similar results were found by Devarajan et al in immortalised cell lines (54-56).

Wang et al have developed an in vivo guinea pig model of treatment related ototoxicity, and have shown by electrophysiological studies that exposure of these animals to Cisplatin this recapitulates the typical pattern of high tone hearing loss seen in children (57). In their model, Cisplatin caused morphological evidence of apoptosis in outer hair cells (OHCs), inner hair cells (IHCs) and non-sensory cells in the organ of Corti. A detailed molecular analysis of the apoptotic pathways involved showed Cisplatin induced the mitochondrial/Caspase 9 and 3 apoptotic pathway. Treatment with Cisplatin caused intracellular activation and redistribution of cytosolic Bax protein and release of cytochrome c from injured mitochondria. Cytosolic cytochrome c leads to the formation of apoptosomes and the consequent activation of the procaspase 9 pathway. Importantly, direct intracochlear installation of specific caspase 9 and 3 inhibitors (z-LEHD-fmk and z-DEVD-fmk respectively) dramatically reduced the ototoxic effects of Cisplatin. It is evident from these in vitro and in vivo studies that Cisplatin initiated apoptotic cell death is caused by free radical induced mitochondrial damage and protection against ototoxicity will be afforded by the use of free radical scavengers.

Young age and total cumulative dose appear to be the most important risk factors for Cisplatin related ototoxicity (58-59). Other factors such as the mode of administration and associated
drugs (aminoglycosides, furosemide) may be less important in children than in adults. However, it is evident that not all children of a given age and treated with the same cumulative Cisplatin doses experience equivalent degrees of hearing loss, raising the possibility that some children may be genetically predisposed to greater or lesser degrees of hearing loss.

The genetic basis of familial and acquired hearing loss is complex with many linked mitochondrial and nuclear loci and with multiple deafness causing genes already known (60). While predisposition to aminoglycoside related hearing loss has been determined by mitochondrial loci, as yet no genes or gene loci have been found for Cisplatin related deafness. In a small study of candidate mitochondrial genes, Peters et al (61) found no mutations present but a weak association was found with a mitochondrial haplotype. In another relatively small study Knoll et al screened affected and control patients for mutations and polymorphisms in 5 deafness associated genes but no association was found (62).

This SIOPEL 6 study presents a unique opportunity to identify the possible genetic determinants of ototoxicity since it will enrol a cohort of young children who will be uniformly treated with single agent Cisplatin and randomised to STS chemoprotection.

7.5 Pharmacological Studies

The platinum drugs are thought to exert their antitumour effects by reacting with DNA to form platinum-DNA intra- and interstrand crosslinks or adducts. The extent of platinum-DNA adduct formation in leucocytes isolated following Cisplatin treatment has been correlated with clinical response in several studies, suggesting that adduct formation in normal cells may parallel that in tumour tissue (63-65). It may also be the case that the formation of platinum-DNA adducts is responsible for host toxicities associated with Cisplatin treatment. We have previously shown that measurement of platinum-DNA adducts in patients may provide a marker for haematological drug toxicity, with higher platinum-DNA adduct formation correlating with increased toxicity (66). This study indicated that platinum-DNA adduct formation is not simply a function of drug pharmacokinetics or exposure but is determined by host-specific cellular factors. These factors may include intracellular inactivation of Cisplatin by glutathione, variations in drug uptake and/or the influence of protein binding in the blood. Results from this study also showed that platinum-DNA adduct levels determined following the in vitro incubation of pre-treatment whole blood with Cisplatin correlated closely with adduct levels determined in leucocytes obtained following Cisplatin treatment. These data suggest that measurement of the extent of platinum-DNA adduct levels formed in pre-treatment blood samples in vitro may be used as a predictive marker of toxicity in a clinical setting.

7.6 Study Rationale

The SIOPEL 2 trial introduced preoperative Cisplatin monotherapy for standard risk hepatoblastoma. The short- and long-term outcomes reported from this trial were excellent for standard risk patients with an overall response rate of 90%, 97% macroscopically complete resections and a 3 year overall survival of 91%.

The SIOPEL 3 protocol compared Cisplatin monotherapy with the Cisplatin – Doxorubicin combination therapy (PLADO) for pre- and postoperative chemotherapy in a non-inferiority trial. Final results are not yet available. Confidential interim results have been submitted yearly to an Independent Data Monitoring Committee, who saw no reason for early stopping and encouraged the SIOPEL group to continue the trial to the final accrual goal of 250 patients.
Ototoxicity of Cisplatin has always been a concern. In SIOPEL 2 and 3, 59% of children reported a Brock grade 1-4 hearing impairment. Such hearing impairment may seriously jeopardize the speech learning and academic achievement capacity. Sodium Thiosulphate (STS) has shown promising otoprotective activity in combination with Carboplatin. The current trial aims to assess the otoprotection of STS in Cisplatin monotherapy.

7.7 Risk-benefit evaluation

7.7.1 Cisplatin

Cisplatin will be given at a maximum dose of 80 mg/m² as an IV infusion. This dose has been used in children since the 1980’s. The time of the infusion has varied between treatment protocols from 20 minutes, through 6 hours, to 24 hour continuous infusion. In previous SIOPEL trials a 24 hour infusion has been used. However there is to date no consensus in the literature as to the most efficient or the least toxic method of administration. Infusions of 6 hours or more are however considered safer to the kidney than shorter infusions. The data on ototoxicity is conflicting and it is not known whether a 6 hour or longer infusion is more or less ototoxic. In this trial a 6 hour infusion will be used, which is the method of administration preferred in America. It has been chosen because the STS needs to be given 6 hours after the end of the Cisplatin infusion. If a 24 hour infusion were used, then the potential chemoprotectant effect of the STS would be lost.

There is a risk that patients randomised to the Cisplatin alone arm will have more renal and hearing toxicity than the experimental arm of the trial. There is also a risk that patients randomised to the Cisplatin plus STS arm will show more progression of the disease under study. Both these risks will be carefully monitored as the trial proceeds.

The risk of not treating the patient with Cisplatin is progression of the disease and ultimately death. Before HB was treated with Cisplatin, the survival was around 30% using surgery alone. Since the introduction of pre- and post-operative chemotherapy including Cisplatin, the cure rate has increased to over 80%.

7.7.2 Sodium Thiosulphate

Based on positive reports of STS as a protectant against Cisplatin-induced nephrotoxicity in human cancers, studies in both animals and humans have been carried out to investigate whether STS had a protective effect against the hearing loss caused by platinum agents.

In an early adult study a dose of 20 g/m² caused transient hypernatremia and grade II nausea and vomiting, the nausea and vomiting was well controlled with antiemetics. STS was administered IV as one (20 g/m²) or two (20 g/m² and 16 g/m²) 15 minute doses, depending on baseline hearing status. There were significant differences between the two STS groups and the historical comparison group at 8 kHz (p = 0.0010) and at 4 kHz (p = 0.0018). STS delayed to 4 hours after Carboplatin significantly decreased time to development and rate of ototoxicity when compared with the historical group.

In another adult study the proportion of patients requiring dose reduction for thrombocytopenia was decreased. Robbins et al reported on patients with advanced squamous cell carcinoma of the larynx and/or pharynx receiving Cisplatin therapy (150 mg/m² weekly x4) with concurrent IV STS (35). No grade III or IV ototoxicity was observed in this series. Thus STS appears to permit increased doses of platinum that may be given in adults by limiting platinum-related toxicity.

In the early 1990’s Neuwelt et al treated three paediatric patients with malignant brain tumours with the intra-arterial carboplatin regimen, without STS. All three patients experienced a
significant decrease in hearing sensitivity following treatment. Based on the safety profile of delayed STS in adults they administered delayed STS to 10 paediatric patients (1.5 to 12 years) treated with the Carboplatin regimen. Three patients (30%) had a significant decrease in hearing sensitivity during treatment.

Hypernatremia has occurred in some patients treated with high dose delayed STS (36), but no significant intervention or modification of subsequent therapy due to transient hypernatremia was required.

The difference in this trial is that the average age of patients will be very low, around 18 months. Neonates under the age of 1 month have been excluded, since their sodium haemostasis is less well developed. The dose of STS to be administered is 20 g/m². This equates to a sodium load of 160 mmol/m², equivalent for a neonate to 10 mmol/kg. This is a large dose but given as a one off infusion in an afebrile child with well established diuresis, well controlled blood pressure and normal stable neurology should be well tolerated (37).

Severe hypernatremia in the neonate is usually attributed to low water and dehydration. Intravenous sodium overload is rarely the underlying cause of hypernatremia (38). Oral salt poisoning which is well documented in infants is usually chronic and in a different league in terms of sodium load.
8 OBJECTIVES AND ENDPOINTS

8.1 Objectives

8.1.1 Primary Objective
- To assess the efficacy of STS to reduce the hearing impairment caused by Cisplatin chemotherapy

8.1.2 Secondary Objectives
- To carefully monitor any potential impact of STS on response to Cisplatin and survival.
- To assess the short- and long-term tolerability of the combination of STS and Cisplatin chemotherapy.
- To prospectively evaluate and validate biological, radiological and pathological features of standard risk hepatoblastoma for future risk adapted management.
- To investigate the effect of STS on the formation of Cisplatin-DNA adducts.
- To prospectively collect patient DNA specifically for the analysis of possible genetic factors that may contribute to the development of treatment related ototoxicity and nephrotoxicity.

8.2 Endpoints

8.2.1 Primary end-point
- Rate of Brock grade ≥1 hearing loss determined after end of trial treatment or at an age of at least 3.5 years, whichever is later (see Appendix 5).

8.2.2 Secondary end-points
- Response to preoperative chemotherapy
- Complete resection
- Complete remission
- Event free survival (EFS)
- Overall survival (OS)
- Toxicity as graded by CTCAE v 3.0
- Long-term renal clearance
- Feasibility of central audiology review
9 TRIAL DESIGN
Randomised phase III clinical trial

9.1 Trial Schema

Assessment of response- in the case of progressive disease, refer to section 9.4
Assessment of response and resectability – in the case of progressive disease, refer to section 9.4. If surgery has to be delayed for any reason, 1-2 further courses of chemotherapy can be administered pre-operatively instead of post-operatively.

Figure 9.1: Trial Schema
9.2 Patient accrual

- Projected accrual: 35 patients/year
- Projected total accrual: 102 evaluable patients (115 randomised patients). Evaluable patients are those who are fully evaluable for the primary endpoint. This will be determined by the medical/audiology reviewer.
- Recruitment period: 3.8 years

9.3 Treatment and Follow-up Plan

- Pre-operative chemotherapy, 4 courses Cisplatin +/- STS
- Definitive surgery after pre-operative chemotherapy (as in all previous SIOPEL trials).
- Post-operative chemotherapy, 2 courses Cisplatin +/- STS (this can be given pre-operatively if surgery has to be delayed for valid practical reasons).
- Long-term follow-up (see section 15.7)

9.4 Treatment failure

- Patients with progressive disease after 2 or more courses of Cisplatin with or without STS will stop trial treatment
- No further STS
- 2-4 courses Cisplatin-Doxorubicin (PLADO) are recommended, followed by definitive surgery when the response is sufficient.
- Ideally 2 courses of PLADO will be given post-operatively reaching a total of 6 courses.
10 PATIENT SELECTION CRITERIA

10.1 Inclusion criteria

- Histologically confirmed newly diagnosed hepatoblastoma
- Standard risk hepatoblastoma:
  PRETEXT I, II or III
  Serum alpha-fetoprotein (AFP) > 100 µg/L
  No additional PRETEXT criteria
- Age ≤ 18 years and > 1 month
- Written informed consent and national/local ethics committee and regulatory approval
- Centre/country willing and able to organize audiology at minimum required quality standard (see Section 15.9 and Appendices 5 and 6)
- Ability to comply with requirements for submission of material for central review (radiology, pathology and audiology)
- For females of child-bearing potential, a negative pregnancy test prior to study treatment is required
- Any patient who is of reproductive age should agree to use adequate contraception for the duration of the trial (males should always use a condom and females should ensure their partner uses a condom, and uses one additional method of contraception for the duration of the period of chemotherapy)

10.2 Exclusion criteria

- High risk hepatoblastoma:
  Serum - Alpha-fetoprotein (AFP) ≤ 100 µg/L
  Tumour involving all 4 hepatic sections - PRETEXT IV
  Additional PRETEXT criteria (see Appendix 3)
  - Extrahepatic abdominal disease (E1, E1a, E2, E2a)
  - Intraperitoneal haemorrhage or tumour rupture (H1)
  - Distant metastases, any site (M1)
  - Lymph node metastases (N1, N2)
  - Involvement of the main portal vein (P2, P2a)
  - Involvement of all three hepatic veins and/or the IVC (V3, V3a)
- Hepatocellular carcinoma
- Treatment starting more than 15 days from written biopsy report
- Abnormal renal function defined as calculated GFR < 75% of the lower limit of normal for age at diagnosis, which over 2 years of age is < 60 ml/min/1.73 m² (see Appendix 8)
- Any previous chemotherapy
- Recurrent disease
- Previous hypersensitivity to STS
- Patient unable to follow the protocol for any reason
11 REGISTRATION, RANDOMISATION & DATA COLLECTION

11.1 Centre participation

Patients will be accepted for registration into the trial from any centre recognised for the treatment of children with cancer that is able to comply with the requirements of the protocol and the particular requests listed below. Participating centres are kindly requested to

- Complete a Form of Participation via http://www.siop.org/clinicians/ either on-line using the electronic form or by printing out the PDF version
- Collect signatures on a printed out copy of the electronic form or PDF version, scan and email to siopel@cineca.it.
- Scan the following approval documents and upload them into the Form of Participation:
  - Institutional Review Board (IRB) or Ethics Committee (EC) approval
  - Health Authority and/or other applicable approval as required by national regulations
  - Or alternatively scan each document and email to siopel@cineca.it
- Once the participation request is accepted, the International Trial Facilitator will allocate user IDs and passwords to access the SIOPEL 6 Remote Data Entry (RDE) system.

Whenever possible centres contributing to the trial should do so as a member of a participating national group for whom a national co-ordinator will be identified and for whom representation on the trial committee will be assured. In each country a lead for audiology should be assigned and the name and contact details forwarded to the International Trial Facilitator. In some countries it will be more appropriate to have the end of treatment audiology done at one or a number of selected National Centres. This will mean that patients may need to travel, after they have finished treatment and are at least 3.5 years old, to this centre for testing. It is important that the parents agree to and understand this prior to randomisation. Centres participating outside the framework of a collaborating national group will be requested to provide a name of a local Principal Investigator and local audiological physician who will be responsible for communication with the International Trial Facilitator.

Participating centres are expected to:

- register all eligible patients with standard risk hepatoblastoma according to the trial protocol
- provide adequate material for central reviews (pathology, radiology and audiology) and, if feasible, for the tissue storage program. See Appendix 16 for a summary of sample requirements and other materials for central review.
- provide required data for analysis by using the RDE system
- obtain written informed consent for the treatment, randomisation and end of treatment audiological assessment from the patients and/or their parents

11.2 Registration procedure

To assist local physicians in the diagnostic work up and entry of a patient into one of the SIOPEL trials, an RDE internet-based online pre-registration system has been developed. After pre-registration of a child with a suspected primary malignant hepatic tumour, the system will guide you through the required pre-treatment investigations and the registration procedure. In order to satisfy all the necessary procedures for adequate diagnostic and pre-treatment work-up and centralisation of trial material, if appropriate, please pre-register
patients on this system as soon as possible, preferably before biopsy, when a malignant hepatic mass is suspected in a child.

If on-line pre-registration is not available, a registration form can be downloaded from www.siopel.org SIOPEL 6 protocol section under the members section, which should be faxed to the International Trial Facilitator on +44-0207 813 8588 as soon as possible, and preferably before biopsy. If you require access to the members section, contact the International Trial Facilitator at siopel@cineca.it.

11.3 Randomisation

Randomisation can only be performed when definitive risk assessment and allocation has been completed but must occur within 15 days of written biopsy report confirming diagnosis. If >15 days, the patient is excluded from this study. Please note, that risk assignment can be difficult in some cases. It is strongly recommended that investigators take advantage of rapid radiology review (RRR) in all patients with suspected standard risk hepatoblastoma. The trial radiologist will help you to assign a correct risk group (for details see section 15.2.3).

In order to make definitive risk assignment (including review process) possible, start of chemotherapy can be delayed for maximally 15 days after diagnosis (date of biopsy report) if the patient’s condition permits.

11.4 Data management

For the SIOPEL 6 trial a web based, remote data entry (RDE) system will be implemented through co-operation with CINECA. Data quality control procedures (common range, logical checks, cross checks etc.) will be applied within the RDE system. Further details and passwords will be provided directly to participating centres on acceptance of the participation agreement.
12 CHEMOTHERAPY GUIDELINES

12.1 Treatments to be administered

12.1.1 Cisplatin

Formulation
Cisplatin is supplied in 10ml, 50ml and 100ml vials containing a 1mg/ml solution. It can also be supplied as powder for reconstitution in 50mg vials.

Stability
Pre dilution: as per manufacturer’s Summary of Product Characteristics.
Post dilution: Cisplatin may be stable for up to a maximum of 7 days in a solution of sodium chloride. Follow the manufacturer’s Summary of Product Characteristics.

12.1.2 Sodium Thiosulphate

Source and Pharmacology
STS is a water-soluble thiol compound with reducing agent properties. Following IV injection, Sodium Thiosulphate is distributed throughout the extracellular fluid. Some Sodium Thiosulphate is converted to sulphate in the liver. Up to 95% is excreted unchanged in the urine. The biological half-life is 0.65 hours (range: dependent on dose 16.5 – 182 minutes).

Packaging and labelling
Adherex Technologies are responsible for ensuring the distributor label each vial with a multi-language booklet label. Each vial will be placed into a 6 vial kit box. Each box will be labelled with a multi-language kit label.

STS drug product is manufactured, labelled, and packaged under GMP conditions. STS is supplied as a 25% (250 mg/mL), preservative free, sterile solution. The drug product formulation contains sodium thiosulfate pentahydrate and sodium borate.

The vial label will indicate the drug product batch number and the initial release until date.

Shipment and storage
Boxes containing 6 (50 mL) vials of STS will be sent to the clinical study site pharmacy in various quantities, determined by the Sponsor according to the site’s projected and actual enrolment.

The clinical study site pharmacy will provide sterile Water for Injection (WFI) for dilution of STS before administration.

STS must be carefully stored at the clinical study site, safely and separately from other drugs, and at a controlled room temperature between 15 to 30°C (59 to 86°F).

STS may not be used for any purpose other than this clinical study.

It must be ensured at each clinical study site that the study drug is not used 24 months after the release date.

The Investigator (or designated pharmacist) must maintain records of the delivery of the study drug to the clinical study site, the inventory at the site, the use by each subject, and destruction at the site.
Upon completion or termination of the study, the Investigator/pharmacist will destroy all unused medication at the site, after approval by the Sponsor, as per the site’s appropriate SOPs. For drug accountability records, the site will provide CINECA with a copy of the Drug Dispensing Record and Drug Inventory Record. If the remaining clinical supplies are destroyed by the site, documentation of their destruction must be provided to CINECA. These can be uploaded onto the CINECA system or faxed to the International Trial Facilitator. The record of destroyed clinical supplies will include information on:

- All administered units
- All unused units
- All units destroyed at the end of the study
- Date of destruction
- Name and signature of the Investigator and staff member responsible for the destruction

**Preparation**

STS is supplied in 50 ml vials containing a 25% (250 mg/ml or 12.5 g/vial) solution. Each ml of the 25% STS to be diluted with 1ml of sterile water for injection (1:1 dilution) to a concentration of 125mg/ml for direct administration. (This has an approximately equivalent isotonicity to a 2.3% sodium chloride solution). The volume from the appropriate number of vials for the dose is combined in a PVC IV infusion bag.

Reconstituted STS for administration consists of a clear solution. There are no preservatives in the formulation. After dilution the PVC infusion bag containing the dosing solution must be placed upside down (inverted with injection and filling ports at the top) at room temperature and used within 8 hours. Any solution remaining in the vial should be destroyed according to institutional procedures.

**12.2 Treatment Schedule**

For all patients:
- Pre-surgery: 4 courses on day 1, 15, 29 and 43 (exceptionally, if surgery is delayed, courses may also be given on day 57 and 71).
- Post-surgery: as soon as possible, but within 21 days: 2 courses (day 1 and 15)

Prior to starting each cycle of chemotherapy establish that the child has a good urine output, serum electrolytes and creatinine are within the normal range for age, stable blood pressure <97th centile for age, normal neurology and is afebrile.

**12.3 Cisplatin**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>For children &gt; 10kg:</td>
<td>80 mg/m² IV infusion over 6 hours</td>
</tr>
<tr>
<td>For infants and children 5-10kg:</td>
<td>2.7 mg/kg IV infusion over 6 hours</td>
</tr>
<tr>
<td>For infants &lt; 5kg:</td>
<td>1.8 mg/kg IV infusion over 6 hours</td>
</tr>
</tbody>
</table>

**12.4 Sodium Thiosulphate**

For children randomised to receive STS:

<table>
<thead>
<tr>
<th>Group</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>For children &gt; 10kg:</td>
<td>20 g/m² IV infusion over 15 minutes</td>
</tr>
<tr>
<td>For infants and children 5-10kg:</td>
<td>15 g/m² IV infusion over 15 minutes</td>
</tr>
<tr>
<td>For infants &lt; 5kg:</td>
<td>10 g/m² IV infusion over 15 minutes</td>
</tr>
</tbody>
</table>
12.5 Administration

12.5.1 Pre-hydration

- At least 3 hours pre-hydration with 2.5% dextrose/0.45% saline
- Run at 200 ml/m²/hr (total volume 600 ml/m²)

12.5.2 Cisplatin infusion over 6 hours

- Cisplatin in 0.9% sodium chloride. Suggested volume for infusion over 6 hours:
  - < 60mg in 60ml, 60mg-120mg in 120ml and > 120mg in 240ml
No fluids other than sodium chloride to be used as a vehicle for Cisplatin in order to prevent chloride depletion, which would lead to increased risk for nephrotoxicity.

12.5.3 Hydration during and until 6 hours post Cisplatin (i.e. 12 hours in total)

- 2.5% Dextrose/0.45% sodium chloride
- Plus 6 g mannitol per 500 ml
- Plus 10 mmol potassium chloride per 500 ml
- Run at 125 ml/m²/hr

12.5.4 Sodium Thiosulphate 6 hours post Cisplatin

- Dilute each ml of the 25% STS with 1ml of sterile water for injection (1:1 dilution) to a concentration of 125 mg/ml for direct administration. (This has an approximately equivalent ionicity to a 2.3% sodium chloride solution).
- Infuse over 15 minutes
- Stop the Cisplatin hydration fluid for 15 minutes during the STS infusion. Restart the Cisplatin hydration immediately afterwards

12.5.5 Hydration for subsequent 18 hours (i.e. until 24 hours after the end of Cisplatin infusion)

- 2.5% Dextrose/0.45% sodium chloride
- Plus 10 mmol potassium chloride per 500 ml
- Plus 5 mmol magnesium sulphate per 500 ml
- Plus 0.3 mmol calcium gluconate per 500 ml
- Run at 125 ml/m²/hr

12.5.6 Summary (please refer to Figure 12.1)

- -3 hr Start pre-hydration
- 0 hr Finish pre-hydration, start Cisplatin infusion + hydration over 6 hours
- 6 hr Finish Cisplatin infusion, continue with hydration for a further 6 hours
- 12 hr Finish hydration, start STS infusion
- 12 hr 15min Finish STS infusion, start post hydration
- 30 hr 15min Finish post hydration

A Bedside Nursing WorkSheet (Appendix 15) has been provided to assist you with the timings of Cisplatin, Sodium Thiosulphate (STS), hydration and anti-emetic administration. The WorkSheet may be adapted to suit your local practice. Paper copies of the worksheet should be kept in the child’s notes ready to be filled in when chemotherapy is given. The worksheet also includes a table to record blood pressure and electrolyte monitoring for patients receiving STS. Please ensure that these assessments are performed and recorded at the bedside so that you can enter this information onto the RDE system.
Figure 12.1: Summary Schema of Treatment, Hydration, Sodium Monitoring, Blood Pressure Monitoring and DNA Blood Sampling with 4 Example Start Times

- Hydration: 200 ml/m²/hr
- Hydration: 125 ml/m²/hr + mannitol + KCl
- Hydration: 125 ml/m²/hr + KCl + MgSO4 + Ca gluconate

<table>
<thead>
<tr>
<th>Time</th>
<th>Serum Sodium</th>
<th>Blood pressure</th>
<th>DNA blood sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>-3 hr</td>
<td>06:00 - 09:00</td>
<td>Prepare STS</td>
<td>Pre-treatment</td>
</tr>
<tr>
<td>0 hr</td>
<td>09:00</td>
<td>After 13:00</td>
<td></td>
</tr>
<tr>
<td>6 hr</td>
<td>15:00</td>
<td>After 15:00</td>
<td></td>
</tr>
<tr>
<td>12 hr</td>
<td>21:00</td>
<td>After 16:00</td>
<td></td>
</tr>
<tr>
<td>18 hr</td>
<td>15:00</td>
<td>After 09:00</td>
<td></td>
</tr>
<tr>
<td>24 hr</td>
<td>21:00</td>
<td>Prepare STS</td>
<td></td>
</tr>
<tr>
<td>30 hr</td>
<td>03:00</td>
<td>After 13:00</td>
<td></td>
</tr>
</tbody>
</table>

- Example Start Times:
  - e.g. 1: 06:00 - 09:00
  - e.g. 2: 08:00 - 11:00
  - e.g. 3: 09:00 - 12:00
  - e.g. 4: 21:00 - 24:00

- Cisplatin

- Hydration:
  - 200 ml/m²/hr
  - 125 ml/m²/hr + mannitol + KCl
  - 125 ml/m²/hr + KCl + MgSO4 + Ca gluconate

- Blood sampling:
  - Pre STS, End STS, 30 mins post, 60 mins post
  - 1 hr post STS
  - 6 hrs post STS
  - 18 hrs post STS

- Blood pressure monitoring:
  - Pre-treatment
  - Post treatment
12.6 Supportive care

12.6.1 Fluid balance and diuresis

A careful record of fluid input and output should be kept during administration of each cycle. If diuresis falls below 3 ml/kg/hr for 2 hours give a bolus of mannitol 0.5 g/kg over 15-30 minutes. Careful monitoring of fluid intake and output is essential to prevent renal toxicity from Cisplatin and fluid overload. Any fluid lost through vomiting should be replaced intravenously. The use of loop diuretics such as Furosemide should be avoided as they are ototoxic.

All patients will need serum electrolytes monitored daily during Cisplatin treatment. Patients receiving STS will also need careful monitoring of their serum sodium. This should be done pre STS, at 1 hour post STS and at 6 and 18 hours. If the serum sodium exceeds 150mmol/l at 1 hour then a bolus of mannitol 0.5 g/kg over 15-30 minutes should be given together with a 10ml/kg fluid bolus of dextrose in addition to the standard Cisplatin hydration. Do not stop the standard hydration as this will increase the risk of renal toxicity by reducing the amount of chloride ions in the renal tubule. Additionally if the serum sodium is 146-150mmol/l at 1 hour, the individual clinician looking after the patient may decide whether or not to give mannitol.

Oral magnesium supplements should be given to all patients if necessary between cycles.

12.6.2 Antibiotics

Nephrotoxic antibiotics such as aminoglycosides and vancomycin should preferably be avoided. If used, serum levels should be monitored with extreme care. The use will be recorded in the RDE system. If the patient has received aminoglycosides ≤ 12 months before start of trial treatment this will also be recorded as it has been shown that up to a year previous aminoglycosides can increase ototoxicity from Cisplatin.

12.6.3 Anti-emetics

Cisplatin is a highly emetogenic drug, therefore adequate anti-emetic therapy is essential. Any anti-emetic regimen should include a 5-HT3 receptor antagonist plus dexamethasone.

In addition, STS also causes nausea and vomiting. The anti-emetic regimen should be scheduled such that a 5-HT3 receptor antagonist plus additional anti-emetics (eg dexamethasone, plus chlorpheniramine and or metoclopramide) are given 30 minutes before the STS is due. Children may feel very thirsty during the infusion of STS. There is no reason why they should not drink water, this is however likely to cause more vomiting. This is not a problem as their hydration is assured intravenously. The acute feeling of nausea during the STS infusion is relatively short lived

The combination of Cisplatin and STS is expected to be highly emetogenic. Please be sure to continue to give multi agent antiemetic cover 6-8 hourly for the first 24 - 48 hours. The emesis caused by the Cisplatin is likely to last up to 5 days. Adequate anti-emetic cover should be continued as long as required.

12.6.4 Contraindicated medications

See Appendix 7 for list of drugs to be avoided where possible, but documented if used.
12.6.5 Vital signs

Vital signs, including blood pressure, are to be recorded before, at the end of the STS infusion, and 30 and 60 minutes after the dose of STS. If the blood pressure is not within the normal range or at subject’s baseline level 60 minutes post dose, notify a physician, and monitor every 30 minutes until two successive blood pressure values are within the normal range or at the subject’s baseline level. Please refer to BP percentile charts in Appendix 14.

12.7 Treatment in case of progressive disease

12.7.1 General notes

If there is progressive disease (see definition in section 16.1) at evaluation after 2 or more cycles of trial treatment, i.e. Cisplatin with or without STS, the trial treatment is stopped. No further STS is to be given to the patient.

12.7.2 Salvage treatment guidelines

It is advisable to give chemotherapy pre-operatively and the combination Cisplatin-Doxorubicin (PLADO) is recommended. This should be given via a double lumen line, thus allowing Doxorubicin with or without dexrazoxane to be administered via one lumen and Cisplatin with hydration to be administered via the other line. In difficult cases please contact the chemotherapy coordinators.

12.7.3 Treatment schedule

Pre-surgery: 2 cycles every 21 days, followed by evaluation and surgery.
Post-surgery as soon as possible, but within 21 days: 2 more courses, every 21 days if response at evaluation.

12.7.4 Starting criteria for PLADO

Absolute neutrophil count (ANC) ≥ 1.0 x 10^9/l and platelet count ≥ 100 x 10^9/l and no active infection

12.7.5 Cisplatin

<table>
<thead>
<tr>
<th>For children &gt; 10kg:</th>
<th>80 mg/m^2 IV infusion over 6 hours</th>
</tr>
</thead>
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<td>2.7 mg/kg IV infusion over 6 hours</td>
</tr>
<tr>
<td>For infants &lt; 5kg:</td>
<td>1.8 mg/kg IV infusion over 6 hours</td>
</tr>
</tbody>
</table>

12.7.6 Doxorubicin

Doxorubicin is given in a 1 hour IV infusion with dexrazoxane or IV infusion for 24 hours

<table>
<thead>
<tr>
<th>For children &gt; 10kg:</th>
<th>30 mg/m^2/day IV infusion for 2 consecutive days</th>
</tr>
</thead>
<tbody>
<tr>
<td>For infants and children 5-10kg:</td>
<td>1.0 mg/kg/day IV infusion for 2 consecutive days</td>
</tr>
<tr>
<td>For infants &lt; 5kg:</td>
<td>0.67 mg/kg/day IV infusion for 2 consecutive days</td>
</tr>
</tbody>
</table>

12.7.7 Cardioprotectant dexrazoxane

The use of dexrazoxane as a concomitant medication administered along with Doxorubicin is recommended. However, it is acknowledged that dexrazoxane may not be universally available.
Dexrazoxane is administered in a 15 minute infusion 30 minutes prior to the infusion of Doxorubicin. Two formulations are available with different dosage:
The dose is 20:1 Cardioxane:Doxorubicin or 10:1 Zinecard:Doxorubicin, however the Cardioxane dose needs to be capped at a maximum of 1000 mg/m² per cycle. This means the dose of Cardioxane will be 500 mg/m²/day on day 1 and day 2 or Zinecard 300 mg/m²/day on day 1 and day 2. The Doxorubicin dose is 30 mg/m² IV given over 1 hour on day 1 and day 2.

12.7.8 PLADO administration with additional dexrazoxane

12.7.8.1 Pre-hydration
    - At least 3 hours pre-hydration with 2.5% dextrose/0.45% saline
    - Run at 200 ml/m²/hr (total volume 600 ml/m²)

12.7.8.2 Dexrazoxane followed by Doxorubicin infusion over 1 hour
    - Cardioxane 500 mg/m² or Zinecard 300 mg/m² over 15 minutes 30 minutes before Doxorubicin
    - Doxorubicin in 0.9% sodium chloride over 1 hour

12.7.8.3 Cisplatin infusion over 6 hours
    - Cisplatin in 0.9% sodium chloride. Suggested volume for infusion over 6 hours:
    - < 60mg in 60ml, 60mg-120mg in 120ml and > 120mg in 240ml
    - No fluids other than sodium chloride to be used as a vehicle for Cisplatin in order to prevent chloride depletion, which would lead to increased risk for nephrotoxicity

12.7.8.4 Hydration during and until 6 hours post Cisplatin (i.e. 12 hours in total)
    - 2.5% Dextrose/0.45% sodium chloride
    - Plus 6 g mannitol per 500 ml
    - Plus 10 mmol potassium chloride per 500 ml
    - Run at 125 ml/m²/hr (including Cisplatin)

12.7.8.5 Hydration for subsequent 18 hours (i.e. until 24 hours after the end of Cisplatin infusion)
    - 2.5% Dextrose/0.45% sodium chloride
    - Plus 10 mmol potassium chloride per 500 ml
    - Plus 5 mmol magnesium sulphate per 500 ml
    - Plus 0.3 mmol calcium gluconate per 500 ml
    - Run at 125 ml/m²/hr

12.7.8.6 Dexrazoxane followed by Doxorubicin infusion over 1 hour (24 hours after the first dose)
    - Cardioxane 500 mg/m² or Zinecard 300 mg/m² over 15 minutes 30 minutes before Doxorubicin
    - Doxorubicin in 0.9% sodium chloride over 1 hour

12.7.8.7 Summary
    - hr 0 Start pre-hydration over 3 hours + Start dexrazoxane infusion over 15 min
    - hr 0.5 Start Doxorubicin over 1 hour
    - hr 1.5 Finish Doxorubicin
    - hr 3 Finish pre-hydration, start Cisplatin infusion + hydration over 6 hours
    - hr 9 Finish Cisplatin infusion, continue with hydration for a further 6 hours
    - hr 15 Start post hydration
    - hr 24 Start dexrazoxane infusion over 15 min
    - hr 24.5 Start Doxorubicin over 1 hour
- hr 25.5 Finish Doxorubicin
- hr 33 Finish post hydration

12.7.9 PLADO administration without additional dexrazoxane i.e. Doxorubicin infusion over 48 hours

12.7.9.1 Doxorubicin 30 mg/m²/day for 24 hours

12.7.9.2 Cisplatin pre-hydration
- At least 3 hours pre-hydration with 2.5% dextrose/0.45% saline
- Run at 200 ml/m²/hr (total volume 600 ml/m²)

12.7.9.3 Cisplatin infusion over 6 hours
- Cisplatin in 0.9% sodium chloride. Suggested volume for infusion over 6 hours:
- < 60mg in 60ml, 60mg-120mg in 120ml and > 120mg in 240ml
No fluids other than sodium chloride to be used as a vehicle for Cisplatin in order to prevent chloride depletion, which would lead to increased risk for nephrotoxicity.

12.7.9.4 Hydration during and until 6 hours post Cisplatin (i.e. 12 hours in total)
- 2.5% Dextrose/0.45% sodium chloride
- Plus 6 g mannitol per 500 ml
- Plus 10 mmol potassium chloride per 500 ml
- Run at 125 ml/m²/hr (including Cisplatin)

12.7.9.5 Doxorubicin 30 mg/m²/day for 24 hours

12.7.9.6 Hydration for subsequent 18 hours (i.e. until 24 hours after the end of Cisplatin infusion)
- 2.5% Dextrose/0.45% sodium chloride
- Plus 10 mmol potassium chloride per 500 ml
- Plus 5 mmol magnesium sulphate per 500 ml
- Plus 0.3 mmol calcium gluconate per 500 ml
- Run at 125 ml/m²/hr (including Doxorubicin)

12.7.9.7 Summary
- hr 0 Start Doxorubicin infusion over 24 hours
- hr 24 Finish Doxorubicin + Start pre-hydration over 3 hours
- hr 27 Finish pre-hydration, start Cisplatin infusion + hydration over 6 hours
- hr 33 Finish Cisplatin infusion, continue with hydration for a further 6 hours
- hr 33 Start Doxorubicin infusion over 24 hours
- hr 39 Start post hydration
- hr 57 Finish Doxorubicin
- hr 57 Finish post hydration
13 TOXICITY AND DOSE MODIFICATIONS

Toxicity should be manageable and is outlined below.

However, due to the nature of this trial using Cisplatin as the only active chemotherapy modality, severe Cisplatin toxicity in an individual situation may lead to alternative treatment being sought. In standard risk hepatoblastoma where surgical resection is possible, by definition at diagnosis, it is possible to discuss alternative approaches to treatment. The child would remain on trial, but the changes in the treatment must be clearly noted on the CINECA RDE system. The gold standard treatment of the SIOPEL group used to be PLADO using both Cisplatin and Doxorubicin, which is cardiotoxic. It should be possible to discuss with the parents the pro’s and con’s of alternative chemotherapy replacing the Cisplatin with Carboplatin and combining this with Doxorubicin together with dexamethasone as suggested for patients with progressive disease. It is important to bear in mind that surgery alone for hepatoblastoma gives a cure rate in the region of 30% (4). It is always considered better to operate on a hepatoblastoma, which has shown response to chemotherapy. Alternative chemotherapy in hepatoblastoma is not as effective but etoposide and ifosfamide have been shown to be active in xenograft models and irinotecan is currently in phase II. Any change in treatment needs to be assessed carefully to show response prior to surgery. Contact the chemotherapy coordinators for advice.

13.1 Gastrointestinal and Metabolic toxicity

Cisplatin causes severe nausea and vomiting and needs to be administered with adequate antiemetic cover. Cisplatin can cause anorexia. It is essential that nutritional status is monitored and naso-gastric feeding be implemented where necessary. It is advised that naso-gastric feeding is implemented immediately for patients presenting in a poor nutritional state.

STS causes nausea and needs to be given with the appropriate antiemetic cover - see above.

In infants, electrolyte disturbances during and after Cisplatin administration are to be expected and can lead to fitting particularly if there are multiple abnormalities. The infusion guidelines are standard, however, in some children the electrolyte substitution may need to be altered. Please monitor electrolytes carefully. Hypomagnesaemia is not a reason to stop Cisplatin. Daily serum electrolytes during and after Cisplatin are suggested.

STS creates a sodium overload estimated at 160 mmol/m², equating to about 10 mmol/kg in neonates, and requires careful monitoring of serum sodium. Patients receiving STS treatment will require monitoring of serum sodium at 1 hr, 6 hrs and 18 hrs after administration see above. STS should only be given to a child who has well established diuresis, well-controlled blood pressure, stable neurology and is afebrile. Should the serum sodium exceed 150mmol/l at 1 hour then the guidelines above should be followed.

STS should be stopped and not given at further treatment cycles if metabolic, vascular, neurological or other, presumed to be related, toxicity of CTCAE grade 3+ is experienced.

STS should not be given to a patient with previous hypersensitivity to STS.
13.2 Renal toxicity

13.2.1 Glomerular toxicity

Nephrotoxicity of Cisplatin in children (as in adults) is dose-related and sometimes severe (46-47). Renal monitoring should be carried out carefully during and at the end of treatment and at follow-up.

Serum creatinine measurements and creatinine clearances are not reliable guides to the degree of Cisplatin-induced renal damage, particularly in children. Careful measurement of Glomerular Filtration Rate (GFR) by isotope clearance or other clearance method is recommended for accurate monitoring of renal status. The GFR monitoring in this trial is requested prior to start of preoperative chemotherapy, after preoperative and after postoperative chemotherapy. However as the Cisplatin will be being administered 2-weekly it is advisable to watch the creatinine carefully prior to each dose and if necessary repeat the GFR. The GFR should not be done when a child is receiving i.v. hydration as the result will not be reliable. Please use the same technique on the same child at every time point. Cr-EDTA-clearance is the preferred technique and involves obtaining the isotope, injecting it into the child and taking 4 blood samples at hourly intervals (the simplified version with 2 blood samples is also acceptable) from an indwelling catheter. It entails less irradiation to the child than daily natural sources. Other clearance methods, for example iohexol clearance, are also acceptable. If for financial reasons this is not available at your hospital, then do a standard endogenous creatinine clearance with a 24 hr urine collection. If the urine collection is not complete, then please repeat it. DTPA and other scans are useful for gathering information on a child’s renal function but are not helpful in a multi-national trial, so please do not submit these results instead of a GFR.

A GFR of CTCAE grade 1: < 75-50% of the lower limit of normal, which over the age of 2 years is < 60 ml/min/1.73 m², should be reported as an AE.

In young children renal function is not fully mature until about the age of 2 years. At diagnosis the GFR can be very low in absolute terms in a particular child. This in itself does not contraindicate the administration of Cisplatin if on subsequent monitoring the GFR improves. However the renal function needs to be carefully monitored and adequate diuresis assured at all times. In children over the age of 2 years at diagnosis where there is a reduction in GFR to <60 ml/min/1.73 m² the long term toxicity should be discussed with the parents and alternative chemotherapy approaches sought.

STS should be stopped and not given at further treatment cycles if renal toxicity of CTCAE grade 3+ is experienced:
GFR of CTCAE grade 3: < 25% of the lower limit of normal, chronic dialysis not indicated.
GFR of CTCAE grade 4: chronic dialysis or renal transplant indicated.

13.2.2 Tubular toxicity

Renal loss of magnesium and consequent hypomagnesemia is expected in nearly all children on this trial and oral magnesium supplementation is recommended for all. Hypomagnesaemia is not a reason to stop Cisplatin. Children can develop renal tubulopathy even though the GFR is improving. Thus, regular long term electrolyte monitoring is necessary in children who have been exposed to Cisplatin treatment. Hypomagnesaemia may persist years after stopping therapy. Infants are at higher risk of Cisplatin induced electrolyte imbalance and consequently the need for regular electrolyte monitoring is particularly important in this age
group. However, Cisplatin does not seem to be more nephrotoxic in infants than in older children.

### 13.3 Bone marrow toxicity

It is unlikely that this regimen will cause aplasia. The treatment should be given regardless of count. Cisplatin tends to lower the haemoglobin and a blood transfusion may be necessary. Blood counts should be monitored and adequate precautions taken should the child develop febrile neutropenia.

### 13.4 Audiological toxicity

The monitoring of ototoxicity has been extensively dealt with elsewhere, however the problem may arise as to whether or not to adjust treatment on the basis of ototoxicity. This is a problem in young children with hepatoblastoma who are prone to more severe ototoxicity and where Cisplatin remains the best chemotherapy treatment. Please contact the chemotherapy coordinators if you wish to discuss a particular case. If the decision of physicians or parents is to alter treatment, then the child will remain on trial but the changes in the treatment must be clearly noted on the CINECA RDE system.
14 SURGICAL GUIDELINES

14.1 Pre-treatment biopsy

14.1.1 Background

Even though the diagnosis of hepatoblastoma can be reached on clinical grounds alone, please note that a diagnostic tumour biopsy is required in all cases regardless of the patient’s age and serum AFP level.

The aim of biopsy is to obtain sufficient tumour material for:
- Histological diagnosis and classification of hepatoblastoma
- Central pathological review
- Biological and translational research facilitated by the SIOPEL Liver Tumour Tissue Bank
- For further reading see references (67-72)

14.1.2 Technique

The preferred method of obtaining a tumour biopsy is by using a percutaneous ultrasound-guided needle biopsy. However, depending on local preference and expertise, alternative approaches include a minilaparotomy via a subcostal incision or a laparoscopic biopsy in which a wedge of tissue is taken or a biopsy needle is introduced percutaneously under camera control.

Diagnostic material can be obtained with a “Tru-cut” or similar needle but at least 3-5 cores of tissue should be collected. Additionally, one core of normal liver parenchyma should also be obtained. With open or laparoscopic techniques a wedge of tumour can be excised, this results in a larger tissue sample, which provides more material.

No attempt should be made at this time to perform radical tumour removal. At the time of the biopsy, placement of a long-term central venous catheter should be considered.

Fine needle biopsy is not acceptable as it generally provides insufficient material.

14.1.2.1 Technique (general)

In the SIOPEL 1 and 2 studies, when open biopsy was used in the majority of cases, no life-threatening biopsy complications were recorded. Complications did occur in 7% of cases (7/96) and were generally minor: bleeding from the biopsy site in 4 patients (1 open, 3 closed), abdominal pain in 2 (1 open, 1 closed), and a wound infection in a child who had an open biopsy. All patients recovered completely. The most important potential immediate complication is haemorrhage.

There is the possibility of seeding tumour cells into an uninvolved segment of the liver, the abdominal wall, or peritoneal cavity. These risks can be minimized by using a percutaneous coaxial technique or, in the case of a laparoscopic approach, using a protective sheath to guide Tru-cut biopsies.

14.1.2.2 Equipment

Diagnostic material should be obtained preferably with a semi-automatic core needle (e.g. Coaxial Temno, Allegiance Healthcare, McGaw Park, IL, USA). These are spring-loaded needles based on the “Tru-cut” type.
14.1.2.3 Operative technique

Severe coagulopathy and/or thrombocytopenia should be resolved before biopsy. Biopsies may be performed under general anaesthesia in children. Real time ultrasound (US) guidance makes liver tumour biopsy safer and easier, and is strongly recommended.

The outer needle of a coaxial core biopsy system should be introduced through a short depth of healthy hepatic parenchyma to minimize the risk of tumour seeding. As it is advanced through normal liver tissue into the tumour, care should be taken not to cross a segment of liver that is likely to be retained at future surgery.

The inner needle is a semi-automatic core biopsy needle, usually one gauge smaller than the outer needle. This is inserted into the outer needle after removal of the trocar, and advanced into a part of the tumour that does not appear to be necrotic, taking care to avoid large blood vessels. It is then fired under continuous US guidance. The inner needle is then removed and the specimen retrieved. The needle is then reinserted, and the process continued until the operator is confident that sufficient cores of tissue have been obtained. Often, particularly with large and necrotic tumours, it is worthwhile confirming the adequacy of the pathologic material samples by immediate frozen section histology.

After the final removal of the inner needle, the biopsy tract may be plugged under US guidance as the outer needle is withdrawn. This can be done with small plugs of gelatine foam (Gelfoam, Pharmacia and Upjohn, Kalamazoo, MI, USA) placed in the barrel of a 2 ml syringe filled with normal saline, and injected down the outer needle. Alternatively a slurry of microfibrillar collagen (Avitene®, MedChem Products, Woburn, MA, USA) can be used.

Biopsy samples should be sent fresh to the local histopathology laboratory. The biopsy tract should be marked by a stitch or tattoo and resected during subsequent definitive surgery.

14.1.3 Biopsies and resections

As immunohistochemistry is an important method in evaluating HB, a sufficient number of unstained sections (if possible at least 10, preferably more) should initially be prepared (on Superfrost Plus slides) in addition to the standard stain (i.e. hematoxylin and eosin).

For the resection specimens, the standard work up should take into account that these specimens may be greatly altered by chemotherapy (extensive necrosis) so that the most 'viable' looking areas should be sampled. It is proposed to macroscopically estimate, on the cut surfaces of the tumour, the area occupied by necrosis (in percent), as this may be relevant for prognosis. A gross photo or digital image of the most representative tumour sector would be very helpful, if feasible.

14.1.4 Centralisation of the histological material

Slides of both the diagnostic biopsy and representative sections of the resected tumour should be referred for central review in order to produce relevant clinical information.

It is still not known if there is an entity such as an 'unfavourable histology HB'. The anaplastic and the macrotrabecular variants may have a more aggressive clinical course and pure fetal histology a more favourable outcome but the evidence is not yet substantive. Only central review of this material will allow us to address this issue seriously.

To unify the centralization process of the histological material, the unstained sections should be on slides allowing immunohistochemistry (e.g. Superfrost Plus). Two unstained sections are the
minimum requirement, however the review group would be grateful to have more unstained sections, if this is feasible.

The specimens should be sent along with a copy of the pathological report direct to:

**Dr Monique Fabre/ Dr Catherine Guettier**  
Département de Bio-Pathologie Médicale  
Institut de Cancérologie Gustave Roussy  
114, rue Edouard Vaillant  
94805 Villejuif Cedex  
Tel: +33 145 212 024/ +33 145 212 024 2465  
Fax: +33 145 2132 81  
e-mail: monique.fabre@bct.aphp.fr/ catherine.guettier@bct.aphp.fr

The results of central pathology will be posted under the patient’s trial number on the remote data entry system.

### 14.1.5 SIOPEL Tumour Tissue Bank

To achieve the biological objectives of this study, SIOPEL kindly requests the submission of tumour material to the Tumour Bank in Zurich. Please contact Dr Michael Grotzer from the SIOPEL tumour banking project for further instructions regarding tissue sampling, preparation and transportation (see Appendix 11 for details).

Submission for the following samples is requested
- fresh frozen tumour tissue (see Appendix 11), from diagnosis if possible and at tumour resection
- formalin fixed paraffin embedded tumour block from diagnosis and resection
- histological sections as noted above
- DNA blood filter on Whatman FTA blood card (available from Tumour Bank) or alternatively use Guthrie paper.

### 14.2 Definitive tumour resection

#### 14.2.1 General notes

The ultimate goal of hepatoblastoma treatment is to achieve complete surgical resection of all residual tumour foci after preoperative chemotherapy. The SIOPEL International Liver Tumour Strategy Group recommends a delayed surgical approach in every case of hepatoblastoma since preoperative chemotherapy facilitates resection by shrinking most tumours and even downstaging some by limiting them to fewer hepatic sections. Only complete tumour resection gives realistic hope of cure for children with hepatoblastoma. This implies that all options should be explored before declaring a tumour unresectable. In this context orthotopic liver transplantation must also be considered for selected cases of standard risk hepatoblastoma.

#### 14.2.2 Technical considerations

In view of the variety of available surgical techniques and technical devices for performing liver resection, it is difficult to give detailed surgical guidelines.

Complete excision of the primary tumour can be achieved either by conventional hepatic surgery (partial hepatectomy) or by total hepatectomy and orthotopic liver transplantation.
In most cases of standard risk hepatoblastoma, hemihepatectomy or left lateral lobectomy (excising segments 2 and 3) is sufficient. Larger and/or multifocal tumours limited to 3 hepatic sections require an extended heptatectomy. In some cases, central liver resections (central heptatectomy as described by LaQuaglia) (69) or an extended atypical left lobectomy (according to Superina) (67) may be needed. These latter techniques require significant surgical expertise.

Blood loss during the phase of parenchymal resection can be minimized by maintenance of a low central venous pressure and by use of the Pringle manoeuvre (compression of the hepatoduodenal ligament). The latter can be applied safely up to 30-45 minutes except in small infants in whom severe bowel congestion may be hazardous.

Because of the rarity of liver tumours, surgery should be performed in specialist centres that are suitably experienced and equipped (e.g. ultrasonic or water-jet dissector, infrared beam or argon coagulator, intraoperative ultrasonography) for hepatic surgery. Even when all these conditions have been met, extensive personal experience of hepatic surgery remains important. Appropriate postoperative care facilities and experienced anaesthesiologists are also essential. Pre-operatively the patient’s cardiac function should be assessed by echocardiography to avoid any unexpected cardiac complication during surgery.

14.2.3 Assessment of resectability

- Accurate assessment of tumour resectability after neo-adjuvant chemotherapy (and before planned definitive surgery) is crucial. This requires optimal imaging studies: spiral CT with contrast administration (including angio-CT reconstruction of hepatic vessels when necessary) and/or magnetic resonance imaging with Gadolinium administration. Ultrasound Doppler examination is particularly valuable in demonstrating the relationship of the tumour to the hepatic vessels and their patency and potential invasion. Ideally, the surgeon should be present during the US evaluation to discuss the findings directly with the radiologist, particularly if there are doubts about resectability of the tumour. However, distinction between real invasion beyond the anatomic border of a given hepatic section and its compression and displacement by the tumour can sometimes be very difficult.

In multifocal tumours one should take into consideration the possibility to resect completely all invaded liver sections (if feasible) as determined by imaging at diagnosis even if they were cleared latterly with preoperative chemotherapy. If not feasible by conventional resection, consider liver transplantation. Otherwise small lesions, undetectable by preoperative imaging, may persist and the chance for local tumour recurrence may be higher.

The completeness of tumour resection should be assured by all possible means. If there is any doubt, frozen section histology from both sides of the resection margin should be obtained. If microscopic residual tumour is reported, the surgeon should explore the possibility of immediate re-resection of the margin taking an “extra slice” of the liver, when applicable and reasonable. The final judgement of completeness of resection is dependent on the definitive pathology report.

14.2.4 Difficult liver resections and the role of liver transplantation

Difficult liver resections with a high risk of residual tumour should be avoided whenever possible. This applies particularly to central tumours in close proximity to the major hepatic vessels which, in order to be preserved, would have to be peeled off the tumour. This is an
infrequent scenario in standard risk hepatoblastoma, but in such cases, primary liver transplantation should be considered.

14.2.5 General notes on liver transplantation

Recent data from the SIOPEL studies and a review of world wide experience demonstrate that liver transplantation is an effective and curative option for those children with hepatoblastoma in whom partial heptectomy is not feasible. The following guidelines help to select those cases suitable for liver transplantation.

14.2.5.1 Possible indications for liver transplantation in standard risk hepatoblastoma

Unifocal centrally located tumours involving mainly the hilar structures or main hepatic veins (especially if arising in caudate lobe), e.g. some PRETEXT II or III V1,V2/P1 tumours. The majority of these tumours should be resectable with standard surgical techniques but those where there is a significant risk of incomplete resection, are best treated by liver transplant. In any patient where the surgeon has doubts about the feasibility of complete surgical resection it is strongly recommended to seek the advice of a paediatric liver transplant surgeon and/or of the transplant/surgical co-ordinator of the trial. Progression of disease during chemotherapy or existence of active extrahepatic tumour deposits are contraindications for liver transplantation.

In case of doubt that an extrahepatic deposit is active or is simply a fibrous residual scar after chemotherapy, it is recommended to perform complete excision of the residue (biopsy if resection not possible) for histological analysis and appropriate decision of further patient management.

Poor tumour response to pre-operative chemotherapy is a relative contraindication, but the final decision for transplantation on an individual patient with this type of disease must take into account not only tumour size but several aspects and finally belongs to each transplant centre.

14.2.5.2 Timing of liver transplantation

The timing of definitive surgery, including liver transplantation, is critical for achieving adequate local tumour control. Liver transplantation should be done as soon as possible on haematological recovery after the last course of neo-adjuvant chemotherapy. Waiting for a deceased donor liver is a good option if the access to a donor graft can be anticipated within this time. Otherwise a living related donor liver transplantation (LRLTX) must be considered.

14.2.5.3 Rescue liver transplantation

Since the results of primary liver transplantation are far superior, in terms of patient survival, to those of rescue liver transplant heroic attempts at partial heptectomy with risk of incomplete resection should be avoided. When partial heptectomy has resulted in a macroscopically incomplete or when intrahepatic recurrence is observed after a previous partial heptectomy, the appropriateness of rescue liver transplantation is controversial, because of the disappointing results observed both in the SIOPEL 1 study and in the world experience. In addition, donor organ shortages necessitate strict selection of these cases.

However the final decision on an individual patient's selection for rescue liver transplantation belongs to each transplant centre.
14.3 Rapid surgical consultation

To assist centres in making the final decision on tumour resectability and issues of operative techniques, the possibility of a rapid consultation with our panel of experts (including a liver transplant expert) is available. To obtain this consultation please send a clinical summary of the patient and the pertinent radiological findings to either:

Dr Piotr Czauderna,
Department of Surgery and Urology for Children and Adolescents, Medical University of Gdansk, ul. Nowe Ogrody 1-6, 80-803 Gdansk, Poland.
Tel: +48 58 302 64 27 Fax: +48 58 302 14 16
Mobile: +48 609 060 774 E-mail: pczaud@gumed.edu.pl
or
Dr Daniel Aronson
Email: aronson.dc@hotmail.com
or
Mr Gordon MacKinlay,
Royal Hospital for Sick Children, Sciennes Road, Edinburgh EH9 1LF, United Kingdom.
Tel: +44 131 536 0660 Fax: +44 131 536 0655
E-mail: g.a.mackinlay@ed.ac.uk
or
Prof Frédéric Gauthier,
Département de Chirurgie Pédiatrique, Hôpital Bicêtre, 78 rue du Général Leclerc, 94 275 Le Kremlin Bicêtre, France.
Tel: +33 1 45 21 31 84 Fax: +33 1 45 21 20 97
E-mail: frederic.gauthier@bct-hop-paris.fr

Colleagues taking advantage of this service are requested to forewarn the doctors concerned via e-mail, phone or fax.

In those cases where liver transplantation is a probable therapeutic option the advice of a liver transplant surgeon must be sought early, preferably directly at the beginning of treatment, to facilitate a timely and accurate decision on the required surgical technique and the necessity of liver transplantation.

Colleagues seeking advice or consultation on LTX related issues are kindly asked to contact:

Dr Jean de Ville de Goyet,
Tel: +39 06 6859 2193 or 2851 Fax: +39 06 6859 3841
Email: deville@obpg.net

or (for South American countries):
Dr Paulo Chapchap,
R. Dona Adma Jafet, 60-6 Andar, CEP 01308-050, Sao Paulo, Brazil.
Tel: +55 11 214 4184 Fax: +55 11 255 9397
E-mail: mchapcha@ibm.net
## 15 TRIAL INVESTIGATIONS

### 15.1 Summary of Investigations Required Before, During and After Treatment

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<th>Course</th>
<th>Screening phase (A)</th>
<th>Pre-operative chemotherapy</th>
<th>Pre-operative assessment</th>
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<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Pregnancy Test (L)</td>
<td>(X)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-HCG (M)</td>
<td>X</td>
<td>(X)</td>
<td>(X)</td>
<td>(X)</td>
<td>(X)</td>
<td>(X)</td>
</tr>
<tr>
<td>Hepatitis B and C serology</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA blood sample (N)</td>
<td>(X)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine Tests</td>
<td>Creatinine, Na⁺, K⁺, Ca²⁺, Mg²⁺ Phosphate (fractionated excretion)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFR</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Audiogram (O)</td>
<td>X</td>
<td>(X)</td>
<td>(X)</td>
<td>(X)</td>
<td>(X)</td>
<td>(X)</td>
</tr>
<tr>
<td>Echocardiogram</td>
<td>(X)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imaging liver US/CT/MR (P)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>(X)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Imaging lungs CT/CXR</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Adverse Events: Record throughout treatment phase and up to 30 days post last administration of medication. SAEs: Immediately on knowledge of event.
X = Required
(X) = If indicated/Optional

A. Randomisation must occur within 15 days of written biopsy report confirming diagnosis.
B. As soon as possible but within 21 days of surgery.
C. 6 hours post completion of Cisplatin infusion.
D. Pathology from diagnosis and surgery must be reviewed centrally.
E. If consent for Biological studies has been obtained, provide FFPE tissue for Tissue banking and biomarkers from diagnosis and surgery, and DNA on a Whatman FTA blood spot card or Guthrie card at screening.
F. Measure temperature prior to each course of chemotherapy.
G. For patients receiving Sodium Thiosulphate (STS): record vital signs, including blood pressure (BP), pre STS, at the end of STS and 30 and 60 minutes after STS. If BP is not normal or returned to baseline level at 60 minutes post, notify a physician and monitor every 30 minutes until 2 successive BP values are normal or returned to baseline.
H. Assessed by mid-upper arm circumference (MUAC) at diagnosis, pre-surgery and end of treatment.
I. During chemotherapy FBC, Creatinine, Na+ and K+ must be assessed weekly.
J. For patients receiving STS, serum sodium must be monitored pre STS, 1 hour post, 6 hours post and 18 hours post STS.
K. During chemotherapy (pre and postoperatively), AFP level must be assessed weekly for at least 5 weeks, then every second week (prior to each course of chemotherapy).
L. For females of child-bearing potential.
M. β-HCG level must be assessed at baseline and then repeated if initially elevated, as indicated.
N. On Course 1 Day 1 of chemotherapy, an optional DNA blood sample (5-10ml) can be taken pre-Cisplatin and 24 hours following the start of Cisplatin administration (see Appendix 13).
O. It is recommended to perform audiometry after every second course of Cisplatin. A reliable hearing test must be performed within 6-12 weeks of end of treatment but not before the child is 3.5 years old.
P. Tumour evaluation must take place in the second week of courses 2 and 4. If definitive surgery is delayed after 4 courses of chemotherapy, a further 2 courses can be administered.
15.2 Investigations at Diagnosis

15.2.1 Pre-treatment assessment of tumour extension (PRETEXT)

The PRETEXT system has been conceived to describe tumour extension before any therapeutic intervention. The classification is based on the Couinaud system of segmentation of the liver and the segments are grouped into four sections, i.e. the left lateral and medial sections and the right anterior and posterior sections. The PRETEXT number reflects the numbers of sections that are involved by tumour. This number is a rough estimate of the difficulty of the expected surgical procedure and is used to allocate a patient to either standard risk (PRETEXT I-III) or high risk (PRETEXT IV).

Furthermore the PRETEXT system includes criteria for tumour extension beyond the liver. The original system assessed involvement of the inferior vena cava and hepatic veins (V), involvement of the portal veins (P), extrahepatic abdominal disease (E) and distant metastases (M). This system has been further elaborated through the 2005 PRETEXT revision to more clearly describe tumour extension with additional criteria (73). However the risk stratification is not changed compared to the SIOPEL 4 protocol.

See Appendix 3 for full description, allowing assignment of PRETEXT category. Diagrams and further explanations of the PRETEXT system are also available on the SIOPEL website (www.siopel.org). In case of difficulties see section 15.2.3 below.

15.2.2 Radiological investigations

The essential investigations are:

- Abdominal ultrasonography with particular attention to the portal and hepatic venous systems
- Contrast-enhanced computed tomography (CT) and/or magnetic resonance imaging (MR) of the abdomen
- CT of the chest

Other imaging investigations should be performed at the discretion of the local centre. Guidelines for radiological investigations are given in Appendix 4.

The extent of disease should be assessed according to the PRETEXT system (Appendix 3). The imaging radiologist should also determine the number of lesions in the liver and their location, as well as maximum diameters of the primary tumour in three dimensions (two is acceptable) and the tumour volume.

Local surgeons and radiologists will be asked to judge the resectability of the primary tumour based on the diagnostic imaging. An advisory radiological panel (ARP) is available to assist local physicians in their decision making.

Central review will take place of all diagnostic, pre-surgery and relapse imaging studies. This includes CT, MRI and ultrasound images.

Images for central review should be sent on CD in DICOM format. Alternatively, hard copy files or CDs with JPEG files are acceptable but not preferred. Images should be sent via post as soon as possible after they are obtained to:

Dr Derek Roebuck
Department of Radiology
Great Ormond Street Hospital
Great Ormond Street
London WC1N 3JH
United Kingdom
15.2.3 Optional Rapid Radiological Review (RRR)

It is acknowledged that there may be difficulties in correctly identifying the precise extension of the tumour within the liver and its operability. The most important problem occurs when it is difficult to judge if a large PRETEXT III tumour compresses or infiltrates the remaining hepatic section. In order to help individual centres arrive at a correct assessment, our Radiological Coordinator Dr Derek Roebuck has agreed to coordinate an advisory radiology panel (ARP). Radiological images (either hard copy films or digital versions) can be submitted for review, and a final opinion on a specific patient’s imaging will be produced. The panel’s opinion will be forwarded urgently (by fax or email) to the referring centre. The Rapid Radiological Review (RRR) form can be downloaded direct from www.siopel.org members section under the RRR area.

The accuracy of radiological review depends strongly on the quality of the imaging available. To submit a case for RRR, as many images as possible should be submitted. If electronic transport of the pertinent radiological studies is not feasible films should be sent to:

Dr Derek Roebuck  
Consultant Interventional Radiologist  
Department of Radiology  
Great Ormond Street Hospital  
Great Ormond Street  
London WC1N 3JH  
United Kingdom

Tel: +44 20 7829 7943  
Tel Home: +44 20 7689 2143  
Air Call: +44 20 7405 9200  
Fax: +44 20 7242 1607  
Email: RoebuD@gosh.nhs.uk

Colleagues sending material for this rapid central review are requested to forewarn Dr. Roebuck by email (above) and also the International Trial Facilitator, Margaret Childs, at siopel@cineca.it (Drs. Kieran McHugh and Øystein Olsen at the same department are available for RRRs if Dr. Roebuck should for any reason be temporarily unavailable).

Please note that complete investigations, including RRR if desired, have to be completed prior to the start of the treatment. In a patient who is unstable it is better to stabilize the child first. There is no evidence that waiting for full grouping and staging is detrimental to outcome provided that this is completed in a reasonable time frame. This implies that central radiological review should be requested as soon as possible after the initial imaging studies have been performed.

15.2.4 Pre-treatment biopsy

Diagnostic tumour biopsy is mandatory in all cases regardless of the patient’s age and serum AFP level. Percutaneous ultrasonic guided core needle biopsy (i.e. Tru-cut type) is preferred but other methods may be chosen. However, fine needle aspiration cytology is not acceptable as it generally provides insufficient material. See section 14.1 for guidelines how to perform biopsy and sections 7.1 and 14.1.4 for pathological guidelines regarding classification and central review. The SIOPEL Liver Tumour Tissue Storage Program is described in Appendix 11.

15.2.5 Serum AFP

- Serum alpha-fetoprotein (AFP) level is to be determined (see Appendix 2).

N.B. In cases of normal serum AFP - ensure that appropriate dilutions have been carried out. Beware of false negative result. True low AFP (<100 µg/L), i.e. level confirmed with at least two consecutive measurements and appropriate dilutions, is a high-risk (and exclusion) criteria.
Infants with AFP normal for age (see Appendix 2) or lower, but >100 µg/L, should be discussed with the study coordinators whether they should be treated according to a standard-risk or a high-risk protocol.

15.2.6 Audimetry

Baseline audimetry is strongly recommended as the main objective of the trial is to study and try to reduce hearing impairment caused by Cisplatin chemotherapy. The youngest children, who are the hardest to test, are the most prone to suffer from ototoxicity. The audiology committee has developed guidelines and test protocols recommended for different age groups, please consult section 15.9 for detailed instructions.

The use of any aminoglycosides ≤ 12 months before entering the trial will be recorded.

Audiologic data will be centrally reviewed by an Audiology Committee. Dr Kaukab Rajput, Consultant Paediatric Audiologist in London is the committee chair. In case there is a need to discuss the appropriate audiological assessment for a particular child, or the choice of appropriate National Centres and necessary audiological facilities Dr Rajput can be contacted by e-mail RajpuK@gosh.nhs.uk

15.2.7 Physical examination

- Anthropometric measurements (weight, height/length and body surface area)
- Nutritional status assessment by mid-upper arm circumference (MUAC) (percentiles or standard deviations (SD))

15.2.8 Laboratory tests

15.2.8.1 Blood

- Full blood cell count
- Liver function including: Bilirubin, ALT, AST, Alkaline Phosphatase, gamma GT
- Serum creatinine and urea
- Serum electrolytes (Na⁺, K⁺, Ca++, Mg++, Phosphate)
- Partial Thromboplastin Time, Prothrombin time or equivalent
- Serum β-human chorionic gonadotrophin hormone (β-HCG) level along with alpha-fetoprotein (AFP) level
- Hepatitis B and C serology
- Blood sample spotted onto Whatman FTA DNA blood filter (available from Tumour Bank in tumour collection kit) or Guthrie card
- A pregnancy test for females of child-bearing potential

15.2.8.2 Urine

- Urinary creatinine and electrolytes (Na⁺, K⁺, Ca++, Mg++, Phosphate) so that, using fractionated excretion, tubular function can be measured

15.2.9 Glomerular filtration rate

By clearance method either ⁵¹Cr-EDTA, iohexol or inulin. The same method should be used for a particular patient throughout. (If completely impossible to obtain before the first course calculated clearance might be used for the first course only.)

15.2.10 Cardiac function

If clinically indicated at diagnosis, otherwise pre-surgery.
- 2D-derived echocardiogram - measurement of shortening fraction and/or left ventricular ejection fraction (LVEF)
- To be repeated only if the initial shortening fraction (SF) is < 29% (or ejection fraction (EF) < 40%)

15.3 During chemotherapy (pre- and post-operatively)

15.3.1 Physical examination

Including weight, height/length, body surface area, temperature, BP and nutritional status assessment (MUAC) before starting each course

15.3.2 Monitoring during Cisplatin and STS

Serum electrolytes (Na⁺, K⁺) have to be carefully monitored according to section 12 and 13. Urine output must also be carefully monitored. For patients on STS: vital signs, including BP, must be recorded pre-STS, at the end of STS and 30 and 60 minutes after STS. If BP is not within the normal range or returned to baseline 60 minutes post dose, notify a physician and monitor every 30 minutes until two successive BP values are within normal range or returned to baseline. Please refer to BP systolic percentile charts in Appendix 14.

15.3.3 Laboratory Tests

15.3.3.1 Day 1

DNA blood samples (5-10 mls) – refer to section 15.8 and Appendix 13 for details.

15.3.3.2 Weekly

Blood: Full blood cell count, serum creatinine, Na⁺, K⁺
AFP level weekly for at least 5 weeks (then every 2nd week)

15.3.3.3 Before starting each course

Blood: Full blood cell count, creatinine, urea, Na⁺, K⁺, Ca++, Mg++, Phosphate, ALT, AST, Bilirubin, Alkaline Phosphatase, gamma GT
AFP level
ß-HCG level if initially elevated

15.3.4 Audiometry

After baseline audiometry has been obtained, before start of the treatment, it is recommended to perform audiometry after every second Cisplatin course, see section 15.9.

15.3.5 Glomerular filtration rate

⁵¹Cr-EDTA clearance (or other valid clearance method) after every second course

15.3.6 Response evaluation during pre-operative chemotherapy

During pre-operative chemotherapy, response and tumour status must be evaluated in the second week of courses 2 and 4 courses of Cisplatin to exclude progression.

15.3.6.1 Measurement of serum AFP

- Weekly during the first two courses until clear response has been documented (for at least 5 weeks)
Before starting each course (i.e. every second week) after a clear response has been documented and until end of therapy.

15.3.6.2 Radiological investigations
- abdominal ultrasound

15.4 Before surgery

15.4.1 Physical examination
Including weight and nutritional status assessment (MUAC). Please note, that surgical complications are more common in children with a poor nutritional status.

15.4.2 Laboratory tests
Blood: Full blood cell count, creatinine, urea, Na⁺, K⁺, Ca++ , Mg++, Phosphate, ALT, AST, Bilirubin, Alkaline Phosphatase, gamma GT
Partial Thromboplastin Time, Prothrombin time or equivalent
AFP level
β-HCG level if initially elevated

15.4.3 Assessment of resectability
- abdominal ultrasound and abdominal CT scan with contrast or MR with gadolinium
- chest X-ray
- other imaging studies (angiography etc.) if indicated

Please note: Careful assessment of the relationship of the tumour to adjacent vital structures and demonstration of the segmental/vascular anatomy of the remaining liver will be required. This may be done by any combination of ultrasound, CT and MR studies. The presence or absence of lung metastases is best assessed by CT (see Appendix 4).

15.4.4 Glomerular filtration rate
51Cr-EDTA clearance or other valid clearance method

15.4.5 Audiometry
After baseline audiometry has been obtained, before start of the treatment, it is recommended to perform audiometry after every second Cisplatin course, see section 15.9.

15.5 After surgery

15.5.1 Tumour/resection status evaluation after definitive surgery:

In case of any doubt the lack of residual tumour must be confirmed with imaging studies performed within 2 weeks after surgery:
- abdominal ultrasound or abdominal CT scan with contrast or MR with gadolinium
- other imaging studies if indicated
- AFP level

See also sections 15 and 16
15.6 At the end of therapy

15.6.1 Physical examination
Including weight, height/length and nutritional status assessment (MUAC)

15.6.2 Laboratory tests
Blood: Full blood cell count, creatinine, urea, Na+, K+, Ca++, Mg++, Phosphate, ALT, AST, Bilirubin, Alkaline Phosphatase, gamma GT
AFP level (see above and section 16)
ß-HCG level (if initially elevated)

Urine: creatinine and electrolytes (Na+, K+, Ca++, Mg++, Phosphate) so that, using fractionated excretion, tubular function can be evaluated

15.6.3 Audiologic evaluation
- bilateral pure tone air conduction thresholds
- otoscopy
- immittance evaluation
- measurement of transient evoked otoacoustic emissions

Please see section 15.9.3 for recommended test protocol for age.
A reliable hearing test must be performed within 6-12 weeks of end of treatment but not before the child is 3.5 years old. Please refer to section 15.7.

15.6.4 Tumour/remission status
- abdominal ultrasound and abdominal CT scan with contrast or MR with gadolinium
- chest X-ray
- other imaging studies if indicated

15.6.5 Glomerular filtration rate
$^{51}$Cr-EDTA clearance or other valid clearance method

15.6.6 Cardiac function
- Only to be repeated if the initial shortening fraction (SF) was < 29% (or ejection fraction (EF) < 40%)

15.6.7 Due to any kind of toxicity
Contact the chemotherapy panel coordinator for any life threatening, lethal, unexpected or unusual toxicity. (See Serious Adverse Events – Section 17)
15.7 Follow-up and primary end-point

If the child was younger than 3.5 years at the end of treatment a reliable hearing test will have to be obtained as soon as that age is achieved and evaluation can be done, see section 15.9.8.

### Table 15.1 Follow-up schedule

<table>
<thead>
<tr>
<th>Time from diagnosis: Relevant Examinations</th>
<th>1st &amp; 2nd year</th>
<th>3rd year</th>
<th>4th &amp; 5th years</th>
<th>Subsequent years**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical examination</td>
<td>Every 2-3 months</td>
<td>Every 6 months</td>
<td>Every 6 months</td>
<td></td>
</tr>
<tr>
<td>Alpha-fetoprotein*</td>
<td>1st year: every month 2nd year every 3 months</td>
<td>Every 6 months</td>
<td>Every 6 months</td>
<td></td>
</tr>
<tr>
<td>Chest-X ray*</td>
<td>Every 3 months</td>
<td>Every 6 months</td>
<td>Yearly</td>
<td></td>
</tr>
<tr>
<td>Abdominal ultrasound*</td>
<td>Every 2-3 months</td>
<td>Every 6 months</td>
<td>Yearly</td>
<td></td>
</tr>
<tr>
<td>Serum magnesium</td>
<td>Yearly</td>
<td>Yearly</td>
<td>Yearly</td>
<td></td>
</tr>
<tr>
<td>GFR: 51Cr EDTA-clearance</td>
<td>One year off treatment - to be repeated yearly if &lt;80 ml/min/1.73 m²</td>
<td>Yearly if &lt; 80 ml/min/1.73 m</td>
<td>Yearly if &lt; 80 ml/min/1.73 m</td>
<td></td>
</tr>
<tr>
<td>Audiometry</td>
<td>Yearly until reliable result obtained with pure tone audiometry (age ≥3.5 years)</td>
<td>Yearly until reliable result obtained with pure tone audiometry (age ≥3.5 years)</td>
<td>Yearly if no reliable result obtained previously or if clinically indicated</td>
<td></td>
</tr>
</tbody>
</table>

* Abnormal findings should be further investigated with MR/CT abdomen and/or spiral lung CT scan.
** According to national profile.
15.8 Biological and Pharmacological Studies

The proposed SIOPEL biological and pharmacological studies are outlined below (and detailed in Appendix 12 and 13).

The following samples will be requested from participating centres:

- Representative blocks of formalin fixed paraffin embedded (FFPE) tumour plus fresh frozen tumour (FT) (wherever possible) collected at time of diagnosis and at tumour resection
- A minimum of 2 representative histological slides from biopsy and tumour resection
- Peripheral blood sample collected on Whatman FTA blood card for preparation of genomic DNA (for analysis of potential genetic determinants of treatment related toxicity)
- Whole blood samples collected prior to Cisplatin treatment and 24 hours after the start of Cisplatin administration (for analysis of platinum-DNA adduct levels as a pharmacodynamic marker), preferably taken during Cycle 1

**Figure 15.1 Summary of Histological, Biological and Pharmacological sampling**

**Figure 15.2 Sampling scheme for measurement of Platinum-DNA adduct formation in leucocytes of patients treated with Cisplatin +/- Sodium Thiosulphate (STS)**

*optional

** See section 12 for age adjusted dose
15.9 Audiometry

15.9.1 General notes

It is accepted that this trial, aimed at reducing ototoxicity in very young patients with hepatoblastoma, presents a challenge to oncology departments to liaise with their respective audiology departments in different countries. Some units will be better served than others. In order not to have to refuse patients entry into the trial, the end point has been made extremely simple and requires pure tone audiometry to be carried out at the age of 3.5 years when the child is off treatment. 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.

It is hoped, however, that a number of centres will be able to monitor the audiology serially at any age. These patients’ results will be analysed carefully to learn all that we can, in spite of the small numbers, about ototoxicity and its prevention in the very young treated with Cisplatin.

In order to make central review meaningful all audiologic evaluations will be completed using standard clinical audiometers, middle ear analyzers, and evoked OAE systems. All equipment will be calibrated in accordance with international guidelines. Baseline hearing evaluations will be completed before the first dose of Cisplatin whenever possible. Definitive audiologic evaluation will be completed for all children at the age of 3.5 years (or as soon as a reliable hearing test can be obtained). The Minimum criteria for entry into this trial is that Pure Tone Audiometry can be done, together with otoscopy and tympanometry, at the end of treatment directly or when the child reaches the age of 3.5 years, if younger at the end of treatment. The audiological testing must take place in a competent department familiar with testing young children. The parents must agree in writing, on the trial consent form, to travel to a centre, in another town in their country, if necessary, in order to get this done. The end of treatment audiological review must then be uploaded onto the CINECA remote data entry system for central review. All participating centres will be asked to give contact details of the Consultant Audiologist or Ear Nose and Throat Surgeon who will take responsibility for co-ordinating and overseeing the quality of the centre considered competent to carry out this end of treatment audiological assessment in their country.

ASHA criteria and OAE guidelines and Brock grading are available in Appendix 5. Detailed VRA and ABR audiology guidelines and references are outlined in Appendix 6.

15.9.2 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 (TEOAEs) and distortion product otoacoustic emissions (DPOAEs), at facilities where this equipment is available
- If a child is uncooperative 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.

Procedures for obtaining pure tone thresholds will 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 will be used to obtain ear-specific thresholds whenever possible. Bone conduction thresholds will 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 will be estimated using an electrophysiologic test procedure, specifically click or tone burst evoked auditory brainstem response (ABR).

Immittance evaluation will 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 will 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 will be measured at 500, 1000, 2000 and 4000 Hz ipsilaterally in both ears.

TEOAEs and DPOAEs will 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 will be deferred until the middle ear pathology resolves. For TEOAEs click stimuli will be presented at 80 dB peak equivalent SPL. The two TEOAE parameters that will 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 one level of L1 and L2 65/55 dB SPL will be done at least at four frequencies. TEOAE is regarded as a better predictor of low frequency and DPOAE of high frequency sensitivity.

15.9.3 The following test protocol is recommended:

15.9.3.1 Evaluation for children younger than 12 months of age should 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 will then be converted to equivalent of hearing level (HL) by the central audiology committee through the RDE web-site to equate to Pure Tones (Appendix 6).
- tympanometry
- measurement of TEOAEs
- measurement of DPOAEs

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

15.9.3.2 Children aged 12-42 months should 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
- TEOAEs
- DPOAEs

If reliable results cannot be obtained by behavioural testing, then ABR or acoustic reflex threshold measurement is recommended.
15.9.3.3 Children 3.5 years and older should have
- evaluation using play audiometry as described above or standard pure tone threshold
- tympanometry
- TEOAEs
- DPOAEs

15.9.4 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 6.

15.9.5 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 OAEs. 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 behavioural 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 will be obtained if evaluation with frequency specific stimuli is not available. ABR thresholds will be determined as the lowest level at which detectable, repeatable wave V responses are obtained. Responses will be labelled only when replicable and compared with normative paediatric 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 thresholds obtained in dB nHL should be converted to HL using the conversion table.

15.9.6 Guidelines for otoacoustic emissions (OAE)
Otoacoustic emissions were first reported by Kemp in 1978, these are sounds that are produced by healthy ears in response to acoustic stimulation. They are byproducts 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 digitized 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 (74).
15.9.7 Monitoring during treatment

After the initial baseline evaluation as recommended in section 15.9.2 before start of treatment, interim audiometry is recommended after every second cycle of Cisplatin. In children younger than 3.5 years of age interim audiometry is strongly recommended.

15.9.8 Definitive evaluation at end of treatment and age 3.5 years

All children will 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 is to be done within 6-12 weeks after the last Cisplatin dose. Ototoxicity will be assessed using Brock grading (5), as well as ASHA guidelines (6) wherever pre-chemotherapy hearing thresholds are available.

For the purposes of the trial, if the children have hearing loss equal to or greater than Brock’s grade 1 on the definitive audiologic evaluation, that will be considered as positive for ototoxicity.

Wherever ASHA criteria can be applied, for the purposes of the trial, ototoxicity will be defined as positive when there is a hearing loss of:
- 20 db 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 will be reported for both ears.

15.9.9 Central review

All audiologic data will be centrally reviewed by the Audiology Committee chair and members. The data will be collected on each child and will be submitted in HL weighting. Data should be submitted for central review as soon as possible after the tests have been performed. The results can be directly uploaded to the SIOPEL 6 RDE system. Or forwarded directly to:

Kaukab Rajput
Consultant Audio-vestibular Physician
Dept of Audiological Med. and Cochlear Implant
Great Ormond Street Hospital for Sick Children
London, WC1N 3JH
United Kingdom
Tel: +44 207 813 8316
Fax: +44 207 829 7877
16 CRITERIA OF EVALUATION AND DEFINITIONS
For all endpoints, all treated patients (i.e. who received at least one dose of trial medication) will be analyzed.

In order to achieve optimal comparability with previous SIOPEL trials, the same criteria as used in the SIOPEL 1, 2 and 3 trials will be applied in the present trial for evaluation of response to pre-operative chemotherapy and tumour status at the end of trial treatment (SIOPEL criteria).

16.1 Response
Complete response (CR): no evidence of disease and normal serum AFP value (for age).
Partial response (PR): any tumour volume shrinkage associated with a decreasing serum AFP value, > 1 log below the original measurement.
Stable disease (SD): no tumour volume change and no change, or < 1 log fall of the serum AFP concentration.
Progressive disease (PD): unequivocal increase in 1 or more dimensions and/or any unequivocal increase of the serum AFP concentration (three successive 1-2 weekly determinations) even without clinical (physical and/or radiological) evidence of tumour re-growth.

Please note:
- Tumour dimensions, especially the primary, should be recorded in 3 planes. 2 planes will be acceptable, if the third cannot be obtained.
- Bear in mind that "no change" or even an increase in "tumour" volume, especially during the first few weeks of chemotherapy, may be the consequence of intra-tumour haemorrhage/oedema. If serum AFP is falling, continue the same chemotherapy for at least one more course.
- Tumour lysis syndrome may lead to an initial rise in AFP before the level falls.
- Sometimes the actual tumour volume does not change in response to therapy, but the AFP decreases; these are 'responses' not cases of stable disease.

16.2 Complete resection
Total macroscopic removal of the tumour as reported by the surgeon and pathologist. In case of any doubt the lack of residual tumour must be confirmed with imaging studies performed within 2 weeks after surgery.

Please note:
- Total hepatectomy followed by liver transplantation will be considered – if accompanied by complete removal of the primary tumour - as complete resection and not as treatment failure.
- Tumour removal with microscopical residual disease (confirmed by pathological investigation) but no macroscopical residue (as defined above) will be considered as complete resection.

16.3 Complete remission
Lack of evidence of residual disease and normal (for age) AFP at the end of trial treatment.
To establish a complete remission all of the following requirements must be fulfilled:
- No evidence of tumour intra-abdominally: negative abdominal (including hepatic) ultrasound or CT scan or MR.
- No evidence of metastases: clear chest X-ray (PA and lateral) for non-metastatic patients. (Normal lung CT scan for patients with lung metastasis at diagnosis, who are high-risk by definition and not treated according to SIOPEL 6).
- Serum AFP level either normal or compatible with age for at least 4 weeks after normalisation. For normal ranges at various ages see Appendix 2.

Please note:
- A disease free status achieved by total hepatectomy followed by liver transplantation will be considered – if accompanied by no remaining tumour lesion on imaging and normal AFP - as complete remission and not failure of chemotherapy.
- Patients who have microscopical residual disease (confirmed by pathological investigation) after tumour resection but no macroscopical residue and a normal serum AFP will be considered as being in complete remission.
- It can take several weeks before a high AFP level returns to normal. If there is no residual tumour left, AFP should decrease until normal levels are reached.
- A minimal rise in the AFP value shortly after surgery may be a sign of liver regeneration.
- A persistently elevated serum AFP level usually indicates persistence of active disease until proven otherwise. It is not uncommon to find a slowly rising AFP level (particularly for AFP level < 100 ng/ml), before actual residual tumour can be identified. In this case abdominal and chest radiological investigations should be repeated until the site of relapse is identified. Occasionally, especially in infants, the AFP level eventually declines spontaneously to normal, without any cause having been identified.

16.4 SIOPEL relapse criteria
Relapse is defined as one of the following situations:
- Recurrent tumour lesion(s) (local or metastatic) detected by imaging techniques and serial elevation of serum AFP (at least 3 consecutive rising values, taken at weekly intervals). In these patients biopsy of the tumour is recommended but not compulsory.
- Recurrent tumour lesion(s) (local or metastatic) with normal AFP, histologically confirmed by biopsy.

In case of elevated serum AFP only, without detectable tumour lesions, repeated measurement of the AFP level is recommended. If it continues to increase, imaging studies must be performed (ultrasound, CT, MR, PET–scan, bone scan etc.) to detect recurrent tumour mass. Past experience indicates that an elevated AFP can precede by many weeks the actual documentation of tumour recurrence. One should, however, wait until the site of recurrence is clear so that local treatment can be performed.

16.5 Overall survival
Overall survival will be calculated from the time of randomisation to death.

16.6 Event free survival
Event free survival will be calculated from the time of randomisation to the first of the following events: progression, relapse, secondary primary malignancy or death.

16.7 Adverse drug reactions
Adverse drug reactions are defined as adverse events, which are possibly, probably or definitely related to the trial treatment. They will be assessed according to NCI CTCAE v 3.0. (See section 17 and Appendix 10.) The full text version is available at: http://ctep.cancer.gov/forms/CTCAEv3.pdf
17 COLLECTION AND REPORTING OF ADVERSE EVENTS (AEs) AND SERIOUS ADVERSE EVENTS (SAEs)

17.1 Coding of adverse events

Details on individual safety measures (treatment and dose modifications and toxicity monitoring) are given in section 12 and 13. The trial will be monitored for excessive toxicity by regular evaluation of toxicity data by the trial committee during the trial. Toxicities will be classified according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events v 3.0 (CTCAE). See Appendix 10 for an introduction to the CTCAE. Full text version is available at: http://ctep.cancer.gov/forms/CTCAEv3.pdf

17.2 Serious adverse events

Serious Adverse Events (SAE), occurring during therapy and until 30 days after the “end of treatment visit”, must be reported immediately on knowledge of the event, using the remote data entry system.

SAEs must also be reported throughout follow up if considered to be related to study related procedures.

A Serious Adverse Event is defined – in accordance with the ICH Harmonised Tripartite Guideline for Good Clinical Practice definitions and additionally - as any untoward medical occurrence that:

- results in death (NB: death by tumour progression is not considered an SAE for these patients).
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- progression of disease
- is an overdosage
- is a second primary malignancy
- results in persistent or significant disability/incapacity
- is a congenital anomaly
- any unexpected grade 3 and 4 toxicity

SAEs will be immediately directed, by the RDE system, to the Safety Review Panel. Following review and consensus opinion each SAE will be categorized and in cases of SUSARS reported within the 15 day mandatory timeframe within the UK to the Medicines for Health Regulatory Agency. The SUSAR notification will also be alerted to all participating sites within SIOPEL 6, together with National Coordinators for reporting as appropriate to comply with their local requirements.

Automated reports will be accessible on the CINECA RDE system for immediate up-to-date review by each National Coordinator, or for individual collaborating clinicians by contact with either Dr Penelope Brock, International Chairman, or Margaret Childs, International Trial Facilitator.

Expected grade 3 and 4 toxicity will include transient hypernatremia for the patients receiving STS, grade 3 and 4 haematological toxicity, grade 3 and 4 nausea and vomiting, anorexia with tube feeding and alopecia. These will not be regarded as SAEs. The trial end points will also not be collected as unexpected events such as grade 3 and 4 hearing loss, hypomagnesaemia or renal toxicity.
Progression of disease should be reported as an SAE. This is to be certain that the numbers of patients progressing in each arm are promptly recorded. This is important to ensure the stopping rules can be properly applied.

It is expected that each national group will report SAEs to their ethics committees and applicable regulatory authorities as required by national legislation.
18 STATISTICAL CONSIDERATIONS

18.1 Introduction

The main objective of the trial is to investigate if the administration of STS simultaneously with the administration of Cisplatin significantly reduces the hearing impairment at the age of 3.5 years or higher, when compared to patients treated with Cisplatin alone.

Hearing impairment can be defined either as a change from a baseline measurement to after the completion of trial treatment (in line with the concept of the ASHA criteria), or as an audiometry result below a chosen threshold value. In trials SIOPEL 2 and 3, 84% of children were aged less than 3 years at the time of inclusion. As a consequence, a reliable baseline measurement is not obtainable from the majority of patients at the time of inclusion. Therefore it is decided to use the absolute hearing threshold at the age of 3.5 for the definition of the primary endpoint for this trial.

From provisional SIOPEL 2 and 3 results (based on centres with a reporting rate of ≥62%) we know that 59% of children report a Brock grade 1-4 hearing impairment (defined as any threshold ≥ 40dB at any frequency between 4000 and 8000 Hz) any time after the end of treatment. All of these children were treated with Cisplatin. This rate of hearing impairment may be a slight over-estimate due to differential reporting.

Definition of primary endpoint: Rate of Brock grade ≥1 hearing loss determined after end of trial treatment or at an age of at least 3.5 years, whatever is later (see Appendix 5).

Patients are randomized 1:1 between the two trial arms. Randomisation is stratified by:
- Country
- Median age (above vs below 15 months)
- PRETEXT (1 and 2 vs 3)

The minimisation method is used as a randomisation algorithm. The randomisation will be central via the CINECA remote data entry system (see Section 11).

18.2 Sample size

The hypothesis to be tested is a reduction of the rate of hearing loss as defined above from 60% under Cisplatin alone to 35% under Cisplatin and STS. The test is a one-sided chi square test with significance level of 5% and power of 80%. A group-sequential design is chosen with two interim and one final evaluation, an alpha-spending function according to Lan DeMets with O’Brien Fleming boundaries. Early stopping for higher than expected difference is foreseen.

Based on these parameters, a total of 102 evaluable patients need to be recruited (PASS 2002, J. Hintze 2001, NCSS, Kaysville, Utah). If 10% of the randomised patients are expected to be inevaluable for the primary endpoint, then the sample size should be augmented to 115. This will be attainable in 3.8 years with a yearly recruitment of 35 pts and allowing for a run-in period of 6 months.
The baseline hearing loss under Cisplatin alone may not be exactly 60%. The trial does however have $\geq 80\%$ power to detect an absolute reduction in hearing loss of 25% over a wide range of other baseline hearing loss values, as shown in the following table:

<table>
<thead>
<tr>
<th>Hearing loss rates</th>
<th>Power</th>
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<tbody>
<tr>
<td>50 $\to$ 25</td>
<td>83%</td>
</tr>
<tr>
<td>55 $\to$ 30</td>
<td>81%</td>
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<tr>
<td>60 $\to$ 35</td>
<td>80%</td>
</tr>
<tr>
<td>65 $\to$ 40</td>
<td>80%</td>
</tr>
<tr>
<td>70 $\to$ 45</td>
<td>81%</td>
</tr>
</tbody>
</table>

### 18.3 Interim analysis and early stopping

Early stopping may be warranted in case of convincing evidence that a reduction in hearing impairment by at least 25% is corroborated. Interim analyses will be conducted at 1/3 and 2/3 of process time, i.e. after 34 and 68 patients are evaluable for the primary endpoint. If the nominal alpha levels for the test of the primary endpoint will be $<0.00069$ (34 pts), $<0.016$ (68 pts), early stopping of the trial will be considered. The final test will be carried out at nominal alpha level of 0.045.

In case of concerns of an adverse effect of STS on the short-term efficacy of the Cisplatin chemotherapy, the trial may be stopped early as well. Interim results from SIOPEL 2 and 3 (both arms combined) indicate a $\sim 95\%$ response rate after 4 courses of Cisplatin, a $\sim 95\%$ rate of complete resection after this induction chemotherapy, $\sim 85\%-90\%$ rate of 2 year progression-free survival, and $\sim 90\%$ survival rate at 2-3 years. Interim efficacy results on response to chemotherapy will be evaluated after every 20 patients and submitted immediately to the IDMC and the trial committee. The IDMC and the trial committee will independently review the results. The IDMC will formulate a recommendation to the trial committee. All endpoints will be evaluated in the confidential annual report to the IDMC, but the primary endpoint will only be compared by a statistical test, as described above. If interim results are lower than observed in SIOPEL 2 and 3, or if the rate of early progressive disease after 2 cycles (section 16.1) leading to salvage chemotherapy (section 12.7) raises concerns, early closure of the trial will be considered.

After each 20 patients (10 per arm), the 95% lower confidence limit for the difference in rates of progression will be calculated. As soon as the lower limit is zero, the trial will be recommended for closure due to a negative effect of STS on response to chemotherapy. This strategy is equivalent to a one-sided test with a significance level of 5% to test if the difference is more than 10%. The following table shows various combinations leading to a recommendation of early closure:

<table>
<thead>
<tr>
<th>sample size per arm</th>
<th>Number of progression CDDP</th>
<th>Number of progression CDDP+STS</th>
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<tr>
<td>10</td>
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<td>4</td>
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<tr>
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<td>sample size per arm</td>
<td>Number of progression CDDP</td>
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<td>18</td>
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Toxicity will also be assessed by the IDMC as well as by the trial committee. The trial will be stopped early if the treatment leads to toxicity considered unacceptable by either of the above.
18.4 Evaluation

For patients who are older than 3.5 years, the audiometry result has to be obtained 6-12 weeks after the administration of the last chemotherapy cycle. Patients who have not received the whole planned chemotherapy will still be included in the evaluation according to the intention to treat principle. The audiometry results will be interpreted irrespective of the treatment received (chemotherapy, surgery) and its outcome. Patients without interpretable audiometry results (inevaluable patients) will be excluded from the analysis of the primary endpoint.

The results of the primary endpoint analysis will be presented as rates with 95% confidence interval (CI), and as a relative risk with 95% CI. In addition, audiograms will be evaluated in detail. Time to event endpoints will be presented as Kaplan-Meier estimators and corresponding graphs. Overall survival (OS) and event free survival (EFS) will in addition be analyzed in an exploratory fashion by multivariate Cox regression taking into account potential prognostic factors. Rates will be compared by chi-square test or Fisher’s exact test as appropriate. The results will be compared in a descriptive manner with those obtained from SIOPEL 2 and 3 (historical controls).

The final analysis will be done once a definitive assessment of hearing impairment is available for 102 patients, or at the latest 3 years after the last patient has been enrolled. OS and EFS endpoints will be continued to be evaluated up to a follow-up of at least 5 years from the enrolment of the last patient.
19 TRIAL MONITORING and IDMC

Reports on available data will be prepared yearly by the Study Statistician, describing accrual of the patients, treatment modalities and toxicity. The trial committee will meet regularly to consider patient accrual, eligibility, treatment, outcome and toxicity to ensure the good conduct of the trial.

An Independent Data Monitoring Committee (IDMC) composed of three international experts will monitor the progress of the trial on ethical and scientific grounds. The interim analysis of accrual rate, outcome and toxicity will be reported to the IDMC. The IDMC may recommend to the trial committee early stopping, continuation or extension of the trial.

20 TRIAL CONDUCT

Before entering into the trial, clinicians must ensure that they have ethical approval to participate in the trial according to their national guidelines. Written informed consent must be obtained from the patient and/or parent after they have received full explanation of the trial and the treatment options. The rights of the patient and/or parent to withdraw from the trial at any time, without giving reasons, must be respected.

The trial will be carried out in accordance with the ethical principles that have their origins in the Declaration of Helsinki (http://www.wma.net/e/policy/pfd/17c.pdf) and the principles of Good Clinical Practice, ICH Harmonised Tripartite Guideline for Good Clinical Practice (E6) (http://www.emea.eu.int/pdfs/ich/013595en.pdf), applicable EC Directives and national regulations.

Participating national groups and centres have to define a local sponsor according to national law and the principles of GCP and forward details to SIOPEL via Margaret Childs, International Trial Facilitator.

21 PUBLICATION POLICY

The SIOPEL Guidelines for Publication and Presentation will be applicable to the trial. A final report of SIOPEL 6 will be written by the trial committee and published with authors on behalf of the SIOPEL group. Additional publications on trial results will be authorised by the trial committee and published with authors on behalf of the SIOPEL group. Participating centres, with the number of patients entered into the trial, will be listed and acknowledged for their contribution.

Participating centres are authorised to publish their own cases of hepatoblastoma after prior consultation with the study chairs. Data, relating to the SIOPEL 6 trial, may, however, only be published by the trial committee.
22 TREATMENT PROTOCOL REFERENCES


# APPENDIX 1: ADDRESSES AND CONTACT DETAILS

<table>
<thead>
<tr>
<th>Position</th>
<th>Name</th>
<th>Organization</th>
<th>Address</th>
<th>Phone</th>
<th>Fax</th>
<th>Email</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
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<td>Margaret Childs</td>
<td>Contact via;</td>
<td><a href="mailto:siopel@cineca.it">siopel@cineca.it</a></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oncologists</td>
<td>Milind Ronghe</td>
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<td>+44 141 201 0857</td>
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<tr>
<td></td>
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<td>+33 3 81 21 87 92</td>
<td><a href="mailto:vlathier@chu-besancon.fr">vlathier@chu-besancon.fr</a></td>
</tr>
<tr>
<td></td>
<td>Laurence Brugères</td>
<td>Département de Pédiatrie</td>
<td>Institut Gustave Roussy 39 rue Camille Desmoulins 94 805 Villejuif Cédex France</td>
<td>+33 1 42 11 41 78</td>
<td>+33 1 42 11 52 75</td>
<td><a href="mailto:laurence.brugieres@igr.fr">laurence.brugieres@igr.fr</a></td>
</tr>
<tr>
<td></td>
<td>József Zsíros</td>
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<td>+31 20 691 7735</td>
<td><a href="mailto:j.zsiros@amc.uva.nl">j.zsiros@amc.uva.nl</a></td>
</tr>
<tr>
<td></td>
<td>Michela Casanova</td>
<td>Istituto Nazionale Tumori</td>
<td>Via Venezian 1 20133 Milano Italy</td>
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<td>+39 02 2665642</td>
<td>michela.casanova@istituotumori @mi.it</td>
</tr>
<tr>
<td></td>
<td>Bruce Morland</td>
<td>Birmingham Children’s Hospital</td>
<td>Steelhouse Lane Birmingham United Kingdom</td>
<td>+44 121 333 8233</td>
<td>+44 121 333 8241</td>
<td><a href="mailto:bruce.morland@bch.nhs.uk">bruce.morland@bch.nhs.uk</a></td>
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<tr>
<td>Surgeons</td>
<td>Jean de Ville de Goyet</td>
<td>Gordon MacKinlay</td>
<td>Jack Plaschkes</td>
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<tr>
<td>Daniel Aronson</td>
<td>C/O Dipart Med-Chir</td>
<td>Royal Hospital for Sick Children</td>
<td>Dept. of Pediatric Surgical Unit</td>
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<tr>
<td><a href="mailto:aronson.dc@hotmail.com">aronson.dc@hotmail.com</a></td>
<td>Epato-Gastro-Nutrizione Padiglione Spellman</td>
<td>Sciennes Road Edinburgh. EH9 1LF United Kingdom</td>
<td>University Children's Hospital</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>R. Dona Adma Jafet 60-6 Andar CEP 01308-050 Sao Paulo Brazil</td>
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<td><strong>Dale Kraemer</strong></td>
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APPENDIX 2: AFP TABLE

Serum AFP values of term babies without additional factors associated with AFP elevation

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<th>AGE (days)</th>
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<th>AFP 95.5% interval (ng/ml)</th>
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APPENDIX 3: PRE-TREATMENT ASSESSMENT OF TUMOUR EXTENSION
(2005 PRETEXT REVISION)

Introduction
The PRETEXT system was designed by the International Childhood Liver Tumour Strategy Group (SIOPEL) for staging and risk stratification in liver tumours (1,2). The intention was to develop a system that could be used to describe tumour extent before any therapy, and thus allow more effective comparison between studies conducted by different groups. This is particularly important for rare tumours such as hepatoblastoma. The PRETEXT system has both good interobserver reproducibility (3) and good prognostic value in children with hepatoblastoma (2-5). Most other study groups now use the PRETEXT system to describe imaging findings at diagnosis, even if this is not their main staging system.

Fig 1A. Exploded frontal view of the segmental anatomy of the liver. The umbilical portion of the left portal vein (LPV) separates the left medial section (LMS) from the left lateral section (LLS). Segment 1 is obscured in this view. Note that the term “section” has been used in preference to “segment” or “sector” (see text below).

The PRETEXT classification is based on the Couinaud system of segmentation of the liver (Fig. 1A) (6). The liver segments are grouped into four sections as follows: segments 2 and 3 (left lateral section), segments 4a and 4b (left medial section), segments 5 and 8 (right anterior section) and segments 6 and 7 (right posterior section). In the original system, the caudate lobe (segment 1) was ignored. The PRETEXT number was derived by subtracting the highest number of contiguous liver sections that are not involved by tumour from four (1). This
number is, very roughly, an estimate of the difficulty of the expected surgical procedure (see below). Pedunculated tumours are considered to be confined to the liver and to occupy only the section(s) from which they originate.

**Definitions of PRETEXT number**
PRETEXT I one section is involved and three adjoining sections are free
PRETEXT II one or two sections are involved, but two adjoining sections are free
PRETEXT III two or three sections are involved, and no two adjoining sections are free
PRETEXT IV all four sections are involved
See text for PRETEXT number of tumours involving the caudate lobe.

In addition to describing the intrahepatic extent of the primary tumour or tumours, the PRETEXT system includes certain criteria for “extrahepatic” extension. These assess involvement of the inferior vena cava (IVC) or hepatic veins (designated V), involvement of the portal veins (P), extrahepatic abdominal disease (E) and distant metastases (M).

The original SIOPEL risk stratification system for hepatoblastoma has already been modified in two ways in the protocol for SIOPEL 4 (see below). Firstly, tumour rupture or intraperitoneal haemorrhage at the time of diagnosis (H1, see below) is now a defining criterion of high risk. Secondly, children with alpha-fetoprotein levels of <100 µg/L are also considered to be high risk. These patients are not eligible for SIOPEL 6 but may be enrolled in SIOPEL 4. The 2005 revision involves no further change in the SIOPEL risk stratification system for hepatoblastoma.

**Risk stratification in hepatoblastoma for current SIOPEL studies.**
High risk = patients with any of the following:
- serum alpha-fetoprotein < 100 µg/L
- PRETEXT IV additional PRETEXT criteria
  - E1, E1a, E2, E2a
  - H1
  - M1 (any site)
  - N1, N2
  - P2, P2a
  - V3, V3a
Standard risk = all other patients

The SIOPEL 6 study will use the 2005 PRETEXT revision. This improves the original definition of the PRETEXT group, clarifies the definitions of the criteria for “extrahepatic” disease, and adds new criteria (see below). The old term “extrahepatic” disease has been replaced by “additional criteria”.

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2005 PRETEXT staging: additional criteria

C – Caudate lobe involvement
C1 Tumour involving the caudate lobe
C0 All other patients
All C1 patients are at least PRETEXT II

E – Extrahepatic abdominal disease
E0 No evidence of tumour spread in the abdomen (except M or N)
E1 Direct extension of tumour into adjacent organs or diaphragm
E2 Peritoneal nodules
Add suffix “a” if ascites is present, e.g. E0a

F – Tumour focality
F0 Patient with solitary tumour
F1 Patient with two or more discrete tumours

H – Tumour rupture or intraperitoneal haemorrhage
H1 Imaging and clinical findings of intraperitoneal haemorrhage
H0 All other patients

M – Distant metastases
M0 No metastases
M1 Any metastasis (except E and N)
Add suffix or suffixes to indicate location (see text)

N – Lymph node metastases
N0 No nodal metastases
N1 Abdominal lymph node metastases only
N2 Extra-abdominal lymph node metastases (with or without abdominal lymph node metastases)

P – Portal vein involvement
P0 No involvement of the portal vein or its left or right branches
P1 Involvement of either the left or the right branch of the portal vein
P2 Involvement of the main portal vein or both left and right branches of the portal vein
See text for definition of involvement
Add suffix “a” if intravascular tumour is present, e.g. P1a

V – Involvement of the IVC and/or hepatic veins
V0 No involvement of the hepatic veins or inferior vena cava (IVC)
V1 Involvement of one hepatic vein but not the IVC
V2 Involvement of two hepatic veins but not the IVC
V3 Involvement of all three hepatic veins and/or the IVC
See text for definition of involvement
Add suffix “a” if intravascular tumour is present, e.g. V3a

PRETEXT grouping
The traditional approach to radiological segmentation of the liver, based on the paths of the hepatic veins, is an oversimplification. This is partly due to the variability of hepatic venous anatomy (7-9). The main problem, however, is the imperfect correlation with segments defined by the branching pattern of the portal veins (7,10-12). Although the plane of the right hepatic vein reliably separates the right posterior and anterior sections (8), the left hepatic vein runs to the left of the boundary between the left lateral and medial sections, which is best defined by the plane of the fissure of the ligamentum teres and the umbilical portion of the left portal vein (Fig. 1B) (13).
**PRETEXT I**
This group includes only a small proportion of primary malignant liver tumours of childhood. From the definition of the PRETEXT number, it can be seen that only tumours localized to either the left lateral section or the right posterior section qualify as PRETEXT I.

**PRETEXT II**
Most PRETEXT II tumours are limited to either the right lobe or the left lobe of the liver. Tumours of the left medial or right anterior sections are also PRETEXT II. Multifocal tumours involving only the left lateral and right posterior sections are classified as PRETEXT II; this pattern is very rare.

Tumours limited to the caudate lobe (Fig. 2) were not classifiable under the original PRETEXT system (1). In the 2005 PRETEXT system, these tumours are classified as PRETEXT II (but see also C below). This is the only change in the PRETEXT numbering system in this revision. There is no change in numbering for tumours involving the caudate lobe and any other part of the liver, which are classified as PRETEXT II (if two or three contiguous sections are free), III (if there are no two contiguous sections free) or IV (if all four sections are involved).
PRETEXT III
The unifocal tumours in this category spare only the left lateral or right posterior section. These tumours are relatively common. In children with hepatoblastoma, great care must be taken to distinguish between invasion and compression of the apparently uninvolved section of the liver, because risk stratification (and/or the need for liver transplantation) may depend on this point.

Anterior central liver tumours involve segment 4 and either or both of segments 5 and 8. Although recent advances in surgical technique permit resection of these tumours without trisectionectomy (14), classification as PRETEXT III reflects the difficulty of these operations. Multifocal PRETEXT III tumours may also spare the right anterior or left medial sections, or two non-contiguous sections. These patterns are rare.

PRETEXT IV
PRETEXT IV tumours involve all sections of the liver. These tumours are often multifocal. Alternatively, a very large solitary tumour can involve all four sections.

Fig 2. The tumour is confined to the caudate lobe (PRETEXT II C1, see text below).

Caudate lobe tumours (C)
The caudate lobe and caudate process (segment 1 or segments 1 and 9, depending on the system of nomenclature) can be resected with either the left or right lobe of the liver (6). For this reason, segment 1 was not considered in the PRETEXT classification in the original system (2). Modern surgical techniques have made resection of segment 1 safer, but these operations remain difficult. Involvement of the caudate lobe is, therefore, a potential predictor of poor outcome. If any tumour is present in segment 1 on imaging at diagnosis, the patient
will be coded as C1, irrespective of the PRETEXT group (see above). All other patients should be coded as C0.

**Extrahepatic abdominal disease (E)**

The assessment of extrahepatic abdominal disease was one of the most confusing aspects of the original PRETEXT system, and clearly needed revision. Originally, there was a requirement for all extrahepatic abdominal spread of tumour (E+) to be proved by biopsy. Modern imaging techniques are capable, in principle, of identifying extrahepatic abdominal tumour extension in many forms. The frequency and significance of these imaging findings is different for different tumour types, and not all patterns are easily biopsied.

In hepatoblastoma, for example, direct extension of tumour into other abdominal organs is unusual. Tumour extension through the diaphragm is uncommon, but can be shown quite convincingly by magnetic resonance imaging (MR) or computed tomography (CT), and biopsy proof may be impractical. In the 2005 revision, patients with direct extension of tumour through the diaphragm or into other organs can be coded as E1 without biopsy proof.

Peritoneal tumour seeding was originally not included this category (2). It probably indicates more advanced abdominal disease than direct extension of the primary tumour. Imaging techniques, especially ultrasound, can often show even small peritoneal nodules clearly, and the differential diagnosis is very limited. In the 2005 revision, peritoneal nodules will be assumed to be metastases, and will be coded as E2. All other patients should be coded as E0.

Ascites is an unusual finding at presentation in hepatoblastoma, but is more common in hepatocellular carcinoma, where it may be an independent predictor of poor prognosis. For this reason, patients with ascites will be coded as E0a, E1a or E2a as appropriate.

Abdominal lymph node metastases, which were previously recorded as E+, are now coded as N (see below).

**Tumour focality (F)**

In SIOPEL 1, multifocal tumours were identified at the time of diagnosis in 18% of the patients with HB where this information was available (4). Univariate analysis showed that the 5-year event-free survival was significantly worse for patients with multifocal tumour (40%) than for those with unifocal tumour (72%) (4). The German Society of Pediatric Oncology and Hematology reported slightly different results (15). In their HB89 study, 21% of patients had multiple well-defined tumours, and these children had a similar disease-free survival (DFS; 87%) to those with a single tumour (86%). However in 20% of children the tumour exhibited a diffuse growth pattern, and these had a significantly worse DFS (21%) (15). Unfortunately, a diffuse growth pattern is difficult to define, and despite the promise that this finding shows as a potential risk factor, it was decided not to incorporate it in the 2005 PRETEXT revision.

Patients with one hepatic tumour should be coded as F0. All those with more than one tumour nodule, regardless of nodule size or PRETEXT stage, should be coded as F1.

**Tumour rupture or intraperitoneal haemorrhage (H)**

It is not uncommon for hepatoblastoma and hepatocellular carcinoma to present with tumour rupture (16,17). Originally, these patients were not automatically included as high risk in SIOPEL studies, because of the requirement that extrahepatic disease (E) be proved by biopsy. Although the data to prove this is not currently available, it seems intuitively likely that tumour rupture (usually manifesting as intraperitoneal haemorrhage) is a risk factor, and these patients
should be coded as H1. Laparotomy or aspiration of peritoneal blood is not necessary for diagnostic purposes if characteristic imaging and clinical findings (such as hypotension and low haematocrit or haemoglobin level) are present. The presence of peritoneal fluid on imaging alone does not imply tumour rupture (but see E above).

Since the opening of the SIOPEL 4 study in September 2004, tumour rupture has become a defining feature of high-risk hepatoblastoma in SIOPEL studies. Patients with no evidence of tumour rupture or haemorrhage, and those with only subcapsular or biopsy-related intraperitoneal bleeding, are coded as H0.

**Distant metastases (M)**

Patients with distant metastases at diagnosis are coded as M1. In hepatoblastoma, these metastases are predominantly found in the lungs. Although the best imaging modality for the identification of lung metastases is currently CT, the defining characteristics of lung metastases in this context have not been specifically studied. It is believed, however, that factors favouring a diagnosis of metastasis include multiple lesions, a rounded, well-defined contour and a subpleural location. In most parts of the world, a single rounded lung lesion with a diameter of >5 mm in a child with a primary liver tumour is very likely to be a metastasis. Patients with these findings on chest CT should be classified as M1. Biopsy is not required for staging purposes, because it is uncommon for other lesions to mimic metastases in this clinical context. The protocols of the SIOPEL studies recommend central radiological review if there is any doubt about the presence of lung metastases.

Other metastases are infrequently found at diagnosis in hepatoblastoma, but are more common in hepatocellular carcinoma. The imaging findings of brain metastases are usually characteristic, and biopsy is not required.

Bone scintigraphy is recommended for staging in children with hepatocellular carcinoma, but not hepatoblastoma. Abnormal calcium metabolism is common in children with hepatoblastoma, and may cause abnormal uptake on bone scintigraphy, especially in the ribs (18), whereas bone metastases are rare (19). Biopsy proof is therefore mandatory for suspected bone metastases in hepatoblastoma, unless the findings of cross-sectional imaging are characteristic and the patient is already in the high-risk category for some other reason, such as the presence of lung metastases.

Bone marrow biopsy is not recommended in children with hepatoblastoma, because bone marrow spread is rare (19). It is not known whether metastases at different sites have different prognostic implications. For statistical purposes, it is therefore recommended that one or more suffixes be added to M1 to indicate the major sites of metastasis: pulmonary (p), skeletal (s), central nervous system (c), bone marrow (m), and other sites (x). A child with lung, brain, and adrenal metastases would therefore be coded as M1cpx. Patients with no evidence of haematogenous metastatic spread of tumour should be coded as M0.

**Lymph node metastases (N)**

Because porta hepatis (and other abdominal) lymph node metastases are quite unusual in hepatoblastoma, SIOPEL trials have always required this form of tumour spread to be proved by biopsy. In fact, benign enlargement of lymph nodes is probably not uncommon, and the accuracy of positron emission tomography is not known in this context. Because biopsy of equivocal lymph nodes inevitably carries some risk, the SIOPEL committee actively discourages this. Biopsy may, however, be required if there is significant nodal enlargement (for example short axis >15 mm) in a child with no other criteria for high-risk hepatoblastoma.
Lymph node metastases are quite common in hepatocellular carcinoma, and biopsy proof is not required if the imaging abnormality is unequivocal. An arbitrary threshold short axis diameter of 15 mm is suggested for this purpose.

Children with no lymph node metastases by these criteria are coded as N0, those with nodal metastases limited to the abdomen (i.e. caudal to the diaphragm and cranial to the inguinal ligament) as N1, and those with extra-abdominal nodal metastases as N2.

**Portal vein involvement (P)**

Involvement of the main portal vein and/or both major branches has been considered a risk factor in hepatoblastoma, because this has obvious implications for the resectability of the tumour. It is also possible that portal vein invasion detected by imaging is an independent risk factor for tumour recurrence (20). The original PRETEXT criteria, however, did not specifically define the word “involvement”.

It is well recognized that a tumour that abuts or displaces a major portal venous branch at imaging performed at diagnosis (Fig. 3A) may shrink away from the vein following preoperative chemotherapy. Imaging evidence of complete obstruction or circumferential encasement (Fig. 3B) is therefore required to qualify as portal vein involvement. Failure to identify the portal vein or one of its major branches in either its normal position or its expected displaced location, on good quality images, is strong evidence of obstruction. The other form of involvement, portal vein invasion (Fig. 3C), is not uncommon, and is often best detected by ultrasound. Various sonographic signs may be present (21,22), and analogous findings can be seen at CT and MR.

Patients with no imaging evidence of involvement of the main portal vein, its bifurcation, or either of its main branches will be coded as P0. Those who fulfil the original PRETEXT definition of P+ (involvement of the main portal vein, its bifurcation, or either of its main branches), as well as those with “cavernous transformation” of the portal vein will be coded as P2. P2, however, represents very advanced disease. For this reason, the category P1 has been created for patients with evidence of involvement of one major branch of the portal vein. In addition, the detection of portal vein invasion should be marked by the suffix a (e.g. P2a).

Fig 3A  Fig 3B  Fig 3C

**Fig 3.** Involvement of the portal and hepatic venous systems.

3.A. When the tumour (grey) approaches or abuts the vein (black), there is no venous involvement, even if the vein is partially encased.
3.B. Complete obstruction or encasement of the vein is one form of involvement. Obstruction of the inferior vena cava by extrinsic compression, however, does not count as involvement (see text).

3.C. Intravascular tumour growth in the portal and/or hepatic venous systems is not uncommon in children with hepatoblastoma or hepatocellular carcinoma.

Involvement of the IVC and/or hepatic veins (V)
The same definitions of involvement (venous obstruction, encasement and/or invasion) used for the portal veins apply to the hepatic veins. A hepatic vein can be assumed to be involved if it cannot be identified at all, and its expected course runs through a large tumour mass. It is important to look carefully for the hepatic veins, preferably with ultrasound as well as CT and/or MR, as they may be displaced from their expected position by the tumour. Complete obstruction of the IVC can occur with mass effect alone, without any tumour extension to the vein itself. Inability to visualize the IVC, and the presence of an enlarged azygos vein, are not, therefore, sufficient criteria for involvement. Patients with no imaging evidence of involvement of the hepatic veins or IVC will be coded as V0.

As for the portal vein, the original classification of involvement (V+) indicated a very advanced level of disease. Intermediate categories have therefore been created. V1 and V2 indicate involvement of one or two main hepatic veins respectively. V3 indicates involvement of either the IVC or all three of the hepatic veins. In addition, the detection of hepatic vein or IVC invasion should be marked by the suffix a (e.g. V2a). The presence of tumour in the right atrium automatically makes a patient V3a.

SIOPEL risk stratification for patients with hepatoblastoma
The SIOPEL risk stratification for children with hepatoblastoma is essentially unchanged by the 2005 PRETEXT revision (23). Patients with any one or more of certain additional criteria are high risk, and potentially eligible for the SIOPEL 4 study. All other SIOPEL patients are standard risk, and would currently be eligible for SIOPEL 6.

Presurgical re-evaluation
Although the timing of surgery will depend on the treatment protocol and the patient’s response to therapy, preoperative imaging is almost always necessary. All of the PRETEXT categories should be reassessed after preoperative chemotherapy, and as close as possible before surgery, and recorded as POSTEXT (post-treatment extent of disease). Comparison of surgical findings with POSTEXT will allow prospective assessment of the accuracy of imaging techniques.

References


APPENDIX 4: GUIDELINES FOR RADIOLOGICAL INVESTIGATIONS

Imaging requirements
The essential investigations for a child with suspected hepatoblastoma are abdominal ultrasonography (US), contrast-enhanced computed tomography (CT) and/or magnetic resonance imaging (MR) of the abdomen and CT of the thorax. Accurate local staging requires that the radiologist identify the number of lesions and the segments of the liver that are involved, as well as any involvement of the portal or hepatic venous systems and inferior vena cava. Other forms of extrahepatic spread, such as lymph node metastases and peritoneal deposits, are unusual in hepatoblastoma, but must also be identified.

Ultrasoundography (US)
US is the best first imaging test in a child with a suspected abdominal mass. It has an important role in the staging of hepatoblastoma because it provides real-time evaluation of vascular anatomy, which may be crucial, both for determining the intrahepatic extent of disease and assessing venous involvement (Fig. 3 Appendix 3). “Second look” US after CT or MR examination may be helpful to confirm or to complete the evaluation of the hepatic and portal veins.

A sector or curved linear array transducer is ideal for confirming the hepatic origin of the tumour. In cases of doubt, real-time examination of the interfaces between the lesion and normal tissues may be helpful. The tumour may be seen to move with the liver, for example, and slide over the right kidney with respiration. These probes are also used for measuring the size of large tumours.

High-frequency (>7 MHz) linear array transducers are often useful for assessment of the portal and hepatic venous systems, and for accurate measurement of small tumours. Colour Doppler imaging and pulsed wave Doppler examination may contribute to the assessment of intravascular tumour extension (1,2). The earliest sonographic sign of tumour growth into the portal venous system is disruption of the normal hyperechoic stripe of the vein wall. The identification of echogenic material in the lumen of the vein, particularly if there is Doppler evidence of arterial blood flow within this material, can be considered diagnostic of venous invasion (Fig. 3 Appendix 3). Colour Doppler imaging may show flow in the residual lumen around the intravascular tumour. The vein may be distended by the tumour, and venous collaterals may be present (1,2).

The usefulness of Doppler techniques in the differential diagnosis of liver masses is not yet known.

Computed tomography (CT)

Abdominal CT
In general, MR is preferred over CT, but the choice will depend on the equipment available and on the expertise of the radiologist. If CT is performed, this should only be done in a paediatric imaging centre, or by radiologists who are familiar with dose reduction techniques. CT may require sedation or general anaesthesia in young children.

Oral contrast is given in some centres. There is probably no justification for more than one acquisition, but if non-contrast images are obtained they should be limited to the liver. The abdomen and pelvis should be scanned during the portal venous phase following intravenous contrast administration. An injection pump should be used for optimised bolus delivery and
timing. The collimation, pitch and tube current can be the same as for standard abdominal CT according to age. A multi-detector (16 row) scanner with collimation of 1.5 mm and dynamic tube current adjustment (20 to 30 mA) is used. Multi-detector CT minimises the need for sedation and allows the generation of multiplanar reconstructions, which are very useful for accurate staging.

**Chest CT**

Interpretation of chest CT may be made difficult by collapse (atelectasis) of the lungs, usually at the bases. This may be related to sedation or anaesthesia and/or to compression by a large liver mass. Careful anaesthetic technique may overcome this problem to some extent (3). Induction of anaesthesia with a gas mixture with a high fraction of nitrogen, “recruitment” of vital capacity by forced expansion of the lungs at high pressure for 7 to 8 seconds, continuing ventilation with a moderate inspired fraction of oxygen ($F_{O_2}$ 0.3 to 0.4) and positive end-expiratory pressure may all be helpful. If there is posterior basal atelectasis despite these measures, the examination can be repeated in the prone position.

**Magnetic resonance imaging (MR)**

### Technical aspects

In general, image acquisition is a compromise between signal-to-noise ratio and motion artefacts, especially in the upper abdomen. The field of view should be as small as possible and the base and phase encoding resolution reduced accordingly in order to maintain adequate signal-to-noise ratio. In small children (less than about two years) it is best to use a head coil. In older children a flexible phased-array body coil is appropriate (4). Scanning protocols are changing rapidly with new technology, but some rough guidelines can be given.

The use of spin echo sequences is limited by motion artefact on standard clinical scanners. If these are used, it is possible to reduce artefacts in the phase-encoding direction by fat suppression, or by presaturation bands. Inversion recovery images are useful, particularly with small lesions, because of their very high signal-to-noise ratio. When breath holding is not possible, it is advisable to apply respiratory triggering.

Balanced steady state free precession provides reasonably high signal-to-noise at short acquisition times, and may be of value. However, because of the mixed weighting, there may be poor contrast between the lesion and background liver.

Volume interpolated spoiled gradient echo images have poor intrinsic contrast and rather low signal-to-noise ratio, but give excellent visualisation after injection of a conventional extracellular contrast agent containing gadolinium. They are fast (10 to 20 s for the whole liver), providing less motion artefact, and also the possibility of dynamic scanning following contrast administration. Voxel size can be made almost isotropic, so that multiplanar reconstruction is possible.

### Other contrast agents

There is currently no clear indication for the use of reticuloendothelial or hepatocellular contrast agents in children with primary liver tumours, however they may have a role in increasing sensitivity to small satellite lesions, which needs to be explored.

**Magnetic resonance angiography (MRA)**

In cooperative older children, and in children of any age who are anaesthetized, breath-holding techniques give the best results. 3D contrast-enhanced MRA can, however, be obtained in sedated children (5). Optimization of contrast injection technique and scan delay is very
important (5). In general, there is a trade-off between using concentrated contrast, which gives optimal enhancement, and dilute contrast, which is more forgiving in terms of scan timing, particularly in young children when the contrast volume is small. Although the images obtained can be striking, and may be helpful for surgical planning, it is not clear that they add anything in terms of accurate staging.

Other imaging techniques

**Angiography**

Angiography is not performed at diagnosis in children with hepatoblastoma, although it is occasionally helpful for planning complicated surgery, or for hepatic artery chemoembolisation.

**Bone scintigraphy**

Bone metastases are rare at first presentation in hepatoblastoma, but some patients have paraneoplastic osteopenia related to abnormal bone metabolism. Because it is very sensitive to bony abnormalities, but not specific for metastatic disease, bone scintigraphy is not recommended. False positive scans may not be uncommon (6).

**Brain imaging**

Brain metastases are also rare at diagnosis (7), although the brain may be a site of relapse (8-11). Brain imaging is not recommended unless the patient has neurological symptoms or signs.

**Other nuclear medicine techniques**

Scintigraphy with $^{99m}$Tc-labelled monoclonal anti-AFP has been proposed as a method of staging children with hepatoblastoma (12). The clinical usefulness of this technique is not yet known. The value of positron emission tomography for staging is also unclear.

References:


APPENDIX 5: BROCK GRADING and ASHA CRITERIA

Please note audiometry guidelines in section 16.10 and Appendix 6.

Brock grading
Grading system for Cisplatin-induced bilateral high-frequency hearing loss (1-4)

<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>

ASHA criteria and guidelines
From the American Speech-Language-Hearing Association Guidelines for the audiologic management of individuals receiving cochleotoxic drug therapy (5) and Guidelines for the audiologic assessment of children from birth to 5 years of age (6). Complete guidelines available at http://www.asha.org/policy

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.

Audiometric Criteria for Cochleotoxicity
Specific criteria for defining drug-induced hearing decrease are controversial (7). 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 (8). Thus, shifts at adjacent test frequencies indicate more systematic change and increase the likelihood of a true decrease in sensitivity (9). 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 (10). 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.
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 pharmacy personnel.

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. Potentiating 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.

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 audiological 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 inter-test 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 (11-12). 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 (13). Cisplatin can cause observable cochleotoxicity following a single course of treatment (14). Thus, it is important to obtain baseline measures prior to the first dose of Cisplatin (15).

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.

Physiologic Assessment
**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 nHL. 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 the age group birth to 4 months of age 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.

**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 (16).

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 (17).

**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, as shown by Kummer et al (18-19) 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 hearing sensitivity and the DPOAE is a better predictor of high-frequency sensitivity (20-21).

**Acoustic Immittance 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.

**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 (22-25).

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 lost.
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 (26-27). 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 trans tympanic 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 (28). Recent advances in multiple-stimulus ABR (29-31) may shorten the test time when such developments are incorporated into clinical instrumentation.

Audiology References


APPENDIX 6: VRA and ABR Guidelines

Great Ormond Street Hospital for Sick Children (G.O.S), London, UK. Audiology Department, April 2003

Protocol for Performing Visual Reinforcement Audiometry (VRA)

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.

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 soundproof, 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 parents knee facing forward, sitting erect at an angle of 45 - 90° to the reinforcers.

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.

Tester 1 (Distracter)
The person at the front should attract and control the baby’s attention using appropriate distraction techniques. (See distraction protocol)
The distraction should not stop when the sound is presented, but should be kept low key.

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
- no 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 minimal response level
Standard of procedure for insert earphone VRA

Obtain reliable sound field at 1kHz

Insert earphones into baby’s ears

tolerates  
does not tolerate

Carry out testing using PTA protocol, (10dB down, 5dB up)

Do not force. Stop and carry out sound field testing

Test Order
1. Sound Field

1kHz  2KHz  4kHz  8kHz  0.5kHz  0.25kHz

2. Insert Ear Phone

Ear(1) 1kHz  Ear(2) 1kHz  Ear(2) 2kHz  Ear(1) 2kHz

Ear(1) 8kHz  Ear(2) 8kHz  Ear(2) 4kHz  Ear(1) 4KHz

Ear(1) 0.5kHz  Ear(2) 0.5kHz  Ear(2) 0.25kHz  Ear(1) 0.25kHz
Comparison of Threshold ABR Protocols (Tone Burst)

The following table summarises the main parameters for threshold tone burst ABR testing and compares the current G.O.S parameters with the recommended protocol as published on the Neonatal Hearing Screening Programme website [http://hearing.screening.nhs.uk/](http://hearing.screening.nhs.uk/)

<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</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(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 (Universal Neonatal Hearing Screening) in the UK [www.nhsp.info/prots.shtml](http://www.nhsp.info/prots.shtml) does not make any recommendations for the use of notched noise to improve frequency specificity.
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 Programme website [http://hearing.screening.nhs.uk/](http://hearing.screening.nhs.uk/):

<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></td>
<td>(1024 in protocol)</td>
<td>(min 1500)</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 at G.O.S. 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 (++ or +) to identify whether responses are threshold or threshold + 5 dB. (Please see attached protocol for details.) Again this method 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.
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 Programme website http://hearing.screening.nhs.uk/

<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>150</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>11.1</td>
<td>49.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19.1 (when recording wave I)</td>
</tr>
<tr>
<td>Sweep Limit</td>
<td>1024</td>
<td>2000 (min 1500)</td>
</tr>
<tr>
<td>Window (ms)</td>
<td>12</td>
<td>18 - 20</td>
</tr>
</tbody>
</table>

Recommended positioning of the bone conduction and electrodes are discussed in the protocol. The main differences between the recommended protocol and current G.O.S. protocols are minimal and are outlined below:

iv) **High frequency filter**
1500 Hz 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. (Please see attached protocol for details). 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.
Conversion factors from dB(A) to HL equivalent (corrections to be added)

<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>10 000</td>
<td>+3</td>
<td>+16</td>
<td>-13</td>
</tr>
</tbody>
</table>

From: Chapter 4 Behavioural Hearing Tests, 6 months to 3 ½ years.
Practical Aspects of Audiology (series)
APPENDIX 7: OTHER OTOTOXIC MEDICATION

The following medications are known to be ototoxic and should be avoided in children treated with Cisplatin. If the use of any of these drugs is judged necessary in an individual child on the trial and the drug cannot be substituted, for a less toxic alternative, the use has to be documented.

Aminoglycosides particularly should be avoided 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
- Desferrooxamine
- Ethacyrinic acid
- Erythromycin (give I.V)
- Furosemide
- Gentamycin
- Hexachlorobenzene
- Interferon alpha 2 therapy
- Kanamycin
- 4-Methylthiobenzoic acid interacts with platinum based medication
- Mercury if ingested
- Mitomycin (topical)
- Neomycin
- Norvancomycin
- Propythiouracil
- Quinine
- Streptomycin
- Streptidine
- Styrene
- Super oxides (Paraquat)
- Teicoplanin
- Tirapazamine
- Tylenol
- Vancomycin
- Vincristine
APPENDIX 8: 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>

Data based on three studies:
APPENDIX 9: DRUG INFORMATION

Cisplatin

Please refer to the Cisplatin CCLG Monograph provided as a stand-alone document with the CTA. The latest monograph should always be referred to for assessment of adverse events.

Sodium Thiosulphate

Sodium Thiosulphate (STS) is reactive thiol compound clinically used and licensed as an antidote to cyanide poisoning at a dose of 7g/m². It has been found to be effective, in much higher doses 16-20g/m², at reducing Carboplatin and Cisplatin induced ototoxicity. It is the Investigational Medicinal Product or IMP in this trial and will be provided by and distributed worldwide through the support of Adherex Technologies, Inc. The American Reagent product will be used and will be supplied free to trial patients.

STS interferes with platinum and thereby reduces ototoxicity, but it could potentially reduce the efficacy of treatment too. If used 6 hours after platinum treatment animal models have shown that it reduces ototoxicity, but not efficacy. However, it would be misguided to use STS, even though it can be bought off the shelf, outside a carefully controlled clinical trial. The aim of the trial therefore is to show that it can reduce ototoxicity without reducing efficacy. The trial will be carefully monitored by an Independent Data Monitoring Committee. Stopping rules are in place and will be actioned if necessary.

Alternative names
Disodium Thiosulfate Pentahydrate
Na Thiosulfate
Sodium Hyposulfite
Sodium Thiosulfate
Thiosulfuric Acid
Disodium Salt
Pentahydrate
Versiclear®

Chemical name
ADH 300001.

Mechanism of action
STS is thought to provide otoprotection by direct binding and inactivation of platinum containing DNA alkylating agents and may also act as a free radical scavenger; other mechanisms of actions have not been ruled out.

Considerations prior to administration
- Adequate anti-emetic therapy
- Urea & electrolytes, particularly sodium since the high one off STS dose means a high sodium load is being administered
- Adequate hydration, which should be guaranteed by the concomitant Cisplatin hydration in this trial
- Blood pressure within normal range for age – this will be monitored before, at the end of STS infusion, and 30 and 60 minutes after the dose of STS.
- A separate i.v. line is required as Sodium Thiosulphate must not be mixed with any other substance
Adverse effects
- Nausea and vomiting
- Transient hypernatraemia which should normalise within hours of administration

Pharmacokinetics
- Biological half-life 0.65 hours (range: dependant on dose 16.5 - 182 minutes).
- Following IV injection, STS is distributed throughout the extracellular fluid. Some STS is converted to sulphate in the liver. Up to 95% is excreted unchanged in the urine.
- Minimal passage across the blood brain barrier.
- Available evidence is inconclusive or is inadequate for determining fetal risk when used in pregnant women or women of childbearing potential. It is unknown whether STS is excreted into human breast milk.

Packaging and labelling
Adherex Technologies are responsible for ensuring the distributor label each vial with a multi-language booklet label. Each vial will be placed into a 6 vial kit box. Each box will be labelled with a multi-language kit label.

STS drug product is manufactured, labeled, and packaged under GMP conditions. STS is supplied as a 25% (250 mg/mL), preservative free, sterile solution. The drug product formulation contains sodium thiosulfate pentahydrate and sodium borate.

The vial label will indicate the drug product batch number and the initial release until date

Shipment and storage
Boxes containing 6 (50 mL) vials of STS will be sent to the clinical study site pharmacy in various quantities, determined by the Sponsor according to the site’s projected and actual enrollment.

The clinical study site pharmacy will provide sterile Water for Injection (WFI) for dilution of STS before administration.

STS must be carefully stored at the clinical study site, safely and separately from other drugs, and at a controlled room temperature between 15 to 30°C (59 to 86°F).

STS may not be used for any purpose other than this clinical study.

It must be ensured at each clinical study site that the study drug is not used 24 months after the release date.

The Investigator (or designated pharmacist) must maintain records of the delivery of the study drug to the clinical study site, the inventory at the site, the use by each subject, and destruction at the site.

Upon completion or termination of the study, the Investigator/pharmacist will destroy all unused medication at the site, after approval by the Sponsor, as per the site’s appropriate SOPs. For drug accountability records, the site will provide CINECA with a copy of the Drug Dispensing Record and Drug Inventory Record. If the remaining clinical supplies are destroyed by the site, documentation of their destruction must be provided to CINECA. These can be uploaded onto the CINECA system or faxed to CINECA. The record of destroyed clinical supplies will include information on:

- All administered units
- All unused units
- All units destroyed at the end of the study
- Date of destruction
- Name and signature of the Investigator and staff member responsible for the destruction

Preparation
STS is supplied in 50 ml vials containing a 25% (250 mg/mL or 12.5 g/vial) solution. Each ml of the 25% STS to be diluted with 1ml of sterile water for injection (1:1 dilution) to a concentration of 125mg/ml for direct administration. (This has an approximately equivalent isotonicity to a 2.3% sodium chloride solution). The volume from the appropriate number of vials for the dose is combined in a PVC IV infusion bag.

Reconstituted STS for administration consists of a clear solution. There are no preservatives in the formulation. After dilution the PVC infusion bag containing the dosing solution must be placed upside down (inverted with injection and filling ports at the top) at room temperature and used within 8 hours. Any solution remaining in the vial should be destroyed according to institutional procedures.

**Doxorubicin (CCLG Drug Monograph)**

**Alternative Names**
Adriamycin hydrochloride,
14- hydroxydaunorubicin
3- Hydroxyacetyldaunorubicin

**Mechanism of action**
Doxorubicin is an anthracycline antibiotic active in all phases of the cell cycle with maximal activity in S phase. It has several modes of action including intercalation to DNA double helix, topoisomerase II mediated DNA damage, production of oxygen-free radicals, which cause damage to DNA and cell membranes, and complex formation with iron or copper via the hydroquinone moieties. Iron Doxorubicin complexes may contribute to cardiotoxicity by toxic free radical generation.

**Considerations prior to administration**
Well-established robust venous access. A central venous catheter or indwelling vascular access port is recommended for prolonged infusions to reduce the risk of extravasation.

- Full blood count
- Liver function tests
- Cardiac function
- Creatinine, urea, electrolytes

**Adverse effects**

**Common**
- Nausea and Vomiting
- Myelosuppression
- Alopecia
- Mucositis
- Red urine
- Diarrhoea
- Severe tissue damage if extravasated

**Occasional**
- Increased bilirubin
- Cardiomyopathy

**Rare**
- Hepatocellular necrosis
- Hyperpigmentation of skin, mucous membranes, nails
- Anaphylaxis, chills, fever
- Renal damage
- Drowsiness
- Conjunctivitis
Recommended routes
Intravenous

CAUTION
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/m\(^2\), and before each course thereafter. If the left ventricular shortening fraction (SF) is < 29% to 30% (depending on precise echocardiographic methodology) temporary withdrawal of Doxorubicin therapy should be considered. If subsequent testing shows an improvement in SF consider reintroducing Doxorubicin. A fall in SF by an absolute value of > 10 percentile units, or a rate of fall of > 2 to 3 percentile units per 100mg/m\(^2\), despite an SF > 29% to 30%, may also represent significant deterioration. If the patient’s hepatic function is significantly impaired, Doxorubicin dosage reduction should be considered.

Dose/schedule
Due to the vesicant properties of Doxorubicin it is strongly recommended that Doxorubicin be given through a central venous line. For ease of administration, to reduce cardiotoxicity, and allow haematological recovery the following schedule is recommended: administration of Doxorubicin together with Dexrazoxane or as a 24-hour infusion (in glucose/dextrose 5% or sodium chloride 0.9%). Administration: as a single daily dose or divided doses fractionated over several days. Cumulative dose of 450 mg/m\(^2\) to 550 mg/m\(^2\), exceeded with extreme caution.

Interactions
Doxorubicin may interact with the following:
- Dexrazoxane (IRCF-187) - reduce cardiotoxicity
- Cardiac irradiation - increased cardiac damage
- Actinomycin, mithramycin - cardiomyopathy
- Mercaptopurine - increased hepatotoxicity
- Mitomycin - increased incidence of late congestive heart failure
- Barbiturates - increased Doxorubicin elimination
- Verapamil - increased Doxorubicin serum levels, reversal of Doxorubicin resistance, reduced absorption of verapamil
- Propranolol - increased cardiotoxicity
- Tamoxifen - reduced Doxorubicin clearance, modulation of Doxorubicin resistance
- Cyclosporin - increased Doxorubicin serum levels and myelotoxicity, modulation of Doxorubicin resistance
- Carbamazepine, phenytoin, sodium valproate - altered anticonvulsant serum levels
- Warfarin - increased warfarin effect
- Cimetidine, Ranitidine - increased Doxorubicin toxicity
- Interferon alfa - altered Doxorubicin disposition, Doxorubicin dose reduction
- Paclitaxel - increased toxicity of Doxorubicin, if administered after Paclitaxel
- Cyclophosphamide - increases AUC and reduces clearance of parent drug and active metabolite

The clinical relevance of many of these interactions is unclear.

Overdose
Doxorubicin overdosage can prove fatal. Manifestations of overdose may include acute myocardial degeneration, severe myelosuppression and delayed cardiac failure. There is no specific antidote. Symptomatic supportive measures should be implemented.

Dilution specification
Preparation
Doxorubicin supplied in:
(i) Vials containing 10 mg and 50 mg freeze dried powder. Reconstitute with water for injection or sodium chloride 0.9 % injection adding 5ml to the 10mg vial and 25 ml to the 50 mg vial to give a 2 mg/ml solution.
(ii) Vials containing 10mg and 50mg as a 2mg/ml solution in sodium chloride 0.9%.

**Dilution**
Doxorubicin is compatible with sodium chloride 0.9% and Glucose/dextrose 5%.

**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 the following: sodium chloride 0.9% at concentrations of 0.1 mg/ml to 2mg/ml: dextrose 5% at concentrations of 0.1mg/ml to 1.25 mg/ml adsorptive losses which may be pronounced at low concentrations can be prevented by storage in polypropylene or when Doxorubicin is used at concentrations of at least 0.5mg/ml. In addition, at least a 7-day expiry can be given to Doxorubicin reconstituted with water for injection to a concentration of 2mg/ml, stored in polypropylene syringes at 4°C.

**Pharmacokinetics**
The pharmacokinetics of Doxorubicin in paediatric patients have been characterised in children, but the large number of protocols and different disease types make it difficult to produce representative summaries. Volume of distribution varies from 20-28 l/kg (approx 609 l/m²). Anthracyclines are ionised and have low lipid solubility and so do not easily cross the blood-brain barrier. Doxorubicin is metabolised to Doxorubicinol, an active metabolite, which may occur at higher concentrations than parent drug in plasma. Excretion of drug and metabolites is via further metabolism and/or biliary excretion, with only 5 to 15% excreted by the kidney. Elimination is triphasic, with no effect of age on clearance when normalised for surface area. Terminal half-life is 14 to 50 hours, with clearance varying from 267 to 1443 ml/min/m². Relatively little difference in pharmacokinetics has been observed in infants, but there was a trend to lower systemic clearance than in older children (790 vs. 1500 ml/min/m², p=0.07). Dosage adjustment has been recommended in patients with impaired hepatic function, although this has not been validated in paediatric patients.

**Pharmacodynamics**
Although some data exists regarding the influence of plasma concentrations on the therapeutic and toxic effects of Doxorubicin, little of this has been obtained in paediatric patients.

**Additional Information**
A number of ways to reduce cardiotoxicity have been suggested but the use of an alternative dosage schedule of weekly rather than 3 weekly, prolonged infusion schedules, adjuvant cardioprotective agents (e.g. ICRF-187) or the administration of Doxorubicin in a liposome formulation, whilst increasingly advocated are not yet of proven utility. Due to the risk of cardiac abnormalities developing many years after Doxorubicin therapy, long term cardiac follow up is recommended.

**Dexrazoxane**

**Alternative Names**
ICRF-187
Cardioxane
Zinecard

There is insufficient evidence to date to make guidelines for the use of Dexrazoxane in the treatment of paediatric malignancies. However, the SIOPEL Group is particularly concerned about the average low age of patients with hepatoblastoma and the long-term cardiotoxic risks. the SIOPEL Group is aware of the results of the POG 9426 study showing an increased incidence of second malignancies with patients with paediatric Hodgkin disease who received Dexrazoxane. Therefore, there is the option of using a cardioprotectant within this study.43
Mechanism of action
Chelates metal ions. It is thought that the uptake and subsequent hydrolysis of dexrazoxane in the myocardium protects against anthracycline induced cardiotoxicity by the scavenging of metal ions from their potentially damaging complexes with Doxorubicin and by preventing the Fe\(^{3+}\) Doxorubicin complex from redox cycling and forming reactive radicals.

Considerations prior to administration
Liver function
Renal function

Adverse Effects
Increased incidence of chemotherapy induced leukopenia and thrombocytopenia
Pain at the site of injection
Skin reactions following direct contact with dexrazoxane
Rarely liver dysfunction
A recent study in Hodgkin patients\(^{43}\) has shown a possible link with increased risk of second malignancies, however, Hodgkin’s patients have a known higher incidence of second malignancy.

Recommended routes
Intravenous

Administration
Over 15 minutes, 30 minutes before Doxorubicin infusion. An infusion of sodium chloride 0.9% is recommended for 10-15 minutes before the Doxorubicin. Doxorubicin should be administered over 60 minutes.

Cardioxane Dose/schedule
20 x the dose of Doxorubicin but not exceeding a maximum of 1000mg/m\(^2\)/course

Interactions
May potentiate the toxicity induced by chemotherapy or radiotherapy

Dilution specification and stability
Reconstitute 500mg vial with 25ml water for injection.
This solution has a pH of approximately 1.6 therefore must be further diluted to avoid the risk of thrombophlebitis
Dilute in Hartmann’s Solution for central line administration to concentration range of between 4-8mg/ml – to be confirmed.
If administering peripherally a phosphate buffer should be used taking care to calculate the concentration and rate of administration of the potassium and phosphate in the final solution.
Administer within 4 hours of dilution.

Zinecard Dose/schedule
10 x the dose of Doxorubicin

Interactions
May potentiate the toxicity induced by chemotherapy

Dilution specification and stability
Reconstitute in buffer provided to give concentration of 10mg/ml.
Further dilute with 0.9% sodium chloride or 5% glucose to a concentration range of 1.3-5mg/ml.
Administer within 6 hours of dilution.
APPENDIX 10: CTCAE CRITERIA

Common Terminology Criteria for Adverse Events v3.0 (CTCAE)
Publish Date: August 9, 2006

Quick Reference
The NCI Common Terminology Criteria for Adverse Events v3.0 is a descriptive terminology which can be utilized for Adverse Event (AE) reporting. A grading (severity) scale is provided for each AE term.

Components and Organization
CATEGORY
A CATEGORY is a broad classification of AEs based on anatomy and/or pathophysiology. Within each CATEGORY, AEs are listed accompanied by their descriptions of severity (Grade).

Adverse Event Terms
An AE is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with the use of a medical treatment or procedure that may or may not be considered related to the medical treatment or procedure. An AE is a term that is a unique representation of a specific event used for medical documentation and scientific analyses. Each AE term is mapped to a MedDRA term and code. AEs are listed alphabetically within CATEGORIES.

Short AE Name
The "SHORT NAME" column is new and it is used to simplify documentation of AE names on Case Report Forms.

Supra-ordinate Terms
A supra-ordinate term is located within a CATEGORY and is a grouping term based on disease process, signs, symptoms, or diagnosis. A supra-ordinate term is followed by the word 'Select' and is accompanied by specific AEs that are all related to the supra-ordinate term. Supra-ordinate terms provide clustering and consistent representation of Grade for related AEs. Supra-ordinate terms are not AEs, are not mapped to a MedDRA term and code, cannot be graded and cannot be used for reporting.

REMARK
A 'REMARK' is a clarification of an AE.

ALSO CONSIDER
An 'ALSO CONSIDER' indicates additional AEs that are to be graded if they are clinically significant.

NAVIGATION NOTE
A 'NAVIGATION NOTE' indicates the location of an AE term within the CTCAE document. It lists signs/symptoms alphabetically and the CTCAE term will appear in the same CATEGORY unless the 'NAVIGATION NOTE' states differently.

Grades
Grade refers to the severity of the AE. The CTCAE v3.0 displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:

- Grade 1 Mild AE
- Grade 2 Moderate AE
- Grade 3 Severe AE
- Grade 4 Life-threatening or disabling AE
- Grade 5 Death related to AE

A Semi-colon indicates ‘or’ within the description of the grade.
An ‘Em dash’ (—) indicates a grade not available.
Not all Grades are appropriate for all AEs. Therefore, some AEs are listed with fewer than five options for Grade selection.

Grade 5
Grade 5 (Death) is not appropriate for some AEs and therefore is not an option. The DEATH CATEGORY is new. Only one Supra-ordinate term is listed in this CATEGORY: ‘Death not associated with CTCAE term – Select’ with 4 AE options: Death NOS; Disease progression NOS; Multi-organ failure; Sudden death.

Important:
- Grade 5 is the only appropriate Grade
- This AE is to be used in the situation where a death
- cannot be reported using a CTCAE v3.0 term associated with Grade 5, or
- cannot be reported within a CTCAE CATEGORY as ‘Other (Specify)’

Cancer Therapy Evaluation Program, Common Terminology Criteria for Adverse Events, Version 3.0, DCTD, NCI, NIH, DHHS
March 31, 2003 (http://ctep.cancer.gov), Publish Date: August 9, 2006
APPENDIX 11: SIOPEL TUMOUR AND TISSUE PROGRAMME

SIOPEL Tumour and tissue storage programme

The primary objective of the SIOPEL Liver Tumour Tissue Storage Program is to facilitate basic and translational research aimed at a better understanding of the biology and treatment of childhood liver tumours.

Specifically for this SIOPEL 6 study, the SIOPEL group has decided to:

- Collect and store a significant number of childhood liver tumour specimens and tumour-derived DNA, RNA and cDNA for future molecular analyses.
- To collect formalin fixed paraffin embedded tumour blocks for the preparation of a liver tumour array to validate candidate biological markers.
- Establish, characterise and store primary cell cultures of childhood liver tumours and derived immortalised cell lines for future related research.
- Provide childhood liver tumour material for approved research projects conducted by investigators collaborating with SIOPEL to achieve the biological objectives of SIOPEL 6 and related studies.
- Collect DNA for the analysis of possible genetic factors predisposing to treatment related toxicity, in particular the identification of genetic determinants of treatment related ototoxicity and nephrotoxicity.

It is anticipated that tumour and tissues collected in SIOPEL 6 by the Zurich Tumour bank (and other national paediatric tissue banks with centres participating in this study) will be used to prospectively study the molecular changes occurring in a large cohort of standard risk hepatoblastoma, and to correlate the biologic findings to the clinical features and outcome. These studies will evaluate whether these changes are of any diagnostic or prognostic relevance and/or whether they can be used for the development of novel treatment approaches. As mentioned above, DNA will also be collected for the future analysis of genetic determinants of treatment related toxicity.

Eligibility in this study:

All patients with a suspected primary malignant liver tumour are eligible, irrespective of whether he/she will definitively be registered on one of the SIOPEL studies, since the definitive diagnosis and/or risk assignment is not (yet) known, in the most cases, at the time of biopsy. Prior to obtaining tumour sample (biopsy), an informed consent with appropriate information about the storage program must be signed by the patient and/or the parents.

Obtaining tumour tissue, sample processing:

Local surgeons, pathologists and oncologists are kindly requested to obtain tumour material from all eligible patients both at diagnostic biopsy and definitive tumour resection.

Diagnostic biopsies can be obtained by closed Tru-cut biopsy or laparoscopic wedge resection. Both approaches allow the surgeon or interventional radiologist to collect sufficient material for an accurate diagnostic classification (by the local pathologist), and for tumour material to be retained for the SIOPEL Liver Tumour Storage Program. If closed Tru-cut biopsies are taken we recommend an additional pass be made for specifically for tissue banking.
We encourage the surgical or radiological clinical team to co-ordinate biopsy procedures with their institutional pathologist to ensure the sample is processed immediately to ensure its viability and preserve nucleic acid, particularly RNA.

At diagnosis we recommend the extra single Tru-cut pass taken for tissue banking is placed in RNALater or if not immediately available it should go into tissue transport media or sterile saline and transported immediately to Pathology services.

Similarly at the time of definitive tumour resection the surgical team should co-ordinate with their Pathologist to ensure the entire resected tumour is transported directly for processing.

In addition to preparing the tumour for routine diagnostic paraffin sections, a generous sample of the resected tumour should be retained for tissue banking as detailed below. A section of fresh tumour should be cut and placed into RNALater, Hams F10 and formalin. In addition a representation section of the formalin fixed paraffin embedded tumour and unstained slides should be sent to the SIOPEL tumour bank. A peripheral blood sample will also be collected onto Whatman FTA blood spot cards (see figure 1).

**Figure 1 Sample collection and processing for SIOPEL tissue bank.**

**Recommended tumour processing and sample collection for the SIOPEL tumour and tissue bank**

- One tumour fragment (approx. 5x10x10 mm) or a biopsy cylinder have to be collected and stored aseptically in RNA Later for future RNA- and DNA- analyses.
- Another tumour fragment (approx. 5x10x10 mm) or a biopsy cylinder have to be collected and stored aseptically in F-10 medium for cytogenetic/FISH analysis and cell cultures.
- Another tumour fragment (approx. 5x10x10 mm) or a biopsy cylinder, to be collected and stored aseptically in formalin for histological examination/quality assurance.
If normal tissue is available, this can be stored in the same way as the tumour. If normal tissue is not available, 5-10 ml Citrate blood should be taken during the surgery.

- Representative block of formalin fixed paraffin embedded tumour
- Whole blood from finger prick or a few drops of a venipuncture blood sample taken at the time of other routine blood testing.

1. **RNALater (Ambion, Cat. No. 7020 & 7021)** is a colourless non-toxic tissue- and RNA stabilisation solution. RNALater permeates tissues and protects cellular RNA in situ. RNALater-treated tissue can be stored for at least 7 days at 20° C without significant RNA-degradation.

2. **Ham's F-10 Medium (Life Technologies, Cat. No. 41550-013)** is a pink culture medium for the propagation of cell lines that - in contrast to other culture media - contains also Fe, Cu, Zn and fatty acids. Tumour cells stay vital in the F-10 transport medium at 20° C for up to 48h and they can be cultured.

3. **Formalin Fixed Paraffin Embedded** block with representative embedded tumour material

4. **Blood sample spotted onto Whatman FTA spot cards** – available from the SIOPEL tissue bank Zurich, or see the addresses below (Catalogue number WB120208 FTA Gene Card).

Alternatively a blood sample can be spotted on to Guthrie paper and forwarded to the SIOPEL Tissue Bank.

### TRANSPORT ARRANGEMENTS

The transport tubes with the tumour samples have to be labelled (3 letters of surname, 2 letters of first name) date of birth of the patient (DDMMYYYY). They should be sent by international priority mail (e.g. FedEx, DHL) together with the tissue banking form (recording that written informed consent has been obtained) to the Oncology Laboratory of the University Children's Hospital of Zurich. Please notify the tumour bank that a sample is being sent to ensure it can be processed appropriately in Zurich. Contact the laboratory by phone, fax or email.
Contact persons are:

<table>
<thead>
<tr>
<th>Name</th>
<th>Email</th>
<th>Tel</th>
</tr>
</thead>
<tbody>
<tr>
<td>David Betts</td>
<td><a href="mailto:David.Betts@ksipi.unizh.ch">David.Betts@ksipi.unizh.ch</a></td>
<td>+41 44 266 71 11</td>
</tr>
<tr>
<td>Dr. Michael Grotzer</td>
<td><a href="mailto:Michael.Grotzer@kispi.unizh.ch">Michael.Grotzer@kispi.unizh.ch</a></td>
<td>+41 44 266 71 11</td>
</tr>
<tr>
<td>Dr. Felix Niggli</td>
<td><a href="mailto:Felix.Niggli@kispi.unizh.ch">Felix.Niggli@kispi.unizh.ch</a></td>
<td>+41 44 266 71 11</td>
</tr>
</tbody>
</table>

All samples banked in the Zurich Oncology laboratory will be anonymised with a unique identifying number. As soon as the SIOPEL Tumour Tissue Bank confirms the arrival of correctly processed tumour material along with the adequately filled in form, the sender will be credited 50 Euro as compensation.

Kits with the transport tubes will be distributed free every 6 months to the participating centres. They can be stored at 4°C. Participating centres are kindly requested to identify a local contact person (surgeon, oncologist, pathologist or research nurse etc) who will be responsible for communication with the tissue bank.

Tumour samples should be sent along with the adequately filled in transportation form:
**SIOP EL TISSUE STORAGE PROGRAM: SPECIMEN INFORMATION**

Instructions: Please fax this form at the date of shipment to:

- Prof. Michael Grotzer, fax: +41 44 266 71 71

Then send the material together with this form by post, or for fresh tissue by express mail (FedEx, DHL, etc.) to:

Dr. Tarek Shalaby,  
Oncology Laboratory,  
University Children's Hospital of Zurich,  
Steinwiesstrasse 75,  
CH 8032 Zurich,  
Switzerland,  
Tel: +41 44 266 71 11

<table>
<thead>
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</thead>
<tbody>
<tr>
<td><strong>Sender:</strong></td>
<td></td>
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<td><strong>Physician:</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Institution:</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Patient identification</strong></td>
<td></td>
</tr>
<tr>
<td>SIOPEL Trial:</td>
<td>SIOPEL 6 ☐ SIOPEL 4 ☐ Trial Number: ☐☐☐☐☐</td>
</tr>
<tr>
<td>First 3 Letters of Patient's Surname:</td>
<td>☐☐☐</td>
</tr>
<tr>
<td>First 2 Letters of Patient's Forename:</td>
<td>☐☐</td>
</tr>
<tr>
<td>Date of birth:</td>
<td>☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐</td>
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<tr>
<td>Gender:</td>
<td>Male ☐ Female ☐</td>
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<tr>
<td><strong>Clinical diagnosis:</strong></td>
<td></td>
</tr>
<tr>
<td>Diagnostic biopsy</td>
<td>☐ Definitive surgery ☐</td>
</tr>
<tr>
<td>Recurrence Diagnostic biopsy</td>
<td>☐ Definitive surgery ☐ Recurrence ☐</td>
</tr>
<tr>
<td><strong>Remarks</strong> (e.g. 2. recurrence, secondary tumor, hepatitis B infection...):</td>
<td></td>
</tr>
<tr>
<td><strong>Tumor material</strong></td>
<td></td>
</tr>
<tr>
<td>Tumor in F-10</td>
<td>☐ Tumor in RNAlater ☐ Tumor in formalin ☐</td>
</tr>
<tr>
<td>Paraffin Block</td>
<td>☐ Blood spots on Whatman card ☐ Tumor in F-10 ☐</td>
</tr>
<tr>
<td><strong>Date and time of tumor biopsy/tumor removal:</strong></td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐</td>
</tr>
<tr>
<td>Time</td>
<td>☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐</td>
</tr>
<tr>
<td><strong>Tumor localisation:</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Date of shipment:</strong></td>
<td>☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐</td>
</tr>
<tr>
<td>☐ ☐ Informed consent obtained to store and scientifically investigate tumor samples, and to work with and share anonymised personal data.</td>
<td></td>
</tr>
<tr>
<td>☐ ☐ Please send further transport kits with tubes containing RNAlater and F-10.</td>
<td></td>
</tr>
</tbody>
</table>
Procedure for providing material for approved research projects

To ensure the principal biological objectives of this study are achieved priority for access to banked tumour material will be given to approved investigators, for approved projects in collaboration with the SIOPEL Liver tumour Strategy group. However consideration will be given to other investigators who wish to access these samples for projects, which are consistent with the goals and objectives of the SIOPEL group and are in keeping with the provisions of ethical review and informed consent.

The process of review and approval of research projects will be overseen by the SIOPEL Biological Research committee under the Auspices of the SIOPEL Strategy Group. The review panel will consist of the Chair of the Biological Research Committee, the Director of the SIOPEL tumour bank and representatives of the SIOPEL Biology Committee. Where a potential conflict of interest is present normal procedure will be followed and final decisions regarding competing research interests will be referred to the SIOPEL Council.

Applications must be submitted to the SIOPEL Biological Studies Committee through the Director of the SIOPEL tissue bank and include sufficient information on the objectives of the planned study, the quantity and kind of requested material and the expected biological and clinical relevance of the planned research project.

Priority will be given to projects that meet the principal objectives of SIOPEL 6 or the broader objectives of the SIOPEL Liver Tumour Strategy Group. Linkage to clinical data will only be possible where the project is in keeping with the ethical approval and informed consent and is adequately powered to satisfy requirements of the SIOPEL group Statistician.

All publications and arising from use of samples released from the SIOPEL tissue bank programme must meet the publication procedures of SIOPEL and be in keeping with the ReMark criteria for reporting biomarkers studies.

Organisation

The SIOPEL Liver Tumour Tissue Storage Program is a joint project of the International Childhood Liver Tumours Strategy Group (SIOPEL) and the Swiss Paediatric Oncology Group (SPOG).

SIOPEL Liver tumour storage programme director:
Dr. Michael A. Grotzer, University Children's Hospital, Steinwiesstrasse 75, CH 8032 Zürich, Switzerland

Location of the Tissue Bank: Oncology Laboratory, University Children's Hospital, Steinwiesstrasse 75, CH 8032 Zürich, Switzerland

SIOPEL Biological Committee Chair and Co-ordinator of SIOPEL 6 Biological Studies:
Dr. Michael J. Sullivan, Director of Research, Children’s Cancer Research Group, Christchurch School of Medicine, University of Otago, 2 Riccarton Ave, Christchurch, New Zealand, Tel: +64 3 3640744, E-mail: michael.sullivan@otago.ac.nz
APPENDIX 12: BIOLOGICAL STUDIES

The proposed biological studies for SIOPEL 6 and other related SIOPEL studies have been planned to meet the broad scientific objectives of SIOPEL. The research will be conducted in laboratory groups of SIOPEL members or other affiliated research groups in collaboration with SIOPEL, under the specific provisions of the informed consent obtained from the participating centres and approved by local, regional or national ethical review committees. Broad informed consent will be sought for these specific projects, and for future unspecified but related research to be conducted under the auspices of the SIOPEL Liver Tumour Strategy Group.

<table>
<thead>
<tr>
<th>Chair SIOPEL Biology Committee</th>
</tr>
</thead>
<tbody>
<tr>
<td>Michael J Sullivan</td>
</tr>
<tr>
<td>Director of Research</td>
</tr>
<tr>
<td>Children’s Cancer Research group</td>
</tr>
<tr>
<td>Christchurch School of Medicine</td>
</tr>
<tr>
<td>University of Otago</td>
</tr>
<tr>
<td>2 Riccarton Ave</td>
</tr>
<tr>
<td>Christchurch, New Zealand</td>
</tr>
<tr>
<td>E-mail: <a href="mailto:michael.sullivan@otago.ac.nz">michael.sullivan@otago.ac.nz</a></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Director SIOPEL tumour and tissue bank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Michael Grotzer</td>
</tr>
<tr>
<td>University Children’s Hospital</td>
</tr>
<tr>
<td>Steinwiesstrasse 75</td>
</tr>
<tr>
<td>CH-8032 Zurich</td>
</tr>
<tr>
<td>Switzerland</td>
</tr>
<tr>
<td>Tel: +41 44 266 71 11</td>
</tr>
<tr>
<td>Fax: +41 44 266 71 71</td>
</tr>
<tr>
<td>Email: <a href="mailto:Michael.Grotzer@kispi.uzh.ch">Michael.Grotzer@kispi.uzh.ch</a></td>
</tr>
</tbody>
</table>

SIOPEL 6 Biological Study Aims and Objectives:

Aim 1: To prospectively evaluate and validate various biological and genetic markers (genes, gene expression or proteins) of standard risk hepatoblastoma for future risk adapted treatment and management

Aim 2: To prospectively study potential genetic determinants of treatment related ototoxicity and nephrotoxicity

Aim 3: To collect and store tumour material and tissue for future related biological studies

Objectives

The biological studies in SIOPEL 6 are planned to permit two broad biological questions to be addressed: the first will be the identification and validation of candidate biological markers for future risk adapted management of hepatoblastoma; and second will be the collection of genomic DNA specifically for the analysis of possible genetic determinants that may contribute to the development of treatment related ototoxicity and renal toxicity.

Background

Since 1990, SIOPEL has conducted three international collaborative clinical trials, SIOPEL 1, 2 and 3, for patients with standard and high-risk hepatoblastoma. While these SIOPEL clinical trials have been remarkably successful, their focus has primarily been clinical, and there have been no associated biological studies to identify prognostic biological markers.
While some independent small studies have looked at prognostic biological markers, these studies have been seriously limited by the lack of available tumour tissue, and have not been part of a large statistically robust clinical trial. SIOPEL has sought the inclusion of prospective biological studies in all new clinical trials.

**Biological Study strategy**

**Study 1: SIOPEL 6 Hepatoblastoma Biomarker study:** prospective evaluation and validation of potential biological and genetic markers for future risk adapted treatment and management

**Aim 1:** This study will validate any biomarkers identified by studies currently underway in the research groups of Drs Sullivan and Buendia. These two research groups are evaluating tumours obtained from patients enrolled in the SIOPEL 2 and 3 studies, using microarray gene expression analysis and tissue microarrays. Any candidate markers from these or other independent studies will be incorporated into this particular study.

**Methods:** Samples of fresh tumour tissue will permit the preparation of high-grade protein, DNA and RNA for genome wide protein, RNA or DNA analysis, using a variety of high throughput analysis platforms. From the formalin fixed paraffin embedded blocks, representative samples of tumour and adjacent normal tissue (if any) will be excised and RNA and DNA recovered using recently developed methods to extract nucleic acids from paraffin embedded tissue. Representative cores of paraffin embedded tumour will also be extracted and used to prepare large scale hepatoblastoma tissue arrays for the validation of candidate genetic or protein markers using in situ hybridisation or immunohistochemistry.

The following methods will be used:
- a. Microarray gene expression analysis for expression profiling
- b. Real time Q-PCR analysis of selected candidate genes within a profile
- c. Hepatoblastoma tissue array for validation by in situ hybridisation or immunohistochemistry
- d. Possible proteomic analysis
- e. Analysis of candidate genes or gene loci for acquired mutations, genome rearrangements or epigenetic modification

**Analysis:** Candidate genes, proteins, loci or other markers identified in this study will be statistically evaluated against other known clinical features such as histological subtype, location, stage, or response to assess clinically their utility.

**Study 2: SIOPEL 6 Determinants of toxicity study:** prospective study of potential genetic determinants of treatment related ototoxicity and nephrotoxicity.

The proposed study will in the first instance be analysed for candidate deafness related genes, and if no association found, proceed to a genome-wide study to identify deafness related gene loci. It is most likely that genetic determinants will be identified as polymorphic genetic variants within the apoptotic cell death pathways.

**Aim 2:** This study will seek potential genetic determinants of treatment related ototoxicity and nephrotoxicity of patients enrolled in the SIOPEL 6 clinical trial. It is anticipated this research will be conducted by laboratories with experience in genome analysis of deafness related genes, in collaboration with SIOPEL.
Methods: DNA from patients enrolled on SIOPEL 6 will be collected on simple Whatman FTA blood spot cards or Guthrie cards. Blood spotted onto FTA cards is air-dried, labelled and can be sent through the normal post. Whatman FTA card will be available from the Zurich Tissue Bank, or

Whatman International
Springfield Mill
James Whatman Way
Sandiling Road
Maidstone
Kent, ME14 2LE, UK
Tel: +44 162 267 6670

DNA will be extracted from the Whatman FTA blood spot card or Guthrie card for analysis using:

a. DNA mutation analysis of genes known to be associated with predisposition to inherited deafness and renal impairment
b. DNA analysis of possible polymorphic variants of genes in the apoptotic pathways that may be directly involved Cisplatin related apoptosis
c. Genome-wide haplotype analysis specifically to identify genetic loci associated with a predisposition to acquired deafness or renal impairment

Analysis: The future analysis of these samples will be confined to the genetic analysis of determinants predisposing to treatment related toxicity. Candidate genes, proteins, loci or polymorphisms identified in this study will be statistically evaluated against the degree, type and onset of Cisplatin related ototoxicity and renal toxicity.

Study 3: SIOPEL Liver tumour and tissue banking programme: collection and storage of tumour material and tissue for future related biological studies

Aim 3: Under the auspices of SIOPEL, the Zurich Childhood Liver tumour bank will collect and store tumour material and other tissues for future unspecified but related research.

Methods: Samples collected on SIOPEL 6 and other SIOPEL studies will as of priority be used to meet the goals and objectives of the associated biological studies. However samples and resources such as the tissue array will be available by other research groups on application to SIOPEL for collaborative biological research relating to the aetiology, diagnosis and treatment of this group of tumours. Broad consent will be obtained for future unspecified but related research.
APPENDIX 13: PHARMACOLOGICAL STUDY

Study to investigate the effect of Sodium Thiosulphate on the formation of Cisplatin-DNA adducts in patients with standard risk hepatoblastoma being treated on the SIOPEL 6 study protocol.

Dr. Gareth J. Veal
Northern Institute for Cancer Research
Paul O’Gorman Building, Medical School
Framlington Place
Newcastle University
Newcastle upon Tyne
NE2 4HH, UK
Fax: +44 191 222 3452
Email: G.J.Veal@newcastle.ac.uk

Study outline

In the SIOPEL 6 study Cisplatin is administered as a 6hr infusion at a dose of 80 mg/m² and patients are then randomised to receive STS (20 g/m²) or no STS treatment. This study design allows us to investigate the effect of STS on platinum-DNA adduct formation in leucocytes of patients at a defined time following Cisplatin administration. Studies have previously shown that platinum-DNA adduct levels in leucocytes can be measured 24 hours after Cisplatin administration in children receiving comparable doses of Cisplatin (50 – 100 mg/m²). If STS interacts with the formation or repair of platinum-DNA adducts then a difference may be observed between those patients randomised to receive STS following Cisplatin treatment and those who receive Cisplatin with no STS. The measurement of platinum-DNA adduct levels in the SIOPEL 6 study will build on previously obtained data relating to the potential clinical relevance of adduct levels in children treated with Cisplatin.

Sampling details

Whole blood samples (5-10ml) should be taken prior to Cisplatin treatment and 24 hours following the start of the first Cisplatin administration. Samples should be collected in clearly-labelled plastic tubes containing potassium EDTA (20mg/tube) and frozen at -20°C or -80°C. If there is a delay between the taking and freezing of these samples they should be stored at 0-4°C prior to freezing. These samples should be sent frozen on dry ice to the Northern Institute for Cancer Research (NICR), Newcastle University, UK, following completion of the study. Centres should contact Dr Gareth Veal (Tel: +44 (0)191 246 4332 or +44 (0)191 246 4412; email: G.J.Veal@ncl.ac.uk) to arrange for the transport of samples and associated costs.
Figure 1. Sampling scheme for measurement of Platinum-DNA adduct formation in leucocytes of patients treated with Cisplatin +/- Sodium Thiosulphate (STS)

References


Cisplatin-DNA Adduct Study Patient Sampling Sheet – SIOPEL 6

**PATIENT INFORMATION**

<table>
<thead>
<tr>
<th>STUDY CENTRE</th>
<th>DATE OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>/ /</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DATE OF BIRTH</th>
<th>INITIALS *</th>
</tr>
</thead>
<tbody>
<tr>
<td>/ /</td>
<td></td>
</tr>
</tbody>
</table>

(* first 3 initials of surname, first 2 initials of first name)

<table>
<thead>
<tr>
<th>STUDY NUMBER</th>
<th>WEIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg</td>
</tr>
<tr>
<td></td>
<td>m²</td>
</tr>
</tbody>
</table>

**TREATMENT DETAILS**

Cisplatin Dose: 

[ ] mg

Cisplatin Infusion: 

Start: ___:___ Finish: ___:___

STS Dose: 

[ ] g

STS Infusion: 

Start: ___:___ Finish: ___:___

Whole blood samples to be taken for analysis prior to and following intravenous administration of Cisplatin +/- STS. Label all tubes with the patient initials/study number, date of study and time of sample or sample number.

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Time from start Cisplatin infusion</th>
<th>Date / time due</th>
<th>Time taken</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pretreatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>24h</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(5-)10 ml of blood to be taken in a 10ml plastic tube containing potassium EDTA (20mg/tube) and stored at -20°C or -80°C prior to transportation to Newcastle for analysis. Contact Gareth Veal or Alan Boddy (details below) to organise for the transport of samples, the cost of which will be covered by the NICR, Newcastle University.

**SAMPLE TRANSPORT**

Samples to be sent by overnight courier packed on dry ice in an insulated container.

Address for delivery:

Gareth Veal / Alan Boddy
Northern Institute for Cancer Research
Newcastle University
Medical School
Newcastle upon Tyne
NE2 4HH
United Kingdom

Contact numbers:

Gareth Veal: +44 191 246 4332
Alan Boddy: +44 191 246 4412
Fax: +44 191 222 3452
Email: G.J.Veal@newcastle.ac.uk, Alan.Boddy@newcastle.ac.uk
# APPENDIX 14: BP PERCENTILE CHART

## Blood Pressure Levels for Boys by Age and Height Percentile

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<thead>
<tr>
<th>Age (Year)</th>
<th>BP Percentile</th>
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<td>Systolic BP (mmHg)</td>
</tr>
<tr>
<td></td>
<td>8th</td>
</tr>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
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<tr>
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<td></td>
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</tr>
<tr>
<td>8</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

SIOPEL 6 International version 4.0_01May_2011

129/138
### Blood Pressure levels for Boys by Age and Height Percentile (Continued)

<table>
<thead>
<tr>
<th>Age (Year)</th>
<th>BP Percentile</th>
<th>Systolic BP (mmHg)</th>
<th>Diastolic BP (mmHg)</th>
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</table>

BP, blood pressure

* The 90th percentile is 1.28 SD, 95th percentile is 1.645 SD, and the 99th percentile is 2.326 SD over the mean.
<table>
<thead>
<tr>
<th>Age (Year)</th>
<th>BP Percentile</th>
<th>Systolic BP (mmHg)</th>
<th>Diastolic BP (mmHg)</th>
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### Blood Pressure Levels for Girls by Age and Height Percentile (Continued)

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<th>Diastolic BP (mmHg)</th>
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</table>

BP, blood pressure
* The 90th percentile is 1.28 SO, 95th percentile is 1.645 SO, and the 99th percentile is 2.326 SO over the mean.
Reference Ranges Males and Females age < 1 Year

Age specific percentiles of BP measurements in girls- birth to 12 months of age; Korotkoff phase IV used for diastolic BP.

Age specific percentiles of BP measurements in boys- birth to 12 months of age; Korotkoff phase IV used for diastolic BP.
Reference Ranges Neonates

Systolic blood pressure at 4-24 hours
(mm Hg)

Systolic blood pressure at 10 days
(mm Hg)

Gestation at birth (weeks)

Gestation at birth (weeks)
APPENDIX 15: BEDSIDE NURSING WORKSHEET

Patient Name: (attach patient label)  Patient Hospital No: (attach patient label)

Trial ID number: ________________

Day 1 – Date: ________________

Pre-chemotherapy assessments:

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<tr>
<th>Height</th>
<th>cm</th>
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<tbody>
<tr>
<td>Weight</td>
<td>kg</td>
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<tr>
<td>Temperature</td>
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<tr>
<td>Blood pressure</td>
<td>mmHg</td>
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<tr>
<td>MUAC</td>
<td>cm (to be measured by the Clinician prior to course 1)</td>
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Summary of Treatment

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<th>Time Point</th>
<th>Action</th>
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<tbody>
<tr>
<td>-3 hours</td>
<td>Administer Ondansetron and Dexamethasone. Start pre hydration 3 hours prior to Cisplatin infusion as per chemotherapy chart.</td>
</tr>
<tr>
<td>0 hours</td>
<td>Administer Metoclopramide before Cisplatin infusion. Administer Cisplatin over 6 hours with concurrent hydration. Nurses checking chemotherapy should check total volume in burette to calculate hourly rate accurately. For example divide total volume in burette by 6 to work out the hourly rate.</td>
</tr>
<tr>
<td>+4</td>
<td>Administer Ondansetron and Dexamethasone.</td>
</tr>
<tr>
<td>+1-6 hour</td>
<td>Check volume in burette hourly to ensure total dose given in exactly six hours, speeding up infusion as necessary</td>
</tr>
<tr>
<td>+6 hours</td>
<td>Administer Metoclopramide. Post hydration as per chemotherapy prescription</td>
</tr>
<tr>
<td>+11 hours 30 mins</td>
<td>Administer anti-emetics prior to STS – Ondansetron, Metoclopramide, Dexamethasone, and Chlorpheniramine <strong>30 minutes prior to STS infusion</strong></td>
</tr>
<tr>
<td>+12 hours</td>
<td>Administer Sodium Thiosulphate (STS) over 15 minutes. <strong>DO NOT START UNTIL 6 HOURS AFTER THE END OF CISPLATIN INFUSION.</strong></td>
</tr>
<tr>
<td>+12 hours 15 mins</td>
<td>STS Batch Number __________________________</td>
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<tr>
<td>Infusion start time and end time for Cisplatin and STS needs to be recorded on chemotherapy prescription chart</td>
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</table>

- **Strict monitoring of fluid balance** - Monitor urine output closely. If urine output falls below 3mls/kg/hr for 2 hours give Mannitol 20% 2.5mls/kg over 15-30 minutes.
- Give multi agent anti-emetic cover 6-8 hourly for the first 24 hours. Children may feel very thirsty during the infusion of STS. There is no reason why they should not drink water, however this is likely to cause more vomiting. This is not a problem as their hydration is assured intravenously.
Blood Pressure and Electrolyte Monitoring for Sodium Thiosulphate (STS) infusion

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Blood Pressure (mmHg)</th>
<th>Electrolyte Monitoring (U&amp;E)</th>
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<td>Pre STS infusion</td>
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<tr>
<td>End of STS infusion</td>
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<td>30 minutes post-end STS infusion</td>
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<td>6 hours post-end STS infusion</td>
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<tr>
<td>18 hours post-end STS infusion</td>
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</table>

Refer to protocol if blood pressure not within normal range 60 minutes post dose

If patient develops a raised sodium > 150mmol/L at one hour give Mannitol 20% 2.5mls/kg over 15-30 minutes and 10mls/kg fluid bolus of 5% Glucose in addition to Cisplatin hydration – refer to protocol for further management.

Please note any adverse events (e.g. vomiting) that occur during the infusions:

<table>
<thead>
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<th>Event</th>
<th>Comments e.g. frequency</th>
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</thead>
<tbody>
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</tbody>
</table>
## APPENDIX 16: SUMMARY OF SIOPEL 6 SAMPLE REQUIREMENTS

<table>
<thead>
<tr>
<th>Protocol Reference</th>
<th>Samples required</th>
<th>Address to send to</th>
<th>Instructions</th>
<th>When to send</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Central Histological Review</strong></td>
<td>Section 14.1.4 Minimum of 2 unstained slides of both the diagnostic biopsy and sections of the resected tumour on slides allowing immunohistochemistry (e.g. Superfrost Plus)</td>
<td>Dr Monique Fabre/ Dr Catherine Guettier Département de Bio-Pathologie Médicale Institut de Cancérologie Gustave Roussy 114, rue Edouard Vaillant 94805 Villejuif Cedex France Tel: +33 145 212 024 or +33 145 212 024 2465 Fax: +33 145 2132 81 e-mail: <a href="mailto:monique.fabre@bct.aphp.fr">monique.fabre@bct.aphp.fr</a> <a href="mailto:catherine.guettier@bct.aphp.fr">catherine.guettier@bct.aphp.fr</a></td>
<td>Send with a copy of the local pathological report after definitive surgery for each patient.</td>
<td>After definitive surgery for each patient</td>
</tr>
<tr>
<td><strong>Biological Studies Non-UK sites</strong></td>
<td>Appendix 11 and 12 Blocks of formalin fixed paraffin embedded tumour Peripheral blood sample on Whatman FTA blood card or Guthrie card Fresh tumour collected at the time of diagnosis and at surgery and placed into RNAlater, Hams F10 and formalin</td>
<td>Dr Tarek Shalaby Oncology Laboratory University Children’s Hospital of Zurich Steinwiesstrasse 75 CH 8032 Zurich Switzerland Tel: +41 44 266 71 11</td>
<td>Label transport tubes with patient initials and date of birth Complete Specimen Information Form (Appendix 11) Fax a copy of the form to: Michael Grotzer +41 44 266 7171 Send the form with the samples by post or if fresh tissue by international priority mail e.g. Fedex, DHL.</td>
<td>After definitive surgery for each patient</td>
</tr>
<tr>
<td><strong>Pharmacological Study</strong></td>
<td>Appendix 13 Collect whole blood samples (5-10ml) ideally during the first cycle: Prior to Cisplatin administration 24 hours after start of Cisplatin administration</td>
<td>Gareth Veal/Alan Boddy Northern Institute for Cancer Research Newcastle University Newcastle upon Tyne NE2 4HH United Kingdom Tel: +44 191 246 4332 or 4412 Email: <a href="mailto:G.J.Vea@newcastle.ac.uk">G.J.Vea@newcastle.ac.uk</a> <a href="mailto:Alan.Boddy@newcastle.ac.uk">Alan.Boddy@newcastle.ac.uk</a></td>
<td>Freeze at -20°C or -80°C If there is a delay between the taking and freezing of these samples they should be stored at 0-4°C prior to freezing Contact Gareth to organise transport of the samples, the cost of which will be covered. Send frozen on dry ice by overnight courier with Patient Sampling Sheet (Appendix 13)</td>
<td>Following completion of the study</td>
</tr>
</tbody>
</table>
### SUMMARY OF SIOPEL 6 OTHER REQUIREMENTS FOR CENTRAL REVIEW

<table>
<thead>
<tr>
<th>Protocol Reference</th>
<th>Requirements</th>
<th>Address to send to</th>
<th>Instructions</th>
<th>When to send</th>
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<tr>
<td><strong>Central Radiological Review</strong></td>
<td>Section 15.2.2</td>
<td>Diagnostic, pre-surgery and relapse imaging. This includes CT, MRI and ultrasound images.</td>
<td>Dr Derek Roebuck&lt;br&gt;Department of Radiology&lt;br&gt;Great Ormond Street Hospital&lt;br&gt;Great Ormond Street&lt;br&gt;London, WC1N 3JH&lt;br&gt;United Kingdom&lt;br&gt;Tel: +44 20 7829 7943&lt;br&gt;Fax: +44 20 7242 1607&lt;br&gt;Email: <a href="mailto:RoebuD@gosh.nhs.uk">RoebuD@gosh.nhs.uk</a></td>
<td>Send images, via post, on CD in DICOM format (hard copy films or CDs with JPEG files are acceptable but not preferred).</td>
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<tr>
<td><strong>Central Audiology Review</strong></td>
<td>Section 15.9</td>
<td>• Baseline audiometry is strongly recommended.&lt;br&gt;• Audiometry is recommended after every second course of Cisplatin.&lt;br&gt;• A reliable hearing test must be performed 6-12 weeks from end of treatment but not before the child is 3.5 years old.</td>
<td>Not applicable</td>
<td>Results can be: Uploaded to the SIOPEL 6 RDE system Or sent directly to: <strong>Kaukab Rajput</strong>&lt;br&gt;Consultant Audio-vestibular Physician&lt;br&gt;Dept of Audiological Med. and Cochlear Implant&lt;br&gt;Great Ormond Street Hospital for Sick Children&lt;br&gt;London, WC1N 3JH&lt;br&gt;United Kingdom&lt;br&gt;Tel: +44 207 813 8316&lt;br&gt;Fax: +44 207 829 7877&lt;br&gt;Email: <a href="mailto:RajpuK@gosh.nhs.uk">RajpuK@gosh.nhs.uk</a></td>
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