ESIOP Neuroblastoma

NEWSLETTER

#6 February 2005

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Vienna, Austria

2005 Annual Meeting ... ATHENS !!!
November 10-11, 2005
under the auspices of the
Hellenic Society of Paediatric Haematology- Oncology

Editorial

Dear friends,

a. ESIOP Neuroblastoma Board

i. During the Annual Meeting held in Krakow, Members of the Group were requested to indicate their preference for a New Chairman and a new Member of the Board (being concluded the mandates of Bruno De Bernardi and Victoria Castel). Two names for new Chairman and five for new Member eventually came out for ballot. We collected 107 evaluable votes from a total 218 members (as resulted from an inquire with National Co-ordinators).

Voting ended on December 24, 2004, with the following results:

New Chairman	**Andrew DJ PEARSON** ***(57 votes)***
New Members	**Roberto LUKSCH and Dominique PLANTAZ** ***(34 votes each)***

Composition of the New Board
Andrea DJ Pearson (Chairman)
Bruno De Bernardi (Past-Chairman)
Ruth Ladenstein
Annabel Foot
Roberto Luskch
Dominique Plantaz

ii. In Krakow the Assembly agreed with the Chairman's proposal of establishing an Enlarged Board, composed of National Co-ordinators, Sub-Committees Chairmen and Chairmen of Clinical Studies.
The first meeting of this Board will be held in **Vienna on June 21-23, 2005**.
Among the issues in the agenda, the discussion of a Statute and the nomination of a Steering Committee, basically overlapping with the actual Board.

b. ESIOP NEUROBLASTOMA MEETINGS,

i. 2004 Meeting - Krakow (Poland), October 21-23

This meeting was very successful! Walentyna has provided a wonderful setting and every aspect of the organisation worked beautifully … We all leaved Krakow with the intention to return in the near future. The scientific programme (see next page) also provided a nice overview of what is going on in our Group.
A number of reports are available logging into SIOPEN-R-NET web-site.

ii. Forthcoming 2005 Meetings

VIENNA June 23 INES Group
June 24, "Enlarged" Board,
June 25 HR-NBL-1/ESIOP Study

ATHENS – November 10-11 ESIOP ANNUAL MEETING

c. CLINICAL STUDIES

i. INES: Trials 99.1 - 99.4 were closed to patient enrolment on June 2004. Summaries of the 4 Trials are reported on next pages and are followed by a communication of the German Paediatric Oncology (GPO) Group on stage 2 and 3 single copy MYCN for whom no upfront chemotherapy was given (reports and communication held in Valencia on January 21-23, 2005). ESIOP NB, GPO and COG are planning to tightly co-operate to find a common basis for treating these patients.

ii. LNESG 2. It has been Activated on October 2004. Protocol Forms management will be on-line, integrated into SIOPEN-R-NET system, probably active in March 2005 (see page 5).

iii. HR-NBL-1 and Unresectable Studies. Reports at page 7-12.

d. Advances in Neuroblastoma Research, 2004 (June16-19, Genova)

Cancer Letters - Special Issue devoted to ANR 2004 will contain 36 Mini-Reviews and will be published within June 2005.

e. International Neuroblastoma Risk Group (INRG)

Andy Pearson, Victoria Castel and Frank Berthold took advantage of ANR 2004 Conference for gathering the major clinical experts with the aim to tight the international co-operation.

Background. The main neuroblastoma co-operative groups have different therapeutic/risk groups based on different prognostic factors. Although, stage, age and MYCN status are universally employed, some groups include other factors, such as DNA ploidy, histology, and 1p and 11q chromosome loss. In addition, there are discrepancies in the techniques used for assessing bone marrow and skeletal disease.

INRG Principal Objectives.

a. To develop common risk/therapeutic groups.
b. To define genetic features to be determined by all groups.
c. To establish common methodologies to evaluate the presence and the response of metastatic disease.

Future directions. The investigators involved will meet in Vancouver on September 2005, just prior the 37th SIOP Meeting, under the auspices and partial sponsorship of Forbeck Foundation.

Bruno De Bernardi
for the ESIOP Neuroblastoma Board

INES - Infant Neuroblastoma European Studies

Trial 99.1 (Hervé Rubie, Hôpital des Enfants, Toulouse)

Objective: To evaluate the efficacy of moderate-dose chemotherapy in infants with localized and unresectable neuroblastoma (LU-NB) and confirm the good results of the French pilot study (Rubie et al, Br. J. Cancer 2003).

Patients and methods: All consecutive infants with LU-NB and no MYCN amplification (NMNA) were eligible in the INES-Trial 99.1. Primary tumour was deemed as unresectable according to imaging data showing any risk of immediate resection. Diagnostic procedures and staging were done according to INSS recommendations. For children providing they had no threatening symptom (ie vital risk or dumbbell NB with neurologic deficit), chemotherapy consisted in low-dose cyclophosphamide (5 mg/kg/d x 5 days) and vincristine (0.05 mg/kg at day 1) – CV and repeated 1 to 3 times every 2 weeks until surgical excision can be safely performed. No post operative treatment was given. Conversely patients received 2 courses of Carboplatin (CBP)-VP16 and possibly Vincristine-Cyclophosphamide-Doxorubicin (CAdO) in case of either a threatening symptom or poor response to CV.

Results: Between November 1999 and June 2004, 120 consecutive infants with LU-NB and NMNA were registered in the study. INSS stage 3 was recorded in 84 and unresectable 2 in 36, 34 children having a symptomatic dumbbell tumor. Among 110 evaluable patients at the time of the present analysis, 37 had a threatening symptom (34 dumbbell) and received CBP-VP16 (n=6) followed by 2 courses of CAdO (n=15) according to the protocol. Among the 73 children having no threatening symptom, CV alone (2-4 courses) was administered in 24, followed by CBP-VP16 (n=17) or CadO (n=11) according to the protocol. Surgery was attempted in most patients. Overall Survival and Event-free Survival are respectively 100 and 91% with a median follow up of 2 years. A relapse was observed in 10 children either local (n=8) or metastatic, of whom 9 are in 2nd CR and 1 in 2nd relapse.

Conclusion: In infants presenting with a LU-NB and NMNA and no threatening symptom, anthracyclins-containing regimes could be avoided in 65% of patients without jeopardizing their long-term outcome.

Trial 99.2 (Mary Gerrard, Sheffield Children's Hospital)

Trial 99.2 was for infants with metastatic disease (excluding bone, CNS or lung) who did not have amplification of MycN. When the study closed in June 2004 133 patients had been registered. The patients were from 8 countries (Austria 15, Belgium 5, France 31, Italy 38, Norway 2, Portugal 3, Spain 18, and United Kingdom 21). The median age was 95 days (range 4-364) with 24 infants diagnosed in the first month of life. Seven were detected by prenatal ultrasound scan. There were slightly more males (71:62). The primary site was abdominal in the majority. According to the data at registration, sites of metastatic involvement included marrow in 53, liver in 106, skin in 24, lymph nodes in 11. The recommendation for this group of patients was that only infants with severe or life threatening symptoms be given chemotherapy, because of the expectation that many infants would have spontaneous regression of disease. In order to determine whether the Philadelphia scoring system described by Hsu et al (Med Pediatr Oncol 27;521-528 (1996) was of use in a prospective study it was recommended that this be used to inform the decision regarding treatment with neonates receiving treatment if the Philadelphia score ^{3} 1 and older infants if the Philadelphia score was ^{3} 2. Treatment - at the time of this report data relating to chemotherapy given is available on 104. A total of 52 did not receive any chemotherapy (50%). 44 (42%) received only etoposide and Carboplatin (17 1 course, 27 2 courses). 8 (8%) received additional CADO courses (5 received 2 and 3 received 4 courses of CADO). Fifty three infants are known not to have had secondary surgery. Fifteen did have secondary surgery; data are not currently available on the remainder.

Treatment appears to have been well tolerated with little serious toxicity; although there are two reported deaths from sepsis (one also had progression of disease)

Outcome - in addition to the two deaths as a result of infection, there have been two as a result of progressive disease. With a median follow up of 1.8 Years (range 0.0 to 5.0 years) the overall survival is 97.3% at 12 months (95% CI 94.4 – 100%) and 95.8% at 2 years 95% CI 91.6 – 100%). Event free survival at 1 and 2 years is 89.6% (95% CI 84.0 – 95.2%)

This study does confirm that the outcome for a significant proportion of infants with metastatic neuroblastoma is good and that they can experience resolution of disease without any treatment or with only limited chemotherapy.

It is crucial that all missing data are returned to the data centre as soon as possible so that full analysis can take place.

Trial 99.3 (Bruno De Bernardi, Istituto Giannina Gaslini, Genova)

Trial 99.3 was designed for infants without MYCN amplification with metastases in the bone (documented by mIBG positivity associated to X-ray and/or CT scan abnormalities), CNS or lung/pleura. In the period November 1999-June 2004, a total of 48 such patients were registered, mainly from France (14), UK (14), and Italy (10). The primary tumour was in the abdomen in 33 cases. Metastases involved the bone in 30 cases, bone marrow in 28, liver in 23, lung in 9, pleura in 6, CNS in 6, skin in 9. Chemotherapy consisted of carboplatin+etoposide (2-4 cycles), and CADO (2-4 cycles) if needed. Primary tumour was resected radically in 23 cases, sub-radically in additional 4 with a total of 6 non lethal complications. At end of therapy, 22 patients were in CR/VGPR, 8 in PR, 1 had NR and 1 developed PD (missing information in 16, 33%). Events include one death for disease at 75 days and 4 relapses (2 in bone, 1 in bone + bone marrow, 1 in skin) at 100-457 days. There was only one severe adverse event. Overall survival after a median observation of 2 years is 98%. Event free survival is 86%.

Conclusions. The recruitment was lower than expected; treatment was well tolerated; provisional results are excellent; lot of data are still missing.

Trial 99.4 ***(Adela Cañete and José Bèrmude, Hospital La Fe, Valencia)***

Infants with *MYCN* amplification in Europe has been treated according to trial 99.4 since 1999. Treatment consisted on four courses of Induction Chemotherapy (IC), alternating carboplatin and etoposide with CADO (Cyclophosphamide, adriamycin and vincristine) , followed by delayed surgery when CR at metastatic sites was reached, leukapheresis, megatherapy (conditioning regimen: BUMEL) and radiotherapy. In year 2000 it was amended to continue treatment with retinoic acid courses as published by Matthay et al.

From 1999 to April 2004 forty six patients have been included, from different European countries (France, UK, Italy, Spain, Austria, Eire and Belgium). The ratio male:female was 1.1:1, median age at diagnosis was 249 days (8,3 months). Thirty eight patients (83%) had metastases at diagnosis and INSS distribution was the following: 3 stage 2, 5 stage 3, 26 stage 4 and 12 stage 4s. Status of *MYCN* amplification has been centrally reviewed by the Biology Neuroblastoma Subcommittee in 39 cases, showing a homogeneous MycN amplification in 34 of them. Up to January 2005 the median follow-up of the whole cohort is 12 months (range: 0.01-53 months). Overall survival for 42 patients adequate follow-up is 36% (SD: 0.12), with median survival time of 23 months and 19 deaths due to disease. Event Free Survival is in the same range, with 19 events related to disease. Patient with homogeneous *MYCN* amplification demonstrated by central review is even worse (20% with SD: 0,15 and median survival time of 12 months). There were no toxic deaths and treatment was well tolerated, without severe toxicities There were no deviations in timing or dosing during IC; in spite of correct adjustment to treatment 10 out of 31 patients evaluable for response to IC progressed or did not respond. Delayed surgery could be performed in 19 patients, with 15 satisfactory excisions. There were not complications following surgery. Megatherapy was performed in 14 patients with only 2 VOD and 1 major infection as complications. Univariant analysis has shown that stage and metastatic sites are of importance, while factors related to therapy (Dose intensity, timing, deviations) have no stastistical power in this population. Therefore, stage 4s patients survived better than stage 4 (*P*=0.0372) and patiens with liver metastases did better too (*P*=0.04). Patients with CNS/lung metastases fared worse than patients without that type of metastases (*P*=0.009). In conclussion, the accrual was as expected, with a very small group of infants with dismal prognosis and treated homogeneously in a multicenter european study. Although it was a very well tolerated treatment, survival is discouraging, and in our hands, induction chemotherapy was not sufficient to produce a rapid and satisfactory response to allow for the possibility of intensive chemotherapy to be given. Although timing and doses in IC and megatherapy were right, there were 30% of failures in the initial part of the treatment and 25% of relapses. We need to look for new approaches and deeper multicenter and international collaboration for this extremely high risk and small group of infants.

INVITED COMMUNICATION
by the German Paediatric Oncology Group (GPO)

Observation in infants stage 2 and 2 single copy MYCN neuroblastoma without chemotherapy

Frank Berthold, Thorsten Simon, Hans-Gerhard Scheel-Walter, Jan Sörensen, Gabriele Benz-Bohm and Barbara Hero, *for the GPO*.

Overdiagnosis of neuroblastoma in screening programmes and “incidental” observation of single cases suggested that the well known feature of regression in stage 4s patients may apply also for localized disease. The GPO decided in 1995 to study that phenomen prospectively in infants with stage 2 and 3 neuroblastoma. Except for therapy of threatening postoperative symptoms, macroscopic residual tumor was observed only by radiological imaging, tumor markers and clinical investigations and no chemotherapy was applied. Between 05/95 and 07/03 135 infants with stage 2 (n=72) and stage 3 (n=63) were studied. 58 patients were observed only (40/18 in stage 2/3), 50 received chemotherapy for dangerous symptoms (14/36) and in 27 infants the tumor could be completely resected at diagnosis (18/9). Of the 135 patients, 33 experienced relapse or progression (24 local only, 1 local + liver, 8 metastatic). 8/50 belonged to the chemotherapy group, 5/27 to the surgically treated infants and 20/58 to the observation only group. The event free 3 year survival rate was 74 ± 4 % and the survival rate was 97 ± 2%. Thus, almost all patients with recurrences or progression are alive with second line therapy. The disappearance of the regressive tumor was complete in only half of the patients and took a surprising long time (median time to the start of regression 6 months, range 2-15 months).

In conclusion, regression is a common feature in stage 2 and 3 infant neuroblastoma and chemotherapy can be completely avoided in a considerable number of patients. The new trial extends the observation group by including older patients than 1 year at diagnosis and utilizes strict molecular exclusion criteria.

SPECIAL FOCUS ...

Maja Beck Popovic, University Hospital Lausanne, Switzerland

LNESG 2 study

GUIDELINES FOR THE TREATMENT OF PATIENTS WITH LOCALIZED RESECTABLE NEUROBLASTOMA AND ANALYSIS OF PROGNOSTIC FACTORS

Second European Study for the treatment of Localized Neuroblastoma, activated on October 1, 2004

Background

The recently completed multicentre European Study for localized NB (LNESG 1) primarily evaluated the safety and efficacy of surgery as the only treatment in the management of INSS stage 2 neuroblastoma without MYCN amplification (Trial patients). Stage 1 and 3 patients as well as those with ganglioneuroma were followed as Study patients.

From January 1995 until September 1999, 905 patients were registered from 9 European countries. There were 740 patients with localized neuroblastoma or ganglioneuroblastoma, of whom 616 eligible for the study and 124 for the trial. Seventy-one patients presented with ganglioneuroma and 94 were ineligible (32 NB, 62 for other diagnosis).

Of the 124 trial patients, 21 relapsed, locally in 12, combined in 8 and only distant in 1 patient. Metastases involved the bone and/or bone marrow in 7 cases. Relapses were treated with a variety of regimens. Seven out of 21 patients who relapsed died of disease, 6/7 after a subsequent relapse. One other patient died from sepsis. The overall survival for the trial patients is 94.8% at 3 years (90.7-98.9) indicating that the majority of INSS stage 2 patients (MYCN negative) can be cured with surgery alone. The RR was 13.2% (CI 7.2-19.3) at 12 months, 15.8% (CI 9.3-22.3) at 24 and 17.8% (CI 90.7-98.9) at 36 months.

Three risk factors for relapse were evaluated in this protocol, that is preoperative LDH, 1p del and histology. Because of a considerable amount of missing data, no statistically significant conclusion could be drawn with regard to one of these factors predicting a higher risk for relapse. Only histology was associated with a significant higher mortality in relapsed patients (5/8 relapsed patients who died had unfavorable histology), but based on very small numbers. So there was no justification to consider a change in treatment for patients with stage 2 MYCN negative neuroblastoma at diagnosis.

Relapse rate was much lower in stage 1 MYCN negative patients with an overall survival of 99% at 36 months.

Among the study patients, there were 7/296 stage 1 patients with MYCN amplification, 5 of whom relapsed with death from disease in 4. Although mortality was very high in this patient group, the very small numbers did not allow any conclusion.

The preoperative evaluation of surgical risk factors was introduced in LNESG1 to avoid aggressive procedures with a predictably high risk of postoperative complications and any operation with a high risk of leaving a macroscopic residue. Operation should only have proceeded when complete excision appeared feasible without damage or risk to the patient. In all stages, the complication rate was higher when the operation was performed in spite of pre-operative risk factors. In stage 2 patients this difference was statistically significant ($p<0.01$). The main complications were serious bleeding, renal ischemia and chylous leakage. Operative complications were also more frequent if risk factors, including tumour size and tumour fragility, were present. This was statistically significant in patients with abdominal primary disease. Patients with thoracic and abdominal tumours had a statistically higher rate of macroscopic residual disease when operated on in the presence of risk factors. Overall and relapse free survival were lower in

patients who were left with macroscopic residual disease after excision, but abdominal primary disease. Among all patients with macroscopic residual disease for whom data were available (n = 50), there was no difference in outcome with regard to the tumour volume remaining after excision.

Objectives of LNESG2

The LNESG2 is based on the results of LNESG1 and has the following objectives:

1. ***Primary objective.*** To expand the information provided by LNESG1 on factors associated with clinical prognosis in localized neuroblastoma, especially preoperative LDH, 1pdel and histology
2. ***Secondary objectives.***

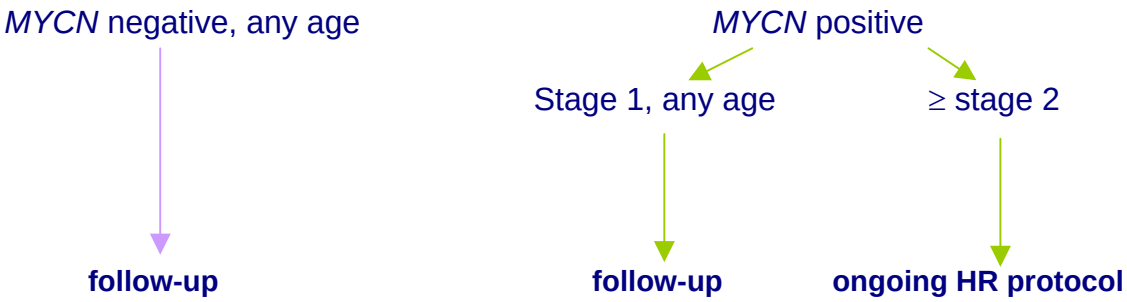
- To maintain or improve EFS and OS when compared to LNESG1
- To improve the quality of management and data collection in patients with resectable localized neuroblastoma without MYCN amplification by
  - A nationally centralized evaluation of the pathological and biological data with secure banking of material
  - Improved data collection, with particular regard to LDH, 1p deletion
- To establish a uniform treatment for relapsed patients

The main points are the respect of presurgical risk factors for operative decision at diagnosis, mandatory preoperative analysis, mandatory biological and metastatic work-up, a central on-line pathology review by the Pathology subcommittee in collaboration with the local pathologist, and mandatory secure of material for future studies.

- Inclusion criteria
- age between 0 and 18 years included
- all patients with resectable INSS stage 1 to 3 NB, without MYCN amplification
- all patients with resectable INSS stage 1 NB, with MYCN amplification
- no previous chemotherapy (except steroids)
- mandatory biological assessment: MYCN amplification, 1p deletion
- mandatory histological assessment: locally and nationally reviewed grading according to INPC classification
- mandatory biochemical assessment: preoperative LDH
- mandatory full metastatic work-up (MIBG at diagnosis included)
- registration within 6 weeks of surgery
- provision of follow-up for up to 3 years
- secure local or national banking of tumour material
- national/local ethical committee approval
- informed consent obtained from parent or guardian.

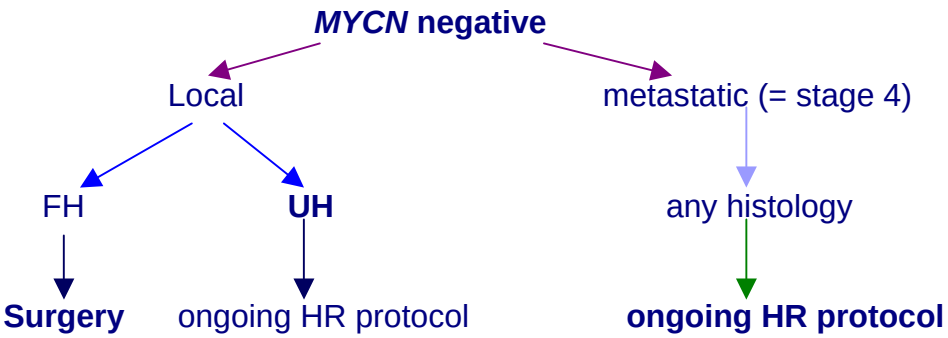
Resected INSS stage 1-3 neuroblastoma

Treatment at diagnosis

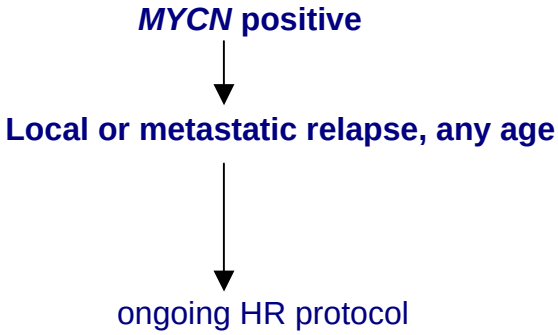


Treatment at relapse

Resected INSS stage 1-3 patients



Resected INSS stage 1 patients



State of the protocol

LNESG2 will be integrated into the SIOPEN-R-NET system. All centres already registering patients in HR-NBL-1 Study can use the same login for registering patients with localised disease. The process of the remote data entry is in development and should be finished during in the coming weeks.

The protocol has been officially activated 1st October 2004 and is undergoing IRB approval in many countries. Two patients have been registered until now.

Ruth Ladenstein, Ulrike Pötschger, Ditha Modritz, Annabel Foot
for the HR-NBL-1/ESIOP Study participants

HIGH RISK CLINICAL STUDY PROTOCOL
(HR-NBL-1/ESIOP study)

In fall 2004 the interim analysis and the progress report on the HR-NBL-1/ESIOP study has been communicated at the Annual ESIOP Neuroblastoma Meeting in Krakow.

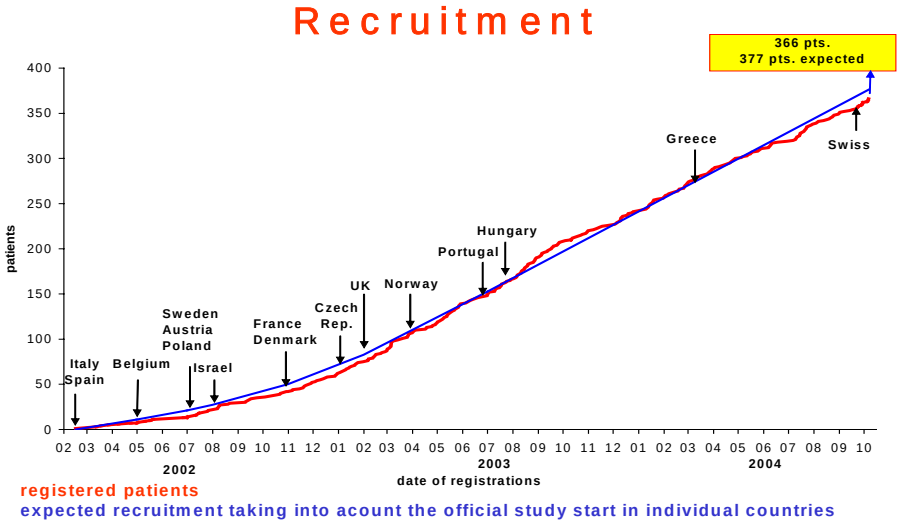
1 Minutes of the Progress Report

1.1 Accrual per October 15, 2004

Recruitment to the study is running very much as predicted with a 366 patients registered on the given date. Median age is 3 years 1 month (1 year – 17 years). Patient distribution: stage 4, 329; stage 2/3 MYCN amplified, 31; not yet specified, 6.

- National ethical approval achieved in all 16 countries
- Slovakia is welcomed as a new participating country
- RECENT NEWS: France has succeeded to re-open the study. ***Welcome back, France*!**

The completeness of data reporting has improved significantly with > 85% chemotherapy data reported and >80% of toxicity data available in October 2004 in contrast to only 60% and 20% in November 2002.



Participants at the study steering committee meeting:
Shifra Ash, Valentyna Balwierz, Maja Beck-Popovic, Bruno De Bernardi, Pavel Bician, Penelope Brock, Adela Canete, Victoria Castel, Aurora Castellano, Andrej Cizmar, Sandro Dallorso, Andrea Di Cataldo, Annabel Foot, Keith Holmes, Igor Jenco, Bertil Kagedal, Anna Keminski, Per Kogner, Ana Forjaz de Lacerda, Ruth Ladenstein, Genevieve Laureys, Roberto Luksch, Wolfgang Marco, Jean Michon, Ditha Modritz, Tom Monclair, Aullneuque Niergode, Aguienlie Othiunueski, Vassillios Papadakis, Andy Pearson, Frida Ponthan, Ulrike Pötschger, Katanyue Powiuntue, Gunther Schreier, Ingeborg Storm-Mathieson, Monika Sujanova, Dominique Valteau-Couanet.

1.2 COJEC induction

The completeness of data has improved for the COJEC induction and is now running at greater than 80%. Toxicity during rapid COJEC is running on average at about 5% Grade 4 (excluding haematological). There are 8% Grade 4 infections reported and only 1% Grade 4 GFR toxicity, which is not excessive. There has been no Grade 4 ototoxicity reported, but 5% Grade 3. Looking at these in more detail 7 of the 8 patients reported come from two countries (UK and Sweden). The question was raised as to whether it is being under-evaluated in other countries who are unable to perform adequate testing at all time points. The most important evaluation is the one prior to R1 and it is emphasised that the child should be well when this is performed. There is a need for feedback as to what is feasible for audiology in each country and consider recommendations/possible workshop for training in the future. Action: Peppy Brock and Per Kogner to take forward the clarifications of audiometry assessments in Europe. It had previously been discussed that there be a recommendation that Grade 3 and 4 ototoxicity should not be randomised to CEM. It will not however be put on the checklist for eligibility. In the interim Peppy volunteered to look at those patients who have Grade 2 ototoxicity prior to CEM and examine their post CEM audiometry evaluations.

Summary

- List of protocol amendments decided at meetings to be circulated officially to national co-ordinators to take forward through their national process (separate from minutes)
- Absence of Grade 3/4 ototoxicity will not be put forward as formal eligibility criteria for R1. However it will be highly recommended that auditory assessment be carried out prior to R1 and if ototoxicity is found to be grade ¾, it will be recommended not to randomise to CEM.

- The 90 day death rate is currently 3%. This is below the stopping rule of 5%.

Action: Volunteers requested to build a working group for a Data Bank Issues and Definitions of Variables to simplify the volume of data collected and hone in on important aspects. Volunteers should identify themselves and email Ruth Ladenstein.

1.3 R0- G-CSF randomisation

A further 20 patients are required for this randomisation. It is therefore predicted that recruitment will be completed early in 2005. These patients should all be evaluable by April/May 2004 when the data will be analysed. Ulrike Pötschger will check in March 2005 if the expected recruitment is achieved and if for this cohort a statistical conclusion has been reached or is to be expected. The ability to close R0 shall be the trigger point for the next major anaylsis and report to the DMC.

1.4 Response following COJEC Induction

The BM response rate (BM pos at diagnosis to BM neg based on reported cytomorphology results of aspirates after 8 cycles of rapid Cojec) is 74% in 204 evaluated patients. The skeletal response rate based on mIBG data in 199 evaluated patients (mIBG skeletal pos at diagnosis) after 8 cycles of Rapid Cojec is 77% (CR rate 44% and PR rate 33%).This is currently based on mainly non-reviewed local investigator judgement. The overall response rate following COJEC (prior to surgery and including the response at the primary tumour site) is running at 68% (4% CR, 18%VGPR, and 46% PR).

1.5 Surgery

There is considerable missing data (37%) with regards to the surgery in these patients (no information on 105 of 307 evaluable patients, although 30 of these had insufficient response and were ineligible to continue the protocol). There have been two surgically related deaths. There is a 7% nephrectomy rate. Stopping rules have not been breached.

1.6 Stem Cell Harvest

Overall there has been an insufficient collection of stem cells in 7% of patients. However it is noted that this rate has fallen over the last year since the amendment to extend the possibility to collect stem cells to 150 days. Before November 2003 it was running at 9% and since that date it is only 2%.

1.7 R1 – MAT randomisation

A total of 128 patients have been randomised in R1 (42%). The main reason for lack of randomisation has been for insufficient response (overall 30%). This rate has increased over the last 12 months from 28% to 40%. This may reflect changes in bone marrow criteria and also enhanced MIBG review. There has also been a small increase in exclusions due to informed consent (from 1% to 7%; overall running at 2%). However there has been an improvement following the extension to 150 days time limit (this has now dropped from a 5% to a 0% rate).

1.8 Toxicities during MAT

Data interpretation is limited as there are currently 31% missing data. However Grade 4 infection has been reported in 6%, Grade 4 renal (GFR) in 3% and Grade 4 liver toxicity in 10%. Bearman Grade 3 VOD has been reported in 5% of patients, pulmonary toxicity in 5% of patients and ototoxicity in 4% of patients. There are no significant differences appearing between CEM and BuMel arms. The death rate within 100 days of BMT is currently running at 4%. No stopping rules have been breached.

1.9 Radiotherapy

One hundred and thirteen patients are evaluable for radiotherapy but here 50% of the radiotherapy data is missing in the data base with an urgent need for update and filling in.

1.10 SAE overview

There is the need to define two types of SAE's clearly – expected and unexpected. The SAE's that had occurred from February to October 2004 according to definitions that had been discussed and decided upon at the meeting in April 2004 were presented. Thirty-seven events have been reported on the database during this time period – 24 of these have been reported as SUSARs. SUSARs by definition should be suspected unexpected severe adverse reactions and these are to be reported immediately to the regulatory authorities. There followed considerable debate regarding definitions, particularly what amounts to an unexpected event. It was agreed that SAE reporting guidelines are required and further work needs to be done in this area following the discussion in the meeting.

Action: Document regarding SAE reporting to be circulated in the near future. Following the DMC report recommendations, fungal infections have been looked at in more detail. Thirty-seven fungal infections have been reported on the database out of a total of 295 patients (13%). Further details were requested on these 37 patients. Data on 7 patients is missing. Seventeen have been reported as severe – 6 cases of candida septicaemia (two died), nine cases of aspergillus (3 died) one aspergillus dermatitis and one fusarium infection. Therefore the incidence of severe fungal infections is 6% - this would rise to a maximum of 8% if all seven missing cases are also severe. The new database will be modified to collect data prospectively

1.11 Monitoring guidelines

a. Toxic deaths during induction >5%
b. Failure to harvest sufficient CD34 cells >15%
c. Surgical event rate > 5%
d. Severe adverse MAT related event rate in one randomised arm > 10%

If any monitoring border is hit, data is to be submitted to Data Monitoring Committee for evaluation. Recommendations are to be brought to the attention of the Trial Committee for decisions on how to proceed. None of these borders were reached in 2004.

2. Antibody Progress Report

In February 2004 the success of recloning and the amplification procedure was approved. By March 2004 a series of new clones were generated. The results presented by Polymun were encouraging. A variety of clones producing ch14.18, were generated, a few were producing quantities sufficient to generate the amount of 200g of product. Further subcloning and productivity and binding testing was ongoing over summer. The last meeting of the antibody task group was held in Vienna on 13 October 2004. Development of a fourth generation sub-clone (which shows superior productivity over the third generation of clone) has resulted in a new master cell bank, but with a delay in the production of two months. Production on a large scale commenced the first week in October. It is estimated that the end of production will be January 2005. A Phase 1 clinical study for equivalence data is planned to satisfy regulatory authorities. This will be a limited dose escalation Phase 1 protocol with pharmacokinetics. It was proposed that this is carried out in three institutions, Vienna, Genoa and Berlin, all involved in the task group. Further volunteering centres are welcome. It is envisaged that this will start during February/March 2005. It is planned that this should be a single course of treatment at dose escalations of 10, 20- and 30 mgs/m2. It is aimed to recruit 14 patients. It is hoped to roll out R2 following this. A cost-share plan is being calculated for new partners – ongoing costs are required for insurance purposes (estimated 135,000 Euros) and also for the distribution of antibody which is costly.

The current production shall easily cover the Phase 1 study as well as the planned part of the Phase III study.

3. Data Base Developments and Issues (ARCs Seibersdorf)

The planned change with migration of the HR-NBL-1/ESIOP study is due to take place on 29/30 November 2004. The database will be off-line for this time period. It

is planned that the new system will be up and running by the 1st December. A brief demonstration of the system was given by Günther Schreier. Various issues were discussed, including the need for a save and sign button. Feedback has been requested and given from national co-ordinators with regards to database issues. However it was also agreed that there is a need for a working party to work on a reduction of the variables requested to simplify the system and make it more "user friendly" as well as efficient. It is suggested that this could be instituted once RO has been completed. Action: Volunteers required for IT Task Group to work on database issues.

4 Protocol Amendments 2004

4.1 Confirmation of previous amendments agreed.

i. **Randomisation 1 (MAT-Question): allowed up to day 150**

ii. **Eligibility Criteria for the R1 Randomisation**

a. Study entry criteria fulfilled.
b. COJEC induction treatment given (strictly no anthracyclines, no investigational anti-tumour chemotherapy)
c. Complete re-staging of disease after completion of induction therapy.
d. CR or PR at metastatic sites after induction treatment: at least 50% reduction in skeletal mIBG positivity and not more than 3 positive, but improved spots on mIBG
e. cytomorphological CR in 2 BM aspirates and no positive BM biopsy
f. Organ functions (liver, kidney, heart, lungs, ears) fulfilling criteria prior to MAT
g. ALT, bilirubin < 3 x normal
h. Creatinine clearance and/or GFR ³ 60 ml/min/1.73m² and serum creatinine
i. <1.5mg/dl. Call study co-ordinator for MAT dose modifications if
j. GFR < 60ml/min/1.73m² and serum creatinine ³ 1.5mg/dl.
k. Shortening fraction ³ 28%, or ejection fraction ³ 55%, no clinical congestive heart failure.
l. Normal chest X-ray and oxygen saturation.
m. Sufficient stem cells available. Minimum required: 3 x10 6 CD34 cells /kg body weight, if a BM harvest was unavoidable at least 3 x 10 8 MNC /kg body weight.
n. Written informed consent for R1, and for minors an agreement by parents or legal guardian.

Any negative answer will render the patient ineligible.

Note:

- Once the patient has fulfilled the eligibility criteria, the patient should be randomised.
- The result of surgery will not influence randomisation.
- The time to randomise for R1 was altered from previously 120 days (from the first day of chemotherapy) to now 150 days since the time previously allowed appeared to limited in sufficient number of patients.
- With a GFR rate ³ CTC- Grade 2 the patient is ineligible for R1. Since recovery of GFR rate is observed in particular post surgery, at least two GFR rates need to be evaluated post surgery if the first postsurgical one shows a GFR grade 2 to 4 prior to day 150 (with at least a two weeks interval but ideally 4 weeks if feasible).
- Ototoxicity not part of eligibility criteria , but if ototoxicity – Grade 3 or 4according to Brock criteria is documented, it is recommended not to give CEM , ie. not to randomise.

III. **Guidelines.** The guidelines for prophylaxis and treatment of fungal infections to be included in the protocol:

Therapy for febrile neutropenia.
The COJEC regimen results in very significant and prolonged neutropenia and has a similar myelosuppressive effect to that which occurs during the therapy of acute myeloid leukaemia. This results in a significant of risk of fungal infection, therefore it is very important that investigators adhere to and follow this approach for therapy for febrile neutropenia.

1. If there is fever (>38OC) and the neutrophil count is less than 1.0 X 109/L, then the centre's usual combination of broad-spectrum antibiotics should be commenced.
2. If fever persists (>38OC) for 48 hours despite broad-spectrum antibiotics, then antifungal therapy should be started, regardless of the clinical condition of the patient. The preferred antifungal therapy is liposomal amphotericin (ambisome) at a dose of 1mg/kg/day. However, if this is not available then amphotericin B 0.5mg/kg for the first dose and then increased to 1.0mg/kg after 24 hours should be given. In the case of impaired renal function liposomal amphotericin is recommended. In addition a chest X-ray (CXR) should be carried out. Other antifungal therapy e.g. fluconazole is not permitted in view of the substantial risk that the underlying fungal infection is aspergillosis and fluconazole will not be active.
3. **If fever persists for a further 48 hours (i.e. a total of 96 hours), without another identified cause then:-**
   a. The dose of ambisome should be escalated to 3mg/kg/day;
   b. If the patient is receiving amphotericin B, then very careful consideration should be given to substituting ambisome for amphotericin B;
   c. Careful consideration should be given to carrying out a CT scan of the chest,

If there are any abnormalities on the CT scan then:

   a. G-CSF should be commenced at 5ug/kg/day;
   b. Consideration should be given to the introduction of caspofungin or another antifungal agent. Itraconazole and voriconazole should not be considered since it should not be combined with vincristine;
   c. Consideration should be given to other specific, appropriate investigations e.g. imaging, biopsies and broncho-alveolar lavarge.
   d. Granulocyte infusions may also be considered.

4. **If the fever persists for a further 48 hours (i.e. a total of 144hours) without CT scan changes) then:**
   a. GSCF should be commenced at 5ug/kg/day;
   b. Consideration should be given to the introduction of caspofungin or another antifungal agent. Itraconazole and voriconazole should not be considered since it should not be combined with vincristine
   c. Further dosage escalation of ambisome to 6mg/kg/day could be considered

The early (after 72 hours of fever) introduction of ambisome or amphotericin is the most important measure and investigators MUST adhere to this. Investigators must have a very high index of suspicion of invasive fugal infections, in these myelosuppressed patients and vigorous, empirical antifungal therapy must be given early in an episode of febrile neutropenia

IV. Thoracic primaries

The point as to whether children with thoracic primaries may be eligible for randomisation had been discussed in April and was again discussed at this meeting. Following further debate it was decided that these patients would be eligible for randomisation. However, there should be a recommendation that caution should be employed when considering radiation therapy following the BuMel arm. This would is particular the case with a large tumour volume and would need careful discussion with the radiotherapist.

4.2 Proposed protocol amendments

4.2.1 HR-NBL 1 ESIOP Study SAE reporting guidelines. The processing of Serious Adverse Events in a timely manner is a regulatory requirement. It is also vital in order to safeguard the safety of patients entered into any trial.

This Standard Operating Procedure covers the following areas: definitions of SAEs, reporting by centres, clinical review of reported SAEs, responsibilities of (National) Trials Units, including reporting to regulatory authorities.

I. Definitions of Events

a. **Adverse Event.** Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

b. **Serious Adverse Events.** The following definitions are standard ICH-GCP definitions and should be included in all protocols. Any untoward medical occurrence that at any dose:
   - *results in death;*
   - *is life threatening;*
   - *requires in-patient hospitalisation or prolongation of existing hospitalisation;*
   - *results in persistent or significant disability/incapacity; or*
   - *is a congenital anomaly/birth defect.*

It is possible, however, to establish exceptions in terms of expedited SAE reporting requirements, as long as these are clearly stated in the protocol.

Thus the definition of serious follows guidelines as stated in the protocol. The sponsor /Chief Investigator (International Study Coordinator) / National Study Coordinator needs to be informed immediately within 24 hours of knowledge of event. An urgent clinical review of SAEs is to be arranged by the National Study Coordinator and to be reported to the Chief Investigator/International National Study Coordinator. The decisions needs to be documented within the given time frames – see below.

Two types of 'SAEs' to be distinguished:

a. *Suspected Serious Adverse Reaction (SSAR):*

These are events that have previously been reported for a given treatment concept and are thus considered as expected. Their occurrence cannot be completely avoided within the context of a given intense treatment concept for a life threatening disease. However, their frequency is to be controlled through the trials monitoring guidelines which set limits (stopping rules) to the frequency of such events. These events are to be reported within the toxicity section and as SAEs within the HR-NBL-1/ESIOP Study data base and, if needed for national rules, as an 'expected SAEs': Thus they clearly reflect expected toxicities within a given intense treatment schedule including induction, surgery, MAT, radiotherapy and minimal residual disease treatment.

Action:

- The sponsor /Chief Investigator (International Study Coordinator) / National Study Coordinator needs to be informed immediately within 24 hours of knowledge of event.
- An urgent clinical review of life threatening SAEs is to be arranged by the National Study Coordinator and immediately to be reported thereafter to the International Study Coordinator (Data Centre).
- The National Coordinator should report these to an Independent Ethics Committees once a year (annual report) or at a frequency according to given national guidelines.
- The SSARs are continually controlled through stringent study monitoring and stopping rules. They should trigger an internal trial alarm system within the given study concept: There may be a need for action such as treatment modifications or specified guidelines to overcome the incidence of SSARs and eventually their frequency may activate stopping rules. If toxicities borders as described in detail in the statistical section are hit, the Data Monitoring Committee (DMC) needs to be notified and eventually modifications of the trial concept will follow or the trial may even be stopped if unacceptable toxicity is encountered. Investigators need to be informed regularly about the occurrence of these SAEs according to the monitoring guidelines.

b. *Suspected Unexpected Serious Adverse Reaction (SUSAR)*

SUSARS are clearly new unexpected SAEs and previously not encountered and not reported SAEs within a given treatment concept.

- Fatal or life threatening: The Sponsor/Chief Investigator (International Study coordinator)/National study Coordinator needs to report SUSARs within 7 days of notification of the event to regulatory authorities, independent ethics committee and investigators concerned (+ 8 days for further information)
- NOT fatal or life threatening: Sponsor/Chief Investigator (International Study coordinator)/National study Coordinator needs to report these SUSARs within 15 days to regulatory authorities, independent ethics committee and investigators concerned.

II. Suggested definition of SSARs within the HR-NBL 1 ESIOP Study. Discussion on how to value the SAEs, final agreement on definitions pending and to be confirmed at next meeting:

a. Progression of disease
b. Death due to tumour progression
c. Requirement of in-patient hospitalisation or prolongation of existing hospitalisation: Prolongations of existing hospitalisations for application of the study medication are expected within the frame of this intense treatment concept to treat expected secondary treatment effects, i.e. infections and complementary supportive care measures.
d. Definition of toxicities (CTC Toxicity Score) as applied in the HR-NBl-1/ESIOP study for severe, but expected events within this intensive regimen

- **GENERAL CONDITION: Grade 4- ICU, very sick**
- **HAEMATOLOGICAL TOXICITY: Grade 4**
- **SKIN: Grade 4**
  - exfoliative dermatitis/necrosis requiring surgical intervention
  - anaphylaxis
- **GASTRO-INTESTINAL TRACT**
  - Nausea/Vomiting: Grade 4 - intractable vomiting
  - Constipation: Grade 4 - ileus >96h
  - Stomatitis: Grade 4 – total parenteral nutrition required
  - Diarrhoea: Grade 4- leading to dehydration
  - Liver: Grade 4 (bilirubin >3 x N) and SGOT/SGPT > 20xN or Bearman Toxicity's Grade III –VOD: Severe hepatic dysfunction with bilirubin < 20 mg%; or hepatic encephalopathy; or ascites comprising respiratory dysfunction
- **URINARY TRACT**
- **RENAL TOXICITY**
  - Any Grade 4
  - All GFR rates ^{3} Grade 2 è patient ineligible for R1. Since recovery of GFR rate is observed in particular post surgery at least two GFR rates need to be evaluated post surgery if the first post-surgical one shows a GFR grade 2 to 4

prior to day 150 (with at least a two weeks interval but ideally 4 weeks if feasible)
    - Hemorrhagic cystitis (Bearman Grade III: Macroscopic haematuria with frank blood, necessitating invasive local intervention with installation of sclerosing agents, nephrostomys or other surgical procedures
- **CARDIAC: any Grade 4**
- **RESPIRATORY TRACT**
    - Pulmonary toxicity (Bearman Toxicity's Grade III)
    - Interstitial changes requiring mechanical support or >50% oxygen on mask and not caused by infection or CHF
- **NEUROLOGICAL**
    - Central Neurotoxicity: Grade 3 Somnolence >50% of time (severe disorientation and hallucinations) and Grade 4- Coma-Seizures
    - Peripheral Neurotoxicity: Grade 4 paralysis
    - Ototoxicity: Brock grade 3 and 4: è patient is not recommended to receive CEM. Evaluation of Ototoxicity has to be performed before and after MAT; follow up of ototoxicity is required
- **INFECTIONS: Grade 3 to 4**
    - Major infection
    - septicemia alone (controlled infection)
    - pneumonia alone
    - urinary infection alone
    - severe soft tissue infection
    - minor localised fungal infection (stool culture pos only, diaper rash and/ or mouth – candida pos)
    - **Life threatening with hypotension:**
        - *septic shock*
        - *proven systemic fungal infection*
        - *blood culture pos or biopsy positive or unequivocal imaging (for prevention of fungal infections please adhere to UK guidelines)*

4.2.2 Infants with MycN amplification.

a. The 99.4 data (up to April 2004) for infants with MycN amplified tumours was presented in Krakow by Adela Canete. There were a total of 39 patients of whom 32 were metastatic. Toxicity during treatment had not been a major feature – there were only four Grade 4 toxicities reported (one VOD and three sepsis).

b. The overall survival was 42% (medial survival time 12 months). However the problem appeared to be that of a group of patients who showed early progression (33% of those in whom response date was available). The aim would therefore be to intensify treatment for these patients. The proposal made is to treat these patients as per the HR–NBL–1/ESIOP protocol.

c. One concern to be considered is the toxicity of rapid COJEC in infants. There is no current data in children under 12 months. The data for children aged 12-18 months on ENSG5 has been reviewed. Out of a total of 15 patients, there was reported toxicity of 13% for fever and 20% for GI problems. There were two toxic deaths. The data for children 1-2 years of age included on the HR-NBL-1/ESIOP protocol has also been reviewed. Out of a total of 35 patients there have been three SAEs reported that have resulted in death. Seventeen SAEs have been reported as infections. These are higher than in older patients. It might therefore be expected that the risk of toxicity of rapid COJEC in infants might be high on this regime. A proposal is therefore made to calculate dosage according to weight rather than surface area. Maximal support would be advocated for these children regarding infection prophylaxis and nutritional support (outcome of R0 awaited).

d. There then followed debate regarding this proposal. The use of rapid COJEC was discussed – although some concerns were voiced about the response rate in older children, in this cohort it is in fact a problem with progressive disease that needs to be addressed. Pharmacokinetic studies may be useful in this group of children – the data from Newcastle does not suggest that there is any difference in infants compared with children on the current etoposide/carpoplatin study. There was also debate as to whether this cohort of infants should constitute an amendment to the high-risk study or form a study in their own right. The former proposal would be a speedier process with current bureaucracy. There was also discussion as to whether these patients should be considered eligible for R1.

Conclusion:

- Up to date analysis of data on the 40 patients included in 99.4 required (for January 2004). This should include analysis regarding the sub-group of patients who show progressive disease as to whether any factors can be identified with this group (such as age, liver involvement etc.).
- Patients with MycN on the high-risk study to be analysed - ? does this sub-group show more evidence of progressive disease on treatment.
- Meeting for National Coordinators planned in Valencia 21/1/05 – final decisions then.

4.2.3 VOD Prophylaxis

The question was raised as to whether the guidelines for VOD prophylaxis should be changed. Action: Volunteers are requested to do a survey on the current usage of ursodiol and defibrotide around centres.

5 Next steps

Next HR-NBL-1/ESIOP Study Steering Committee Meeting 25th, Vienna 2005 together with the next Infant Group Meeting June 23rd and the enlarged board meeting on the 24th including a Grant Strategy Meeting with Subcommittee chairs.

A new progress report together with a DMC report will be prepared as soon as R0 has closed recruitment.

The Phase I Antibody bridging study shall start ideally in March/April 2005.

Unresectable Neuroblastoma Protocol

Report October 2004

Recruitment by year (1)

	2001	2002	2003	2004 (10/12)	Total
Austria	-	1	-	-	1
Belgium	2	1	2	-	5
Eire	-	-	-	1	1
France	-	7	19	8	34
Italy	10	7	4	1	22
Norway	-	1	1	-	2
Portugal	1	1	1	-	3
Spain	2	7	7	9	25
U.K.	1	12	5	4	22
TOTAL	16	37	39	23	115

Although recruitment is as predicted, unfortunately many patients are ineligible. In 11 Myc-N was either amplified or unknown, and in one there was a staging error (not eligible* in table 2).More significantly, in 15 the registration form was received more than 30 days after diagnosis. Thus only 81 patients are eligible by study criteria, and another year of accrual is planned.

Eligibility status (2)

	Pre-Reg	Too early	Not eligible*	Late Reg	Eligible
Austria	1	-	-	-	1
Belgium	5	-	-	1	4
Eire	1	-	-	-	1
France	34	1	1	3	29
Italy	22	-	5	1	16
Norway	2	-	-	-	2
Portugal	3	-	-	2	1
Spain	25	4	4	5	12
U.K.	22	2	2	3	15
TOTAL	115	7	12	15	81

Of 44 patients who have completed 6 courses of treatment, 30 are in CR and 8 VGPR. There has only been one patient with progressive disease in this group, and one death (due to surgery). In a further 13 patients who received less that 6 courses of chemotherapy, there were 6 CRs and 2 VGPRs.

8 SAE forms were submitted, of which only 4 met the study criteria. These included the one surgical death, and 3 episodes of severe sepsis.

5 patients have relapsed.
The writing group recommend continuing the study in 2005 with the aim of accruing 100 eligible patients. The response rate is satisfactory and adverse events and relapses (as reported) acceptable.

Data is often submitted late and many forms are incomplete. We would appreciate a major effort from everyone to complete this study satisfactorily in the next year!

Günter Schreier & Wolfgang Marko, Seibersdorf research For the SIOPEN-R-NET IT team

Dear SIOPEN-R-NET partners!

the past project year has been very intense in terms of IT developments. Besides the integration of the web-based High Risk Study system into the new SIOPEN-R-NET (the so called “Migration”, which will be discussed in more detail below) we mainly dealt with sub-study issues (the Biology and the Nuclear Medicine and Physics sub-studies are already in use by their respective members) and put image management into operation.

During the ongoing third project year our focus will be on the three C’s, i.e. correction, consolidation, and completion, in particular with respect to providing additional subcommittees with IT structures to have their sub-studies electronically supported and to complete the development and rollout of image management.
In addition, the LNESG2 trial will be made available to the participating members of the network in the first half of 2005.

In December 2004 we undertook the migration of the High Risk Study to the SIOPEN-R-NET system. It required considerable efforts and also significantly more time as originally estimated. This migration, however, is the basis for a unique and modular IT infrastructure for the future, on which the ESIOP group can build on, and is intended to increase both, the quality and the efficiency of your scientific work. It also gives us the possibility to implement a number of innovations, i.e.:

a. A dedicated patient registry for all Neuroblastoma patients treated by the ESIOP group
b. Infrastructure for multiple clinical trials with homogeneous user interface
c. Enhanced role concept for better right management
d. Effective interconnections between the main clinical trials and the sub-studies (notifications, reviews, etc.)
e. Improved Electronic Data Capture (EDC) concept (enhanced SAE management, data query support, built-in data consistency and completeness checks, workflow support, etc...)

Some of these features are already incorporated, some will become available in the near future.
Let us take the opportunity to give you some hints which may be important for your daily work in the new SIOPEN-R-NET:

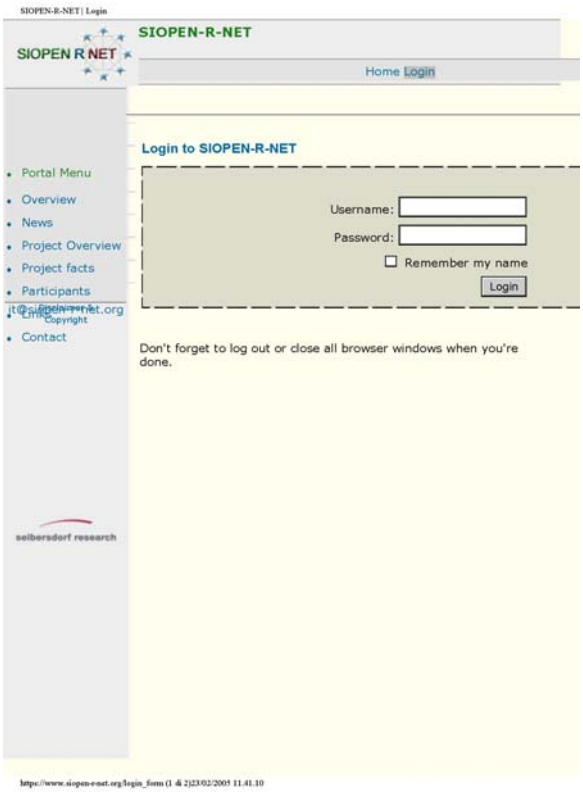
1. Please use (and bookmark) the following links to access the new

a. SIOPEN-R-NET system: https://www.siopen-r-net.or
b. the corresponding SIOPEN-R-NET test site: https://test.siopen-r-net.org

*The test site is equivalent in functionality to the main site except that it only contains data for learning and testing purposes. Please use this test site to get yourself familiar with the system without the danger of causing any harm. An additional "*** TEST ***" on top of the site indicates that it is the test site which you have on the screen. Initially, all user accounts from the main site (usernames and passwords) are valid for the test site as well. (The sites will have different passwords, once you change your password in one of them.)*

2. Login:

a. All personalized accounts remain valid. This is



the case for study physicians, study assistants, statisticians, and subcommitee members. That means that your username and password can be used to login to the new system as well.

b. All non-personalised accounts are no longer valid. That means, if you previously had only an account of the type "NC-XX" or "NA-XX" (XX = your country's code), you now have a new, personalized, account. This is the case for national coordinators and national assistants who did not have an additional account as study physician at the time of the migration.

3. Basic help: Immediately after successful login you will encounter the so called “personal desktop”.
This desktop informs you about new internal messages, a system similar to e-mail but with a recipient list limited to the members of the network.
The main navigation items are now on top of that personal desktop. The rightmost item is a link to the Help section, where you can find the links to manuals for:

a. Use of the registry (How to register a new patient, enter AMED data, submit study eligibility criteria and final enrolment into a study)
b. User guides for the "new" HR-NBL1 trial (and then also the LNESG2 trial)

Since the latter also explains the general principles of the new SIOPEN-R-NET web system, it is worth reading not

only for National Coordinators and Study Physicians/Assistants but also for members and chairs of the subcommittees.
There are additional user manuals available for the sub-studies which are located in the specific help sections of the respective sub-studies.

4. Changes to workflow:

a. The "old" High Risk study system only allowed for saving of data to make your entries permanent. This did not indicate whether a form was finalized or just filled with an initial set of data (and many data queries were sent out to verify if datasets were indeed complete). The new system now allows you to also "Sign" a form, indicating finalisation of data entry. Please try to use the new functionality, but do so only if you are sure that everything has already been filled in. The system will also check for completeness of all mandatory fields and instruct you accordingly. Once signed, no changes can be made any more. In case you have signed a form too early, however, the IT support will be able to "unSign" it upon request.

b. The current implementation of the rights management allows only the last user to make changes to a given form. In the "old" High Risk study however, a physician could make changes to a form where an assistant made initial entries. This was especially important in the case of the Randomisation forms. Since there is not yet a final decision on the distribution of rights and the ability to transfer rights on forms, we urge physicians to fill out these Randomisation forms by themselves or allow their assistants to perform the randomisation only with their explicit consent.

If there is the need to make changes to a form, where the user who saved it the last time is no longer available, send a request for a rights transfer to us.

5. Users and Institutions Management. National coordinators/assistants had the ability to create new institutes and users. This is not yet available in the new system. If the need arises to create a new institute/user, we will do this upon your request. Please contact us by e-mail and include the following information:

i. name, title
ii. e-mail address (absolutely mandatory - since the new account data will be sent to this address!)
iii. institute (if not already existing, we will send you a list of the required data)
iv. optional phone numbers

The data of the new account (username and password) will be sent directly to the e-mail address of the new user!

6. Support and user request handling. In case of any problems, questions, and suggestions please use the following communication channels:
E-mail: it@siopen-r-net.org (use the link on the bottom of the left side menu)
Tel: +43 316 586 570 - 33
Fax: +43 316 586 570 - 12

We are well aware that there will be some initial issues and small bugs related to the new SIOPEN-R-NET IT infrastructure. We will do our best to resolve these issues as quickly as possible, but with the given complexity of the system, such fixes can no longer be done on an ad hoc basis but can be provided only in the course of routinely scheduled upgrades after careful testing every couple of weeks.

As always, we look forward to receiving your comments and suggestions since those are the basis for all improvements that we can possibly make.

Surgery

Sub-Committee Report

MEMBERS

Keith Holmes (chair), Giovanni Cecchetto, Antonino Rizzo, Ernst Horcher, Tom Monclair, Ivo de Wever, Antonio Gentil Martins, Carl-Magnus Kullendorff, Adam Bysiek, Leopoldo Martinez, Sabine Sarnacki.

Last meeting Krakow, 22 October 2004
SAE and Database Review, June 2005
Next meeting Vancouver, September 2005

National representative met in Cracow with the following objectives:

- to review serious adverse events;
- to review protocol compliance;
- to monitor and contribute to protocol development;
- to promote uniformity of surgical approach and 'standard operating procedures';
- to develop future strategies.

Serious adverse events.

High-risk study – 211 operations.

Mortality: The mortality rate is small enough to allow detailed individual patient discussion and is less than 1%. The committee extended its sympathies to the families and team concerned and did not find any reason to criticise patient management.

Two patients died as a result of surgical complications since the start of the study. Each event was discussed with the team involved. Both patients had extremely difficult tumours and were cared for by experienced surgeons. One tumour involved the coeliac axis and its removal was followed by hepatic necrosis, the second involved both renal arteries and removal was followed by loss of both kidneys.

There have not been any deaths in 2004.

The committee felt that this mortality rate was as low as anticipated and was not a reason to alter the protocol.

Morbidity: A total of 13 'events' were reported, (overall <7%). Two of the events are included above. Of the remaining 11, six involved serious bleeding. Five involved renal insufficiency, most of whom also suffered from serious bleeding. The other events did not interfere with subsequent treatment.

Nephrectomy: While not categorised as a serious adverse event this issue prompted a lot of discussion. The incidence was 7%, 14 in 211 operations. The key points of the discussion and recommendations are outlined in the report of the Joint Meeting with Radiotherapy and Nuclear Medicine

Serious adverse advent reporting: There are two elements to this issue: one is clinical and the other is an obligation to the European Union. There are events the severity of which is beyond question. There are others, which have little clinical relevance but are reported under the category of 'damage to other organs'. The sub committee recommended that all events are reported to the Chair who would take responsibility for determining clinical importance and reporting to the EU. This issue will be discussed further with Ruth Ladenstein.

Protocol compliance

In spite of the intensity of the induction protocol, most operation could be scheduled at the correct time. Surgeons felt that the best way to ensure this was that they be involved in the evolution of patient progress from the beginning of treatment. This is particularly important if a patient is transferred from one institution to another for operation. It is permissible to delay operation until after myelo-ablative chemotherapy and radiotherapy in exceptional circumstances but the sub-committee thought it unlikely that operation would be rendered less hazardous.

Protocol development. LNESG 2: The new protocol was accepted. The surgical guidelines have been 'softened' to allow operation in a situation where strict adherence to LNESG 1 would have contra-indicated surgery and indicated chemotherapy, arguably more toxic than operation.

The variables tumour size and fragility, did not emerge as independent variables in LNESG 1 and have not been included as risk factors. These factors were usually found in association with more objective criteria such as vascular encasement.

Standard Operating Procedure. The difficulty of defining this for surgery was again acknowledged. The operative video has been approved as an illustration of surgical strategies and will be available on the SIOPEN-R-NET website in the near future.

Summary. The new protocol did not present insurmountable challenges to the surgeon. Surgical morbidity and mortality were low. Joint meeting with Radiotherapy and Nuclear medicine.

Renal preservation. All three elements of the high risk protocol: chemotherapy, surgery and radiotherapy cause renal damage. The delegates attempted to rank therapy in order of toxicity and efficacy as treatment progressed through the protocol. A synopsis of the aims of the different stages in the protocol follows. The goal of induction chemotherapy is to eradicate metastatic disease and reduce tumour volume to increase the efficacy of operation. The goal of operation is complete tumour excision. The goal of myelo-ablative chemotherapy (MAT) is to eradicate minimal residual disease. The goal of radiotherapy is to eradicate minimal residual disease in the primary site. There are no data on the incidence of late renal loss following operation around the renal pedicle but surgeons have encountered this. Survival is not enhanced by nephrectomy as a planned procedure to facilitate more complete tumour excision, nevertheless this strategy has been accepted. There are no data to indicate a maximum tumour volume which can be eradicated by radiotherapy but the consensus was that this should be as small as possible. MAT presents a huge physiological stress to the patient and good renal function is important for recovery.

Although radiation will impair renal function this effect is not manifest for three to five years after treatment.

Consensus: After trying to balance the disparate risks of the different therapies the committee felt that preservation of renal function for the period of MAT was paramount.

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

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

Future strategies

There was no consensus on the introduction of functional renal studies as routine. The UK groups will develop a pilot study involving functional studies before and after operation and radiotherapy.

Bone Marrow

Sub-Committee Report

MEMBERS
Klaus Beiske (Chair), Katrien Swerts (Secretary), Peter Ambros, Walentyna Balwierz, José M Fernandez, Nicole Gross, Marta Jeison, Jerzy Klijanienko, Dyanne Rampling, Roswitha Schumacher-Kuckelkorn, Angela Sementa, Ales Vicha

Last meeting Paris, Janury 21-22, 2005
Next meeting Paris, April 29-30, 2005

Detection of disseminated neuroblastoma cells using immunocytology/cytogenetics

The demonstration of disseminated tumor cells in bone marrow (BM) or peripheral blood (PB) is important for clinical staging and risk assessment at diagnosis and for monitoring therapeutic response during treatment. In addition, screening of autologous stem cell preparations is crucial since the reinfusion of contaminated stem cell products could lead to systemic recurrence.
According to the International Neuroblastoma Staging System, conventional cytology is still the only accepted technique for the detection of disseminated NB cells. However, the sensitivity and specificity of this approach is limited. Therefore, the development of more sensitive and specific detection methods is indispensable.
In connection with the SIOPEN HR-NB protocol, the Bone Marrow Subcommittee (BMSC) developed, optimized and standardized an immunocytochemical assay based on the detection of the neuroblastoma specific GD2 disialoganglioside. This antigen is highly and consistently expressed in neuroectodermal tumors and is not expressed by normal BM or PB cells.
As long as the members of the BMSC stained their slides using different immunocytochemical staining methods and analyzed their results according to individual morphological criteria, considerable discrepancies were observed. The evaluation of stained samples by the whole group using a multiheaded microscope, clearly demonstrated the urgent need for developing a standardized immunocytochemical staining protocol and introducing morphological and immunocytological criteria. Consequently, the BMSC agreed upon one staining method and formulated morphological and immunocytological inclusion and exclusion criteria for the interpretation of the results. Only cells with a round nucleus, often, but not always larger than that of small lymphocytes, a granular chromatin and a limited amount of cytoplasm are considered positive. In addition, a strong, deep red staining localized to the entire cell membrane and cytoplasm must be present.
However, when applying these criteria, the BMSC discovered that a small proportion of immunocytochemically stained cells could not unequivocally be classified as positive (i.e. NB cells) because they did not fulfill all postulated morphological and immunocytological criteria. Therefore, it was decided to categorize all immunocytochemically stained cells into three groups: Criteria Positive Cells (CPC's) fulfilling all postulated criteria, Not Convincingly Interpretable cells (NCIC's) displaying some but not all inclusion criteria and Negative Cells (NC's) which, in spite of their staining, were identified as non-malignant hematopoietic cells.
The BMSC members also decided to introduce a workflow including two additional analytical steps. Firstly, samples with 1 to 10 CPC's and samples containing only NCIC's are simultaneously reviewed by all BMSC members. Secondly, if no consensus is reached, the genetic profile of the doubtful cells is checked by automatic immunofluorescence plus FISH to disclose the identity of these cells. If the genetic aberrations in the doubtful cells correspond to those found in the primary tumor, the cells are called FISH positive cells (FPC). Finally, the morphological and immunocytological features of these FPC's are carefully studied in order to refine the standardized evaluation criteria.
The standardized staining protocol, the morphological and immunocytological criteria and the work flow were evaluated during multicenter quality control rounds, organized among the nine members of the BMSC. The concordance between the different observers, with regard to the staining and the evaluation of the immunocytochemical results was assessed. After standardization, a significant decrease in the number of discordant results was reported. In addition, the range between the highest and the lowest reported result was reduced by half and discordant results were only found in samples with less than 10 CPC's per 1x106 mononuclear cells.
Based on our results, it is evident that the standardization of the immunocytochemical staining method, the formulation of morphological and immunocytological criteria and the design of the work flow resulted in a higher reproducibility, sensitivity and specificity.
Very preliminary results of the comparison between cytomorphological and immunocytochemical results of BM analyses in HR patients show a higher sensitivity of the immunocytochemical assay at the end of induction when the detection of a few tumor cells is concerned. The future analysis of larger numbers of patients enrolled in the HR study will show weather reproducible tumor cell quantification, as provided by this method, will be of prognostic significance.

Therefore, the standardized immunocytochemical assay is regarded as an appropriate method for the reliable detection of disseminated NB cells.

MEMBERS

Sandro Dallorso (Chair), Volker Witt , Josè Fernandez Navarro, Justyna Kanold, Jonas Abrahamsson, Yosi Kapelushnik, Gergerly Kriván, Bart Vandekerckhove, Nuno Miranda, Alicja Chybicka, Henrik Schroeder, Arcangelo Prete

Last meeting Krakow, October 21-23, 2004

Next meeting, QUALCOR meeting (Manual for PBSC collection)

National members held their SC meeting in Krakow with the following agenda:

- questionnaire on apheretic attitudes: results presented by Volker Witt
- UK experience in PBSC collection in the HR-NBL-1 Study context by D. Leverett
- Tumor contamination and purging: Clermont-Ferrand by Justyna Kanold
- Joint meeting with the RT-PCR Sub-Committee.

Summary of questionnaire's results

- 83% of centres have a paediatric program with upper limitation range from 15 to 21 years
- in the 88% of centres the supervisor of the apheretic procedure is the paediatrician and as consequence the apheresis are performed mainly in the oncological ward (78%)
- only 6 centres have an apheresis program specific for children
- ten centres have national or international accreditations; for the majority of the remaining this is in progress
- 50% of centres manipulate the apheretic product "at home", the others need the help of a third party institution
- no doubts that rh-G-CSF is the widely used cytokine for mobilisation and that the rising value of circulating CD34+ cells is the best method for starting collection
- several methods of CD34+ cell coutn are used
- COBE Spectra and Fenwall CS 3000, and the standard volume apheresis are the procedures more often used
- clotting risk and hypocalcemia are uniformly treated
- the arterial access is rarely used
- great differences have been detected in: use of sedation and/or anaesthesia during the procedure, MRD evaluation, CD34+ cells count, role and techniques of purging.

UK experience

Data regarding 21 deeply analysed patients have been shown, 16 of them performed the PBSC mobilisation. Five children did not undergo mobilisation for persistent marrow infiltration (4 cases), or fungal infection (1 case).

Out of the 16 patients receiving filgrastim for mobilisation, 3 failures occurred (suspected fungal infections and unknown causes), in 1 case very few CD34+ cells were collected. Twelve were able to collect a sufficient number of CD34+ cells (including 1 child who did not receive the 8th COJEC cycle). In all children the successful harvest (range 2.7-11.1, median 3.8 CD34 x 10^6/kg) was performed in a very narrow window (days 79 and 82), it has always been heralded by a CD34+ raise at least above 10/mL.

Clermont-Ferrand experience

Justyna Kanold presented the Clermont-Ferrand experience in PBSC collection, the greatest in France for "small" children (weight <15 kg, age < 2 yrs).

Along with a short review of the literature (Kushner, Matthay, Kawano, Handgretinger, Grupp) and after a short summary of their activity in MRD detection (measured by RT-PCR for TH-mRNA), she presented historical and updated results in clinical application of CD34 selection as purging method, using CellPro and Miltenyi devices.

The conclusions were:

- CD34+ selection of G-CSF mobilized and collected in 1 or 2 leukaphereses (4 folds blood volume processed) PBSC provide sufficient number of CD34+ cells (>10 x 10^6/kg) to ensure normal hematopoietic reconstitution (neutrophils in 11 and platelets in 20 days, respectively)
- immunomagnetic CD34+ selection of PBSC provide 2.6 log depletion of neuroblastoma cells
- clinical significance of infusion or no-infusion of tumor cells contaminating the graft is unknown.

Some data (reported in full in BMT 2002) regarding the ex-vivo CD34+ expansion as purging method have been discussed. The final part of the communication was devoted to the clinical value of quantitative real-time PCR for TH-mRNA in patients with advanced stage 4 neuroblastoma. Thirty children entered the study, 22 performed an ABMT with a median follow-up of 47 mos (range. 11-90). BM, blood, PBSC and PB CD34+ samples were prospectively collected and studied at various points, even after therapy completion. Some conclusions can be drawn:

Patients at higher risk of disease progression are those

- with increased tumor cell content in BM and PB at diagnosis
- with substantial amount of residual disease in BM and stem cell products after initial chemotherapy:

This method is also useful to assess the efficiency of tumor cell depletion in autologous harvests and additionaly enables to monitor regression or progression of the disease.

Joint Meeting with RT-PCR Sub-Committee

The Stem Cell SC's 2005 goal is to better understand the activities going on in Europe concerning purging and to investigate if there is a role for a purging program in children with poor risk NBL, for which the RT-PCR SC (and Bone Marrow SC) are natural partners. Objective of this first informal Joint Meeting is to explore if there are common areas of activity in the field of MRD. Following a brief summary of the purging pros and cons, several papers have documented that harvest can be quite often infiltrated, contamination can contribute to relapse and that circulating cells are clonogenic. On the other hand, it has to be notaed that these data are mostly about bone marrow, they go back to 10 years, and that we are not yet able (till the end of HR-NBL-1 Study) to know the rate of PBSC contamination after COJEC. Some concerns raised about the No. of CD34+ that can be collected to allow a purging procedure (the PBSC collection at the end of COJEC is not particularly "rich"). In addition, the best detection method is not defined (negative or positive selection, combined methods, ex-vivo expansion, others) and the procedure is expensive and not widely applicable.

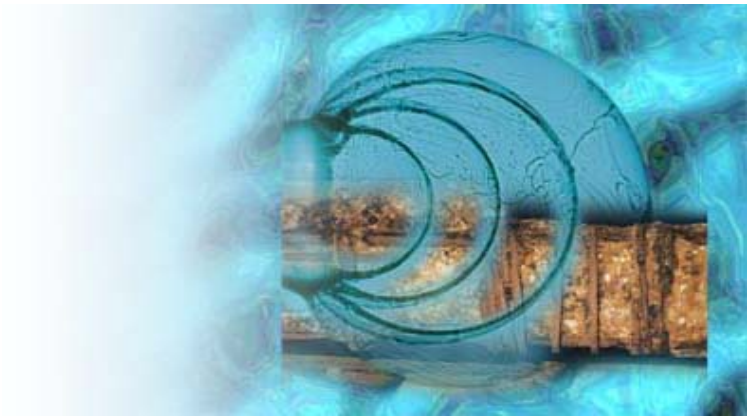
Considering that purging is not allowed in the HR-NBL-1 Study but that approxamately 1/4of children completing COJEC are not eligible for R1 due to persistent marrow involvement, purging can be taken into account for patients entering a rescue Protocol (such as the recently proposed TVD). Propedeutic steps are: to define a validated and approved method for MRD detection, to choose the purging method, and to identify the persons interested in this program.

Objectives for year 2005 !!!

The SC is working on the SOP Manual for PBSC collection in children. Authors of several chapters have been identified, other contributors are requested and welcome. The deadline for the first preliminary draft is end of March 2005. The interest regarding the purging strategy is quite modest. This could mean that this tool is not considered of interest in the treatment of high-risk neruoblastoma. The SC will try to revitalize the discussion before stating that a purgino program is devoid of interest in the European conext.

Pharmacology

Sub-Committee Report



MEMBERS

Gareth Veal and Gilles Vassal (Chairs), Claude Ardiet, Joachim Boos, Pierre Canal, Etienne Chatelut, Moustapha Hassan, Paolo Montaldo, Jose Esteban Peris, Riccardo Riccardi, Brigitte Tranchand

Report and call for the establishment of a European Clinical Pharmacology Laboratory Network

Background

The HR-NBL-1/ESIOP protocol incorporates studies to investigate the pharmacokinetics of busulphan, melphalan, carboplatin and etoposide in patients being treated on randomized high dose myeloablative therapy. These studies have been designed to build on previously obtained data to define the therapeutic window of systemic exposures (AUC) of these high-dose chemotherapy regimens, by studying the relationships between AUC and acute toxicity. In addition, variations in the exposures to these drugs in various dosing groups specified in this protocol, including dosage adjusted based on renal function and body size, will be determined. It is hoped that data obtained from these studies will allow us to establish the use of drug monitoring and dose adjustment, in order to reduce inter- and intra-patient variability in the systemic exposure of these anticancer drugs. Clearly this is an important area, with the incidence of toxicity a significant cause for concern when treating patients with high-dose myeloablative therapy on this protocol. This will require the organisation of a pharmacological laboratory network able to assay drug plasma levels within two days of the first dose, in order to be able to adjust the dose before the end of the high dose treatment in future prospective studies.

Patient Recruitment

There are 24 centres currently registered as eligible to participate in the pharmacology group studies (11 United Kingdom, 4 France, 3 Belgium, 2 Poland, 1 Austria, 1 Spain, 1 Italy, 1 Israel) and a total of 22 patients have been studied to date. Eleven of these patients were studied on the busulphan/melphalan arm of the R1 randomization and eleven during treatment with carboplatin/etoposide/melphalan. Samples are being analysed for carboplatin, etoposide and melphalan at the Northern Institute for Cancer Research (NICR) in Newcastle, UK and preliminary data have been obtained on this initial patient cohort. Busulphan analysis of samples obtained to date, in addition to future samples, will be carried out at the Institut Gustave Roussy, Villejuif, France. Much time and effort have been expended in establishing a framework for carrying out this work and an improved rate of recruitment of patients is now required in order to ensure that meaningful data is generated from these important studies. We strongly encourage centres to consider recruiting patients to the pharmacology part of this study whenever possible.

European Pharmacology Laboratory Network

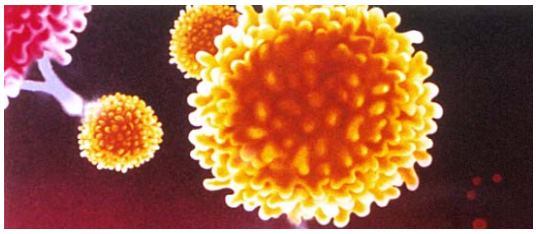
When the SIOP Neuroblastoma Pharmacology Subcommittee was instigated, a major goal was to establish a network of laboratories to allow the future monitoring of high-dose chemotherapy in children being treated on European neuroblastoma trials. To date, work has focused on those laboratories directly involved in the analysis of samples being collected as defined in the HR-NBL-1/ESIOP protocol. We are now in a position to expand this area to incorporate additional laboratories with the analytical facilities to measure drug levels in clinical samples obtained from patients on European Neuroblastoma studies.

The following key advances need to be made in 2005 :-

1. The identification of laboratories and individuals wishing to be involved in the analysis of the following drugs in future European clinical trials: Busulphan, Carboplatin, Etoposide, Melphalan.
2. Cross-validation of assays between the laboratories identified in (1) above. Blind samples will be sent from an independent laboratory for analysis at each of the reference laboratories defined for a particular drug.
3. The organization of a QC meeting to discuss the results from laboratory cross-validation, preliminary results from the HR-NBL-1/ESIOP pharmacology study and the organization of future clinical pharmacology studies.

Centre Involvement

Could any centres interested in recruiting patients to the pharmacology studies associated with MAT treatment on the HR-NBL-1/SIOP study please contact Gareth Veal (G.J.Veal@newcastle.ac.uk) to discuss the practicalities involved. In addition, we are now calling for centres to be involved in expansion of the clinical pharmacology laboratory research network as described above. Please contact Gareth Veal (G.J.Veal@newcastle.ac.uk) or Gilles Vassal (gvassal@igr.fr) to discuss this exciting opportunity.



Last meeting Krakow, Joint meeting with Novel Therapeutic Strategies Sub-Committee, October 22-23, 2004

The main focus of the immunotherapy subcommittee was placed on three major topics.

1. Completion of the ch14.18 antibody production

After a long odyssee the production of 180 grams of ch14.18/CHO antibody was accomplished by Polymun. Since the last newsletter, there was one meeting with Polymune in October 2005 just before the annual Neuroblastoma meeting in Krakow in order to review all the data from recloning of ch14.18 antibody in CHO cells. The conclusion of this meeting was to proceed with the large scale production. The large scale production process went very well and the purification of 180 grams ch14.18/CHO from the bulk harvest was finished by the end of December 2004, which is enough material for the high risk study. After filling of the purified protein in January 2005, we are now waiting for test results of the "end of production" cells, sterility and a qualitiy control run for the final product.

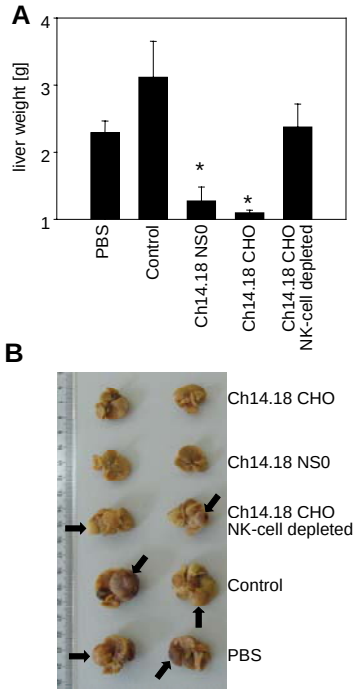


Figure 1. Anti-neuroblastoma activity of ch14.18 preparations against established experimental liver metastasis. Mice (n=6/group) were inoculated with 1x105 NXS2 cells by i.v. injection. Treatment was initiated 3 days after tumor cell inoculation and consisted of 5 daily i.v. injections of 300 µg ch14.18/CHO, ch14.18/NSO and anti-CD20 antibody. In one group, mice were depleted of NK-cells as detailed in materials and methods. Mice were sacrificed 28 days after tumor cell inoculation.

(A) The liver weight was determined on fresh specimen. The y axis starts at 1g corresponding to the average normal liver weight. The differences in average liver weights between experimental groups treated with ch14.18/CHO and ch14.18/NSO and all control groups (PBS, ch14.18/CHO with NK cell depletion and anti-CD20) was statistically significant (* p< 0.05).
(B) Two representative liver specimen of each experimental group (n=6) are shown. Arrows indicate the location of macroscopic liver metastases.

2. Publication of the characterization of ch14.18/CHO antibody

One important aspect during the production process on ch14.18 antibody after recloning in CHO cells was to compare the biofunction of this antibody to previous batches produced in SP2/0 cells and NS0 cells used in clinical trial. This study was very important, because it provides the necessary data in front of ethical committees and regulatory authorities in order to get the approval to use it in clinical trials.

For this purpose, we demonstrate identical binding of ch14.18/CHO to the nominal antigen disialoganglioside GD2 in vitro compared to ch14.18/SP2/0 and ch14.18/ NS0. Binding was GD2 specific, since all precursor- and metabolite-gangliosides of GD2 tested were not recognized by ch14.18/CHO. Second, the functional properties of ch14.18/CHO were determined in complement-dependent cytotoxicity (CDC) and antibody dependent cellular cytotoxicity (ADCC) reactions against GD2 positive neuroectodermal tumor cell lines in vitro. There was no difference in CDC mediated specific tumor cell lysis among the three different ch14.18 antibody preparations. Interestingly, ch14.18/CHO showed superior ADCC activity at low antibody concentrations. Third, the efficacy of ch14.18/CHO was evaluated in the NXS2 neuroblastoma model in vivo. Importantly, the ch14.18/CHO preparation was effective in suppression of experimental liver meatastasis in this model (Figure 1). In vivo depletion of NK cells completely abrogated this effect, suggesting that the mechanism involved in the ch14.18/CHO induced anti-neuroblastoma effect is mediated by NK-dependent ADCC. The results of this study are accepted for publication in Molecular Immunology.

3. Prepartation of a Protocol for a Phase I bridging study for the new ch14.18/CHO antibody

Although the antibody-gene transfer into CHO and SP2/0 was done with exactly the same plasmid assuring an identical protein sequence, changes in the glycosylation of the final protein product may occur since the glycosylation pattern varies between different production cell lines. Glycosylation is important for the immunological effector function of the antibody and the pharmacokinetics in patients. Therefore, this change is considered to be a major change in production requiring the reassessment of the new product in a Phase I bridging study.

The primary objective of this trial is the re-evaluation of toxicity and pharmakokinetics of the new ch14.18/CHO antibody. This is ultimately followed by the secondary objective including the determination of immunostimulation in patients receiving ch14.18/CHO therapy. This involves particularly the determination of activation of immune effector cells and complement during and after application of ch14.18/CHO. Subsequently, we will evaluate the clinical effect of this treatment on the course of the disease.

The nature of this phase I trial is a bridging study for a medicinal product subjected to a major change in production according to the guidelines provided by the "Committee for Proprietary Medicinal Products" (CPMP) of the "European Agnecy for the Evaluation of Medicinal Products (EMEA) (Document Number CPMP/BWP/3207/00). The protocol for this study was prepared and reviewed by the board and all participating centers and will be finalized for approval by ethical committees.

Pathology

MEMBERS
Emanuele S.G. D'Amore and Michel Peuchmaur (Chairs), Claudio Gambini, , Gabriele Amman, Samuel Navarro, Klaus Beiske, Catherine Cullinane

Last meeting Valencia, October 2004
Next meeting Padova, May 2005

In 2004 the ESIOP continued its teaching activity, concluded the study on the prognostic value of INPC classification in localized stages II neuroblastomas, and organized a program for a new round of case review focused on the infant protocol.

The educational activity, which began in November 2003 in Paris, France, was continued in Vien, Austria, in April 2004. In fact the classification of neuroblastic tumors is complex and has changed over time, mainly with a profound redefinition of the Nodular ganglioneuroblastoma; in addition there is still a problem of interobserver agreement, particularly in the evaluation of the mitosis - kariorrhectic index (MKI), which is an important parameter to evaluate in order to construct the histoprognostic categorization. For this reason the ESIOP pathology panel has undertaken a teaching programs mainly addressed to already experienced pediatric pathologists who wish to refine their skill through confrontation and discussion on a multiheaded microscope of difficult cases. An update on recent advanced in neuroblastic tumor pathology was also provided by a 2 hours introductory course held by members of the pathology panel.

A second meeting took place in Valencia, Spain, in October 2004. Two days were spent to finalize a paper (submitted for publication to the JCO) reporting the results of a study on the prognostic value of the International Neuroblastoma Pathology Commettee (INPC) classification in localized resectable peripheral neuroblastic tumors. 121 patients with stage 2A and 2B neuroblastic tumors without N-myc amplification were reviewed. 116 were evaluable and assigned to a favorable (91 cases) or unfavorable (25 cases) category. The 60 month survival rate was 97.8% in favorable cases compared to 73.8% in unfavorable cases (p=0.0001). EFS analysis showed that a 60 month relapse rate of 13.2% and 32% in favorable and unfavorable cases (p<0.025), respectively. This European study shows for the first time that INPC prognostic categorization has a significant impact on outcome prediction in stage 2 localised peripheral neuroblastic tumors.

Finally, next pathology meeting will be held next April either in Italy, either in Padova or in Genova. A blind review of cases enrolled in the infant protocol, including non resectable stages, is planned. The review will be performed blindly by all the participants at a multiheaded microscope: after individual evaluation of each case, the discussion will be open to check for discrepancies and to find, if possible, a consensus diagnosis. In addition one session will be dedicated to the proposals of new study projects by each pathologists. One study has already been preliminarly discussed and is currently under evaluation; it concerns a simulation, performed on wholly resected specimens after image digitalization of all the slides, to determine the minimun amount of material that is necessary for a correct histoprognostic categorization.

Ana De Lacerda
Istituto de Oncologia, Lisboa

Does platelet count count to start 13-cisRA therapy?

A 3 ½ year-old girl was diagnosed with stage 4 neuroblastoma in August 2003 (abdominal primary and invaded bone marrow). The tumour was aneuploid and did not show *MYCN* amplification or 1p deletion. At presentation, LDH was 2440 IU/L, NSE level was 1070 ng/mL and ferritin was 380 ng/mL.
She was treated as a pilot patient according to the HR-NBL-1 Protocol. After completion of induction phase, tumour resecation was carried out, together with right nephrectomy.
Histology revealed a poorly differentiated ganglioneuroblastoma, regional lymph nodes were infiltrated. Due to this and delay in admission to the ABMT Unit, she was further treated with 3 CADO cycles.

Prior to ABMT (BuMel, May 2004) thorough re-evaluation showed complete remission of disease. She completed treatment with local RT, according to protocol guidelines, in August 2004. Again, disease evaluation was completely negative. When all the criteria required by the protocol to start 13-cisRA were fulfilled (Table 1), she was started on this drug. To note, the only abnormal finding was the low platelet count. Five days later she came in for a routine visit, when she suddenly fell sick, vomited and complained of headache. Laboratory evaluation was similar to the previous one (see Table 1); physical examination showed retinal haemorrhages. There was no history or external sign of trauma. A cranial CT scan revealed two extra-axial left sided masses (Fig.1), suggesting neuroblastoma metastases. 13-cisRA was discontinued and treatment with dexamethasone was instituted with immediate clinical improvement. A week later a MRI (Fig.2) suggested that the lesions could be haemorrhagic and dexamethasone was therefore discontinued. Meanwhile, disease re-evaluation was again negative. Follow-up MRI, 2 months later, was completely normal. In January 2005 this girl had a normal laboratory evaluation and was doing very well.
This case raises the issue of whether complete recovery of the CBC (namely platelet count) should be introduced as another criteria for starting differentiation therapy with 13-cisRA. May it be that this compound can cause pseudo-tumor like intracranial hypertension, as ATRA does, and therefore we should be more careful on avoiding adding two factors that may cause a potential life-threatening event to our patients? However, to date there are no descriptions of ATRA causing hemorrhagic events such as the ones described here.

TABLE 1

	Sept 9, 2004 (start of 13-cisRA)	Sept 14, 2004 (event)
Hb (g/dL)	8.8	9.1
WBC (x 10^3/µL)	2.41	3.1
ANC (%)	42	50
Platelets (x 10^3/µL)	50	67
Creatinine (mg/dL)	0.6	0.5
LDH (UI/L)	516	460
Sodium (mmol/L)	-	141
PT (%)	-	98.8
APTT (seconds)	-	36.6
Fibrinogen (g/L)	-	2.35

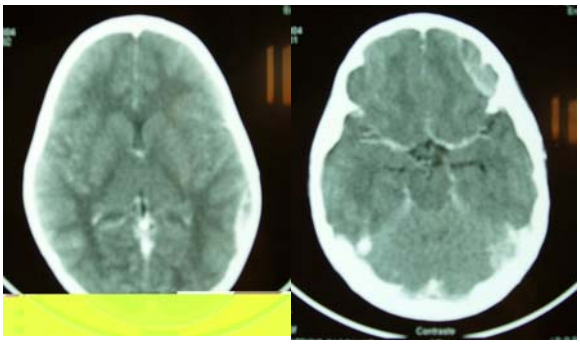


Fig. 1 = CT scan, Sept 14, 2004

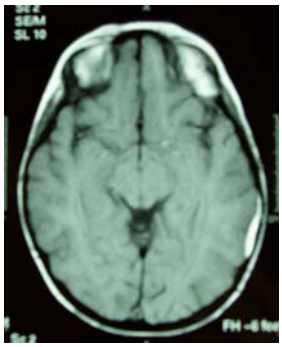


Fig. 2 = MRI Sept 20,2004

8704 Vol. 10, 8704–8719, December 15, 2004 Clinical Cancer Research

Induction of Apoptosis by Flavopiridol in Human Neuroblastoma Cells Is Enhanced under Hypoxia and Associated With *N-myc* Proto-oncogene Down-Regulation

Maura Puppo,[1] Sandra Pastorino,[4] Giovanni Melillo,[3] Annalisa Pezzolo,[2] Luigi Varesio,[1] and Maria Carla Bosco[1]

Conclusions …

Flavopiridol growth -inibitory and apoptotic activity against advanced-stage neuroblastoma is worthy of further investigations

7978 Vol. 10, 7978–7985, December 1, 2004 Clinical Cancer Research

Detection of Neuroblastoma Cells in Bone Marrow and Peripheral Blood by Different Techniques: Accuracy and Relationship with Clinical Features of Patients

Maria Valeria Corrias,[1] Lawrence B. Faulkner,[5] Angela Pistorio,[2] Cristina Rosanda,[3] Francesco Callea,[6] Maria Serena Lo Piccolo,[3] Paola Scaruffi,[6] Cinzia Marchi,[5] Laura Lacitignola,[5] Marzia Occhino,[1] Claudio Gambini,[4] Gian Paolo Tonini,[7] Riccardo Haupt,[2] Bruno De Bernardi,[3] Vito Pistoia,[1] and Alberto Garaventa[3]

Conclusions …

Immunocytochemistry may represent a useful adjunct to conventional morphological techniques, especially in view of its potential prognostic role in patients with localised disease. Longitudinal multicenter studies are warrented to definitively established the clinical usefulness of TH – RT PCR.

Results of a phase I study utilizing monocyte-derived dendritic cells pulsed with tumor RNA in children with stage 4 neuroblastoma

Denise A. Caruso, Lisa M. Orme, Gerlinda M. Amor, Alana M. Neale, Fiona J. Radcliff, Peter Downie, Mimi L. K. Tang, David M. Ashley

Conclusions …

Monocyte derived dendritic cells (RNA) vaccine were both safe and feasible in stage 4 neuroblastoma patients. Humoral responses to tumor were detected, although remained immunosuppressed at the time of administration, limiting efficacy.

E-SIOP Neuroblastoma Newsletter #6 has been edited by Sara Calmanti and was supported by the Italian Neuroblastoma Association