# S I O P E N

## Newsletter #10

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# Editorial

## *Message from the Chairman*

Since the last newsletter a number of exciting things have happened in the SIOPEN group. Firstly we have formed a SIOPEN Association, which became a legal entity in February of this year. Membership of this association will be available online and this process is gradually going live. The establishment of the association will enable the SIOPEN group to securely accept and keep track of funds, coming in from different member countries and charities, to support the work of the group. The SIOPEN treasurer is Dr Isaac Yaniv who will work closely with the myself and the SIOPEN association administrator based at the CCRI in Vienna monitoring the finances, regularly updating the SIOPEN executive and annually reporting to the members.

It was decided at the SIOPEN Board meeting in Brussels that while awaiting further charity and grant funding to support the clinical trial work of the group that each country would be asked to give financially. The money requested would be to support the entry into open clinical trials, HR NBL 1 and TVD, of their own national patient cohort and to support the development of the database for forthcoming trials particularly LINES and AYA. The statisticians help was requested to work out a fair approach to how the money was divided per country, taking into account large and small populations as well as numbers of patients entered onto trial per country. This distribution data was reviewed and agreed by the executive committee, at the February meeting in London.

Secondly the progress on the analysis of the 14,18 chimeric antiGD2 antibody up to EMEA Phase III trial testing has been excellent. The stability, sterility, retroviral testing and animal modelling are complete and have shown the appropriate level for approval by regulatory authorities. All of this data is being collated onto CD's to be sent out to national Co-ordinators for approval in their countries. The amendments to the HR NBL 1 trial are being prepared so that the trial can be completed in a period of 3 to 4 years reaching a successful end point for R1 as well as including the use of the antibody. The exact form of the antibody question is under revision taking into consideration the latest data from the COG group.

Many of the sub committees and trial writing committees have been active since our meeting in Lausanne and a number of publications are either out or close to publication. I look forward to a productive annual meeting in Paris from April 1st to 3rd.

With warm wishes to all our members,

Ruth Ladenstein
http://www.kinderkrebsforschung.at/

# Clinical Trials' Corner

## EUNB

*Treatment of children over the age of one year with unresectable localised neuroblastoma without MYC-N amplification*

**Janice Kohler**

**Opened (UK): January 2001**
**Closed: February 2007**

**Registered patients by country:**

| Country | Eligible |
|---|---|
| **Austria** | 1 |
| **Belgium** | 5 |
| **Eire** | 1 |
| **France** | 49 |
| **Italy** | 38 |
| **Portugal** | 3 |
| **Norway** | 2 |
| **Sweden** | 2 |
| **Spain** | 35 |
| **U.K.** | 29 |
| **TOTAL** | **165** |

**Relapses:**
There are 30 reported relapses, 9 local and metastatic to bone or bone marrow, and two to lymph nodes. The rest (19) were all at the primary site.

**Deaths:**
There have been 14 deaths. One was treatment related (surgery). The rest were following relapse. There have been no deaths in children less than 18 months at diagnosis, nor in those in the favourable INPC group.

**Serious Adverse Events:**
There has been one death (surgical)
There are 6 other SAEs, mainly septic or gastro-intestinal

**Analysis:**
Currently the OS for the whole group is 83% and EFS 75% at 4 years. There is still a great deal of missing data, so the curves are unstable beyond 4 years. Children less than 18 months have the best survival (100% OS and only 2 relapses), followed by those aged between 19 and 24 months. EFS and OS for children aged over 24 months is unsatisfactory, especially for those with adverse histological features.

Of 104 patients whose tumours have been centrally reviewed, those classified as favourable by INPC had an EFS and OS of 91% and 100%, whereas those classified as unfavourable have an EFS and OS of 62% and 63%.

Current recommendations are that:
1. Children >18 months of age with poorly/undifferentiated neuroblastoma should receive local radiotherapy at the end of treatment, and 13 cis retinoic acid.
2. Children with unresectable ganglioneuroblastoma (intermixed) should receive no chemotherapy (very good prognosis).
3. Children under the age of 18 months at diagnosis should be treated on the future 'low risk' protocol.

# LNESG2

*Guidedlines for the treatment of patients with localized resectable neuroblastoma and analysis of prognostic factors*

**Maja Beck Popovic**

**Background.**
LNESG2 is the second European study on localized neuroblastoma. In the previous study the presence at diagnosis of high LDH serum level, deletion of 1p and unfavorable histology according to Shimada criteria were apparently associated with a greater propensity to relapse. However, a statistical value was not reached mainly due a great amount of missing data prohibiting justification of immediate treatment of these patients. A decision was taken to treat patients with unfavourable histology more aggressively following relapse.

**Objectives**
To maintain the good results in the cure of localized neuroblastoma without MYCN amplification by surgery alone, to improve surgical morbidity by respecting the presurgical risk evaluation and to define a subgroup of patients at higher risk of relapse.

Primary objective: to expand the information provided by LNESG1 on factors associated with clinical prognosis in localized neuroblastoma, especially preoperative LDH, 1p deletion and histology.
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,*
    - *improving data collection, with particular regard to LDH and 1p deletion*
- to establish a uniform treatment for relapsed patients.

**Update**
Accrual. Patient accrual has started effectively in August 2005 and 195 pts have been registered until October 30th 2008: 131 INSS stage 1, 60 INSS stage 2 and 4 INSS stage 3 patients.

**Data completeness and review**
There are 100% results in the data base for preoperative LDH, 100% for MYCN amplification, 82% for local pathology, 75% for IDRF, 88% for surgery and 44% for surgical outcome. This is much better than in the earlier protocol, but still needs to be improved.
Histology review by members of the pathology subcommittee is completed for all the cases, other biological analysis are performed within the biology group and are masked.
Data on preoperative imaging and imaging at 1 month after operation are being collected locally, but the central review has not started yet.

**Relapses**
There have been 7 relapses until now, 4 stage 1 and 3 stage 2 patients. Among them 2 had a MYCN amplification and 1 unfavorable histology. On the whole, there have been less relapses than estimated statistically based on the LNESG1 experience.

**Future**
Accrual continues for at least for 2 more years, as there are still less INSS stage 2 patients recruited than expected. Data collection has improved with the data query system.

# AYA

*From the Lausanne Meeting*

**Isaac Yaniv**

During the Lausanne meeting, after discussion of the various options within the treatment components, a draft proposal has been discussed.
In the meantime there have been several suggestions made by various members and during the spring COG meeting we plan to work on a proposal for approval.

The outline of the protocol is:

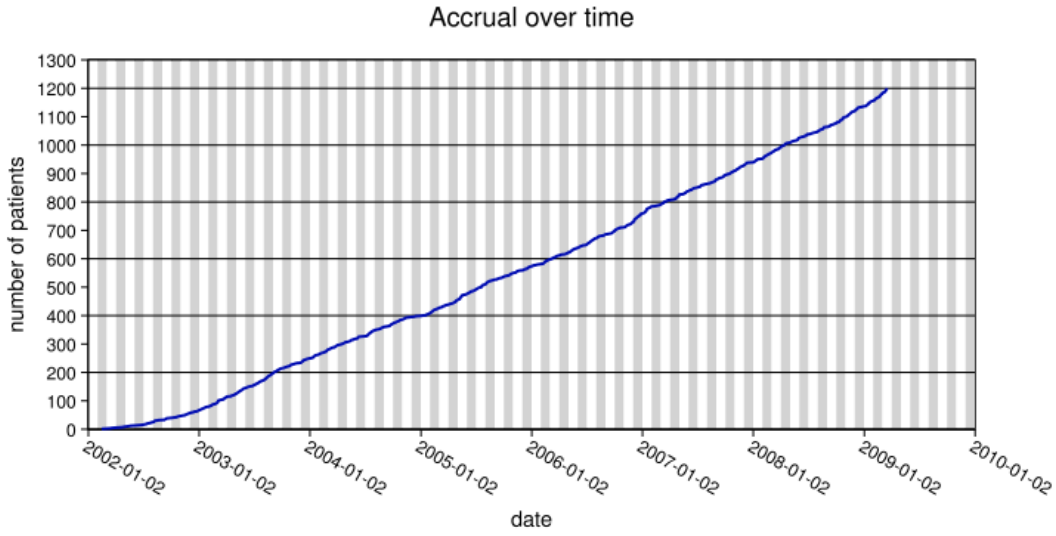
- L1 patients: Surgery and XRT
- L2, M & relapsed L1
- age ≥10y

*Induction with 6 cycles of combination chemotherapy
*Stem cell collection to allow 3 infusions
*Surgery
*1-2 courses of MIBG-TOPO followed by stem cell infusion
*MAT following Bu-Mel conditioning
*Radiotherapy
*Maintenance with Cis-RA and Avastin
*For progressive disease: Phase I-II or Irino- Temodar

- ☒ Two identical protocols will run in parallel in Europe and in the US.
- ☒ The proposal will be submitted to the COG, and upon approval of the concept a follow up meeting will be held in the US.
- ☒ A writing committee will be established including members of Surgery, Radiotherapy and Statistical specialty committees.

## Recruitment

The High-risk neuroblastoma trial is recruiting well **and has now reached a total of 1200 patients.**




### The R0 randomisation

The R0 supportive care question is answered and the article ready for publication. All patients are now getting G-CSF during induction.

### The R1 randomisation

There are currently 468 patients randomised to R1. The R1 question should be answered effectively in 3 to 4 years.

### The R2 randomisation

The antibody question is being revised in the light of new data from the COG high-risk neuroblastoma trial to enable an R2 randomised trial to reach completion together with the closure of the R1 randomisation. The meeting in London in February to discuss the use of the antibody was constructive particularly due to the positive results of the antibody testing which reaches all the required health regulatory requirements. But also statistically, by enlarging the entry criteria into R2, to include all patients who have successfully completed high dose therapy whether randomised or not, we should be able to answer a randomised question with good power with 400 patients.

Ruth Ladenstein

# Committees' corner

# Surgery

## Draft Minutes
## Lausanne 18th October 2008

**Participants**
Stefano Avanzini (Paediatric surgeon - Genoa, Italy), Piero Buffa (Paediatric surgeon - Genoa, Italy), Giovanni Cecchetto (Paediatric surgeon - Padua, Italy), Anna Maria Fagnani (Paediatric surgeon - Milan, Italy), Keith Holmes (Paediatric surgeon - London, UK), Jean-Marc Joseph (Paediatric surgeon - Lausanne, Switzerland), Dejan Kafka (Paediatric surgeon - Belgrade, Serbia), Carl-Magnus Kullendorff (Paediatric surgeon - Lund, Sweden), Tom Monclair (Paediatric surgeon - Oslo, Norway), Sabine Sarnacki (Paediatric surgeon - Paris, France), Roly Squire (Paediatric surgeon - Leeds, UK), Shifra Ash (Oncologist - Tel Aviv, Israel), Bruno De Bernardi (Oncologist - Genoa, Italy), Geneviéve Laureys (Oncologist - Gent, Belgium), Paula Pereira (Radiotherapist - Lisbon, Portugal), Joáo C Silva (Paediatric radiologist - Lisbon, Portugal)

**1. LNESG1** outcome paper is near completion. (KH, GC and TM). A draft will be circulated to members of the Surgery SC before Christmas. **Action KH**

**2. LNESG2** accrual is good, 147 operation data sets. Close to 90% of patients have no risk factors. 75% of operations resulted in complete or near complete excision. The complication rate was 5%. J-MJ will lead on surgery analysis.

**3. High risk study**. Accrual remains good, 626 operation data sets. Complete or near complete excision was achieved in 74%. Complication rate was 11%. Nephrectomy or renal damage was 10%

**4. Unresectable localised Nb (EUNB).** 164 operation data sets have just been analysed by Riccardo Haupt. Complete or near complete excision was achieved in 74%, complication rate was 13% with one death. Further analysis is in progress. **Action KH**

**5. LINES** There was a closed meeting of this group which from which a number of SIOPEN members were barred. RS and SS did eventually join the meeting and will represent Sugery SC. The Surgery SC hoped that the protocol would be available for review soon and that it would be INRG compatible. **Action RS and SS. KH will ask Board to be more precise in defining "open and closed" meetings.**

**6. Adolescent and Young Adult AYA.** Contrary to discussion at the Surgery SC this is to be a short duration study a COG/SIOPEN collaboration. Following the meeting KH was invited to represent Surgery and accepted. Any member wishing to join would be welcome.

**7. Composition of Surgery SC.** The format of two members from each country was accepted. The current membership is listed below. **Action ALL - please inform KH of any change.**

| | |
|---|---|
| Adam Bysiek | (Poland) |
| Giovanni Cecchetto | (Italy) |

| | |
|---|---|
| Enrique Freud | (Israel) |
| Antonio Gentil-Martins | (Portugal) |
| Keith Holmes | (UK) |
| Ernst Horcher | (Austria) |
| Jean-Marc Joseph | (Switzerland) |
| Carl-Magnus Kullendorf | (Sweden) |
| Leopoldo Martinez- | (Spain) |
| Tom Monclair | (Norway) |
| Andras Pinter | (Hungary) |
| Lars Rasmussen | (Denmark) |
| Michal Rygal | (Czech Republic) |
| Sabine Sarnacki | (France) |
| Marian Vidisak | (Slovakia) |
| Ivo de Wever | (Belgium) |

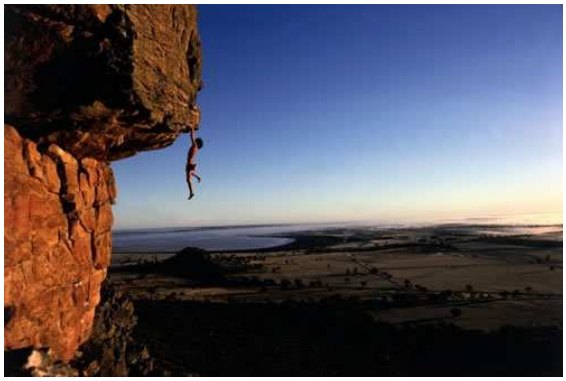
**8. Minimally Invasive Surgery (MIS).** The SC accepted that MIS would have a place in Oncology Surgery and stressed the importance of following oncology guidelines. We have no evidence to write more specific guidelines at present. All future protocols should include "type of operation" (open or MIS) and record sufficient information to test the effectiveness of MIS compared with open procedures. **Action SS and GC to report from the French and Italian registers. KH to ask Board to develop / modify protocols as appropriate.**

**9. Relationship with other SCs.** The surgeons were comfortable with relations in general. We missed closer collaboration with Radiologists. **Action TM agreed to develop this with Herve Brisse. KH will ask Board to be more precise in defining "open and closed" meetings.**

**10. Publication policy** The Surgery SC and other members of SIOPEN expressed their disappointment that the recent BJC paper on LNESG1 did not acknowledge the contributions of European Surgeons to the study. B de B, the first author, represented the co-authors, heard the Surgeons' sadness and apologised for the omission. **Action KH to report to the Board and stress the importance of a Publication Policy.**

**11. Complications of operation.** J-MJ suggested that we use the CHUV Lausanne definition: "any surgery related incident which interferes with a normal postoperative course". This should be used in addition to the precise description of the incident in future protocols.

**12. Future meetings** The spring "Extended Board Meeting" will be in Paris. We do not normally have a Surgery SC meeting at this time but can do if you wish. The next "open" SIOPEN meeting will probably be in Italy. **Action ALL tell me if you want a Surgery SC in Paris Spring 2009.**



# Bone Marrow

## EXTENSIVE VALIDATION OF A STANDARDIZED IMMUNOCYTOCHEMICAL TECHNIQUE FOR MINIMAL RESIDUAL DISEASE DETECTION IN NEUROBLASTOMA: A SIOPEN BONE MARROW SUBCOMMITTEE STUDY

Standardized analysis procedures are indispensable tools for multicenter studies evaluating the clinical significance of minimal residual disease in neuroblastoma. European reference laboratories therefore developed SOP's for the immunocytochemical (IC) detection and quantification of disseminated neuroblastoma cells. In order to guarantee the unambiguous identification of rare tumour cells, the SIOPEN Bone Marrow subcommittee decided to validate the standardized detection technique by comparing results of the IC assay to those of automated immunofluorescence plus FISH (AIPF), a technique which verifies the genetic make-up of detected cells.

In this multicenter study, 195 bone marrow samples or PBSC preparations (Israel: n=146; Czech Republic: n=49) from 46 SIOPEN high-risk neuroblastoma patients were analysed by immunocytochemistry (according to SOP's) and AIPF in a blinded way.

Using immunocytochemistry, in 33/195 samples "criteria positive cells" (CPC's) were detected. In 25/33 samples also "FISH positive cells" (FPC's) were found. Thirteen samples scored positive for AIPF although no CPC's were found. The number of detected neuroblastoma cells in these discordant samples was low. Only in 4 samples more than 10 FPC's per 105 mononuclear cells were detected.

In 18/195 samples, solely IC NCIC's (not convincingly interpretable cells) were found. The malignant nature of the NCIC's was confirmed by AIPF in 3/18 samples. Again, the number of NCIC's detected in the AIPF- samples was generally low (< 10 NCIC's), indicating that the discordance may be caused by sampling error. Finally, the correlation between the results of both techniques was calculated taking only IC CPC's and AIPF FPC's into account. A Bland and Altman plot showed that the results of both techniques were highly comparable (only 2 clear outliers). No bias or trend was found.

Within a trained review panel, there is an acceptable concordance between the results of both techniques suggesting that most cells detected by IC are NB cells. Discordances often result from sampling errors. The standardized technique is reliable, relatively simple and cost-effective and enables the reliable identification of disseminated neuroblastoma cells in a multicenter setting.

# Molecular Monitoring Group

**Membership**
Sue Burchill, United Kingdom (chair), Maria-Valeria Corrias, Italy (vice-chair), Sandro Dallorso, Italy, Bertil Kagedal, Sweden, Katrien Swerts, Belgium, Andrei Tchirkov, France, Aleš Vicha, Czech Republic Virginie Viprey, United Kingdom (vice-chair)

Yania Yáñez Peralta has joined the group as the new representative from Spain and will help maintain sample collection according to SOPs in this country.

**Meetings**
The MMG had a recent meeting on Thursday 16th October 2008 in Lausanne, Switzerland, coinciding with the ESIOP NB Annual Meeting.
Thank you to Maya and Nicole for providing us with facilities and making the group so welcome in beautiful Lausanne.

**Recruitment**
At the time of entry into the QRT-PCR minimal disease study recruitment within the UK and Italy is very similar; 55% (114/207) and 61% (133/217) respectively. Recruitment according to SOPs in Belgium and Sweden is 42% (15/36 and 8/19 respectively), 32% in Czech Republic (7/22). France has re-entered the study in 2006 and is now recruiting 17% (32/185) of total children entered since the start of the trial; this is improved compared to the 4% last year.
All clinical samples from countries outside those where there is a reference centre are processed and analysed in the reference centre in the UK, with the exception of future samples collected in Slovakia which are to be collected and analysed in the reference centre for the Czech Republic and in Norway where samples are to be collected and analysed in Belgium. In Greece, Dr Vassilios Papadakis has helped secure samples from 23% (5/22) of children, and in Austria samples from 7% (3/46) of children entered into the trial have been recruited into this biological study with the help of Professor Peter Ambros. Thank you to Vassilios and Peter for help with recruitment. Yania has now joined the group and we anticipate she will collect samples according to SOPs from children in Spain entered into the high risk protocol; these samples will be analysed in the UK.

At the time of writing, at entry into the study samples from 317 children have been collected, and after induction chemotherapy samples from 245 children.

**Analysis**
Data on tyrosine hydroxylase (TH) mRNA detection in clinical samples is currently being entered into the SIOPEN-R-NET database.
In addition to TH, Phox2B and DCX mRNA may be useful targets for the detection of minimal disease in children with NBL (Viprey et al., J Pathol 2008; NCRI 2008), we have therefore established SOPS for the detection of Phox2B mRNA by QRT-PCR across all reference centres. SOPs for the detection of the third target DCX have been designed and are currently under evaluation across the whole group.
Several observations using TH mRNA to detect NBL cells were presented by Dr Maria-Valeria Corrias on behalf of the group at the ANR 2008,
i) Increased frequency of TH positivity in peripheral blood and bone marrow at diagnosis using SOPs and QRT-PCR; 89% and 87% respectively.
ii) TH detection in peripheral blood and bone marrow is reduced after induction chemotherapy; 24% and 55% respectively.
iii) Clearance of TH mRNA from PB is more effective than from BM.

At the recent Lausanne meeting, we presented a preliminary comparison on the level and frequency of positivity for TH and Phox2B transcripts in bone marrow and peripheral blood at diagnosis and through out treatment. The log reduction of TH mRNA level detected in BM after rapid induction therapy may be a useful indicator of response to treatment. The clinical significance of the data presented at the ANR2008 and at the recent Lausanne meeting remains unknown. In the next year we aim to evaluate the clinical significance of tumour clearance after induction therapy; this will be informed by the DMC.

SIOPEN-R-NET database: An automated email system has been developed to alert reference

centres when samples are required from specific patients. The RT-PCR database is being modified to include an additional field to record analysis of samples for additional markers (Phox2B, DCX). Thank you to Mario Drobics from the Austrian Institute of technology who is working with the group to implement this.

**Funding**
Funding has been secured from The Neuroblastoma Society to help ensure collection of valuable clinical samples across Europe. Funding from Cancer Research UK has also been secured to continue coordination of the work on minimal disease in children with neuroblastoma within the UK. Monies for analysis of samples have been secured in France, Belgium, Italy and Czech Republic.
Thank you to all funding bodies supporting this activity.

Publications on neuroblastoma by members of the group in 2008 relating to the field:

*Burchill SA. PCR-based methods for the detection of cancer cells in blood, lymph nodes and bone marrow for clinical diagnostic assays. In "The PCR Revolution" edited by Stephen A. Bustin. Cambridge University Press, 2008.*

*Corrias MV, Parodi S, Haupt R, Lacitignola L, Negri F, Sementa AR, Dau D, Scuderi F, Carlini B, Bianchi M, Casale F, Faulkner L, Garaventa A. Detection of GD2-positive cells in bone marrow samples and survival of patients with localised neuroblastoma. Br J Cancer. 98: 263-269. 2008*

*Kanold J, Paillard , Tchirkov A, Merlin E, Marabelle A, Lutz P, Rousseau R,*
*Baldomero H and Demèocq F. Allogeneic or haploidentical HSCT for refractory or relapsed solid tumors in children: toward a neuroblastoma model.*
*Bone Marrow Transplantation. 42: S25-S30. 2008.*

*Trager C, Vernby A, Kullman A, Ora I, Kogner P, and Kagedal B. mRNAs of tyrosine hydroxylase are specific for neuroblastoma minimal disease and predicts outcome for children with high-risk disease when measured at diagnosis. Int J Cancer. 123: 2849-2855. 2008.*

*Viprey V, Lastowska M, Corrias M, Swerts K, Jackson M and Burchill S. Minimal disease monitoring by QRT-PCR: Guidelines for identification and systematic validation of molecular markers prior to evaluation in prospective clinical trials. The Journal of Pathology. 216: 245-252. 2008.*

*ANR 2008*

*Corrias MV, Dallorso S, Kagedal B, Oltra S, Swerts K, Tchirkov A, Vicha A, Viprey V, Ladenstein R, Burchill S. High frequency of minimal disease detected by QRT-PCR in bone marrow and peripheral blood from children with high risk neuroblastoma.*
*Abstract number TR110*

*NCRI 2008*
*Viprey V, Lastowska M, Corrias M, Swerts K, Jackson M and Burchill S. Minimal disease monitoring by QRT-PCR: Guidelines for identification and systematic validation of molecular markers prior to evaluation in prospective clinical trials.*
*Abstract number B151. (Awarded the CCLG prize for best poster).*

# Pharmacology

Gareth Veal, Gilles Vassal

The SIOP Neuroblastoma Pharmacology Subcommittee was established to promote the design and instigation of studies to learn more about the clinical pharmacology of agents used for the treatment of neuroblastoma. Initial challenges included the setting up and establishment of a European pharmacology laboratory network, involving the cross-validation of several analytical assays. A number of laboratories in different European countries have been identified as potential reference centres for analysis of drug levels in clinical samples obtained from current and future clinical studies and a number of these centres are currently analysing clinical pharmacology samples obtained from European studies. The group has established common protocols to ensure appropriate and standardised withdrawal, handling, storage and transport of blood samples for pharmacokinetic analysis and was also involved in defining appropriate guidelines for the treatment of patients with carboplatin on the high-dose myeloablative (CEM) arm of the ongoing European high-risk neuroblastoma study (HR-NBL-1/ESIOP). Since its initial founding, members have been involved in a number of studies and projects which are very much in line with the overall aims of the group. Recent activities include:

• Ongoing recruitment to clinical pharmacology studies associated with HR-NBL-1/ESIOP, investigating the pharmacokinetics of agents used in the high-dose myeloablative therapy treatment arm (BuMel and CEM). A total of over 60 patients have been recruited to the study to date, involving the collection and analysis of >500 clinical samples. Clinical data on patient characteristics, drug treatment, concomitant therapy and drug toxicity/adverse events, which will be essential for determining relationships between clinical parameters and drug pharmacokinetics, are being collected for all patients. Data relating to certain aspects of this study are now being analysed with a view to the submission of manuscripts in the near future.

• Based on recently published data (Vassal et al., Cancer Chemother Pharmacol 61: 113-123, 2008), oral administration of busulphan has been replaced by the use of i.v. busulphan (Busilvex®) in the HR-NBL-1/ESIOP protocol. Clinical pharmacology studies are now ongoing to obtain Busilvex® pharmacokinetic data to compare with results obtained from patients who have received oral busulphan within the same study protocol.

• A study investigating the pharmacokinetics of etoposide and carboplatin in patients treated on the European Infant Neuroblastoma study (INES) has now been completed, data analysed and the results written up for publication.

• A collaborative study has been initiated between the UK CCLG and French SFCE Pharmacology groups to investigate the clinical pharmacology of a number of anticancer drugs in infants and very young children. This represents a patient population where very limited pharmacokinetic data exists and will generate data which may help to establish more appropriate dosage regimens for future treatment.

• Clinical pharmacology studies incorporated into the HR-NBL-1/ESIOP protocol have now been expanded to include pharmacokinetic and pharmacogenetic studies relating to treatment with 13-cis-retinoic acid following high-dose myeloablative therapy. This follows the publication of data from a pilot study investigating 13-cis-retinoic acid pharmacokinetics in neuroblastoma patients (Veal et al., Br J Cancer 96: 424-431, 2007). Further advances will be achieved through the opening of a clinical study to investigate the impact of 13-cis-retinoic acid adaptive dosing in neuroblastoma patients in 2009.

• Funding has recently been obtained for two European FP7 projects involving countries in the UK, France, Germany and Italy. The first of these is led by Professor Gilles Vassal and involves the development of novel oral drug formulations of temozolomide and cyclophosphamide in children with cancer. The second study is headed by Professor Alan Boddy and will focus on the clinical pharmacology of the anticancer drug doxorubicin in a paediatric patient population. Both of these studies will be taken forward in the coming months with a view to initiating patient recruitment late in 2009.

Over the past 5 years, we have made significant strides to achieving the long-term aims of the group. These aims include the future implementation of drug monitoring and adaptive dosing approaches to optimise the use of chemotherapy, with a view to improving the immediate tolerance, efficacy and long-term survival for children with neuroblastoma and other tumour types.

# Nuclear Medicine

Val Lewington

To develop and implement new standards for objective nuclear medicine image reporting

Background
An international expert panel was convened to review the status of nuclear medicine scan reporting within the SIOPEN R NET high risk neuroblastoma [HRN] trial. A specialist team developed and tested a new semi-quantitative method to standardise reporting of diagnostic mIBG images. The new, straightforward method avoided pitfalls that have limited the usefulness of approaches used previously and reduced the time required for image review. The method was validated by 6 independent specialists against 328 scans stored electronically on the SIOPEN R NET database and proved highly robust with excellent intra- and inter-observer concordance.

**More definite and amplified objectives**
A meeting will be arranged in Spring 2009 to demonstrate the revised score method to nuclear medicine specialists in participating SIOPEN countries. Individual national representatives will then be well placed to re-commence the on-line scoring process for the HRN trial.

**Meetings**
After the launch meeting in Spring 2009, committee representatives will meet at the annual European Nuclear Medicine meeting in Barcelona.

**Achievements**
Developing and validating the new reporting method was a major undertaking for all involved. Preliminary results from the Expert Panel review were reported at the ANR in Chiba and a manuscript has been prepared for publication submission at the end of February. A further abstract has been submitted for oral presentation at the 2009 US annual nuclear medicine conference.

Current on going work
In addition to on-line image review, an atlas of teaching images is in preparation to illustrate the semi-quantitative score method.

**Future plans**
The main aims for 2009 are
i. to recruit and train a new group of nuclear medicine specialists to
ii. perform on-line mIBG scan reporting using the revised score method
iii. to liaise with the medical physics community to recommend and implement new standards for digital image storage on the SIOPEN R NET database, facilitating more rapid image analysis.
iv. to review current practice in paediatric mIBG imaging in the light of the expert panel review and improve quality standards for data acquisition

Requirements to undertake the work of the committee
Funding is needed to support an annual committee meeting and to allow at least some committee members to attend the 2009 SIOPEN annual meeting.

Administrative support is essential to coordinate the committee's activities, liaise with new members and to retrieve and upload image data for timely review

# Radiology

Marcus Hörmann, Claudio Granata

By initiative of the radiotherapy group and the study centre in Vienna (Ruth Ladenstein) a joint meeting with the radiology group last December was held in Vienna. The aim of this meeting was twofold:

1.Quality assurance of the radiotherapy as delivered to the patients treated in the study so far, based on the radiotherapy and radiology data electronically available in the database.
2. A funding from the government in Austria was to be justified first by a report, and further on by a publication of the radiotherapy and radiology group. The data collected during this meeting therefore will be used for publication of a paper.

In preparation of the meeting it was necessary to upload images of as many patients as possible. Karin Dieckmann was very successful in gathering data from the centres.
Unfortunately a lot of examinations were only available as hard prints, which have to be scanned. At the Medical University of Vienna a volunteer was found who will step by step scan hard copies and burn the images on a CD-Rom to further be disposable for upload on the data base (for future meetings).
In the future we will once again circulate calls in order to complete the data base.

During this joint meeting with the radiology and radiotherapy committees, we wanted to use the available data to test for the first time if this system indeed allows us to perform quality assurance with an electronic platform. In addition to reviewing the data stored on the system, we also had the opportunity to review data available on CD-ROM but not yet stored on the server.
Each person present at the meeting had a computer with internet access to explore the stored data. Two computers were equipped with beamers so that radiology and radiotherapy data could be projected side-by-side for direct comparison and measurement. We went through all the patients for whom we hoped we had complete data either on the system or on CD.

The results of the evaluation will be presented by the radiotherapy committee in this newsletter.

Several technical problems with the database were encountered:

1. when images have been uploaded without a .dcm tag, they cannot be viewed with the viewer provided, despite being in a DICOM format. Consequently some of the images had to be reviewed on the CD-ROM sent to the study centre.

2. uploading images is a time consuming procedure, as well as downloading them again for assessment. This problem may be of significant importance in future as the number of images obtained by CT or MRI is steadily increasing. The server went down twice during the meeting, after the upload of too many files in a short time.

3. the radiotherapy section of the database allows us to see patients in whom radiotherapy images are stored, and also the total number of patients registered in the study. However, we cannot tell from this which of the total patients have actually received radiotherapy. Therefore the completeness of data cannot be adequately assessed at this point.

<u>Imaging assessment:</u>
Images which were assessed for the evaluation of the radiation field were obtained either by CT (multi-slice CT) or MR imaging.

<u>Imaging protocols were according to the study protocol for CT:</u>
Multi-slice technique with acquisition of a volume scan with reconstruction in at least two orthogonal planes (axial and coronal). Reconstructions
were performed in 3mm slices thickness. Administration of contrast agents was not

demanded but in most examinations performed.

MR Imaging was performed on 1and 1.5 Tesla (high field) units. Contrast agent was administered in all patients. T1- and T2-weighted sequences as well as fat suppressed sequences were obtained in all cases. The centres were free to do additional sequences like MR-Angiography and diffusion weighted sequences. Slice thickness was depending on the type of the machine: 2 to 5 mm with different intersection gap less than 1mm.

At least two orthogonal planes were obtained, which were in the axial and coronal orientation, additionally in some patients a third plane in sagital orientation was obtained.

Image evaluation:

A team consisting of radiologists and radiotherapists in consensus description evaluated images. The extent of the tumour and description of the margins as well as the determination of the size was undertaken by the radiologist and the assessment of the size of radiation field was undertaken by the radiotherapist. For assessment of the radiation field we evaluated examinations performed at the milestone "presurgical imaging".

Future:

Further efforts need to be made by direct contact with radiologists and radiation oncologists in individual centres to improve the completeness of image upload. This relates to patients who have no data on the system, and also those with some data. This is because there are numbers of patients with incomplete datasets, either radiology only or radiotherapy only. Limited additional data would make a complete dataset available for analysis.

A further meeting of the committee should have been held in March 2009, but seemed to be postponed

Two manuscripts will be prepared. The first will focus on the process and the difficulties encountered. The second paper will relate to clinically relevant issues of quality control when more patients have been evaluated.

# Pathology
## *Guidelines for LINES*

**Signature**
**Amann Gabriele, d'Amore Emanuele S.G., Beiske Klaus, Cullinane Catherine, Gambini Claudio, Navarro Samuel, Peuchmaur Michel**

Rationale for the role of pathologists and for the procedure to obtain tumor material would be given in the introduction or ad hoc chapter of the LINES protocol.

The pathologist should be made aware of a possible inclusion of the patient in the LINES. The material obtained, in a majority of cases by needle biopsies or surgical open biopsies, and in a minority of cases by surgical resection (complete, with minimal residual disease or incomplete) must be sent fresh without any preservatives, fixatives or formalin and as quickly as possible to the pathologist. The division of tumor material is performed by the pathologist with adequate frozen tissue banking for MYCN FISH and molecular studies on the one hand, and formalin fixation for morphological assessment on the other hand.

**1. Needle biopsies**

a. When needle biopsy is indicated instead of surgical procedure, the same general principles must be applied for handling the material, i.e. secure immediately both formalin fixed and frozen material.

b. To date, there is no data to indicate the minimal volume of tumor material required to permit application of the INPC classification. Empiric data consider that only 2cmx2cm or greater surgical biopsies allow INPC characterization. For these reasons, sufficient tissue must be obtained without taking any risk of adverse effect or complication for the patient, ideally from two different areas of the tumor, and/or with several needle core biopsies to provide sufficient tissue for diagnostic studies. Note that the amount of tissue for biological studies will be depend on overall amount sent to pathologist who will have to consider the pathological features more critically than just confirming NB.

c. This implies that, in case of needle biopsies the INPC histoprognostication (Favorable/Unfavorable), and INPC morphological characteristics independent of the age (i.e. differentiation and MKI) developed in the INRG risk grouping scheme will be determined on a prospective basis, with mention of the size of the formalin fixed tumor material submitted to histological evaluation. The differentiation and MKI will be determined in the stroma poor component of the tumor. These criteria should be assessed only if a minimum of 5000 tumor cells are present in the studied material.
In the "Pathology Form", it will be specified that in the case of a stroma rich tumor, it is not possible to exclude a nodular component in another area of the same tumor, requiring a correlation with clinical and radiological data.
In case of multiple core biopsies, if one or more cores are occupied by a stroma rich tumor and other core(s) by a stroma poor tumor, a diagnosis of "ganglioneuroblastoma stroma mixed nodular" could be proposed.

d. This prospective study will be coupled with two on-going not yet achieved studies, one aim of these two studies is to validate the possibility of applying INPC criteria to needle biopsies: 1/The virtual biopsy project: A European multi-centre study based on the analysis of virtual needle biopsies. 2/ The European Unresectable Neuroblastoma (EUNB)

e. The accurate volume of frozen material for biological studies (Nmyc determination, molecular evaluation) is determined in cooperation with the biologist. The non frozen material must be formalin fixed.

**The virtual biopsy project: A European multi-centre study based on the analysis of virtual needle biopsies**

**Background**

Parallel to the broader availability of ultra-sound/CT-guided and needle-based biopsy techniques, pediatric oncologists in a number of European countries, appear to prefer histological needle (i.e. tru-cut) to open (i.e. surgical) biopsies, when the primary diagnosis of a non-L1 stage peripheral neuroblastic tumor is to be established. Needle biopsies are minimally invasive, and are believed to represent a lower risk of tumor cell spread compared to surgical operations. To assess the prognosis of peripheral neuroblastic tumors, pathologists can make use of the INPC, a histoprognostic classification system, based on the amount of Schwannian cell stroma, neuroblastic differentiation, mitosis-karyorrhexis index (MKI) and age of the patient. For a conclusive histoprognostic evaluation, the analysis of at least 5000 tumor cells is required. Therefore, the application of this system has so far been restricted to sections from resected tumors or surgical biopsies.

**Aim of the study**

***In general:***

To investigate by means of virtual needle biopsies, prepared from histological sections of resected tumors, how many needle biopsies of a given standard size are necessary

- to facilitate the analysis of at least 5000 tumor cells
- to reach the same histoprognostic conclusion as obtained by analysis of slides from the resected tumor

***In detail***:

- To analyse the inter-observer concordance between 7 investigators screening virtual biopsies. This is an important initial step to make sure that inter-biopsy discrepancies are not caused by possible assessment variability between individual investigators, but reflect the biological heterogeneity of the tumor.
- To analyse (i) the concordance between the (consensus) diagnoses of all virtual biopsies belonging to the same tumor and (ii) the relationship to the original diagnosis based on previous review of tumor sections.
- To calculate the statistical probability of establishing a correct histoprognostic classification based on a given number of needle biopsies.
- The final report should contain:
    - Virtual biopsy number
    - Estimated number of investigated tumor cells
    - For stroma-poor NB: NB-UD/PD/D, MKI-L/I/H
    - For GNB: GNB-N with nNB-PD/D, MKI-L/I/H
    - GNB-I.

**The European unresectable neuroblastoma (EUNB):**

The SIOPEN Pathology Speciality Committee focused on the histopathological review of tumours enrolled in the protocol for European unresectable neuroblastoma (EUNB).

By the end of August 2008, 127 tumors were reviewed. Of these, 122 were histologically evaluable and classified morphologically and prognostically according to the International Neuroblastoma Pathology Classification (INPC). Data collected during the review included not only the INPC histoprognostication (Favourable/Unfavourable), but also INPC histological category and subtype because these are elements of the recently developed INRG pre-treatment risk grouping scheme.

Although based on small numbers of patients, the results of this preliminary analysis seem to show a clearly better prediction of survival (especially EFS) by INPC (histology + MKI + age) and by MKI alone as compared to the prediction obtained by histological grade alone (undifferentiated + poorly differentiated versus differentiating), both in all patients and in those >18 months as proposed in the INRG risk grouping scheme.

**2. Surgical open biopsies and Surgical resections (complete, with minimal residual disease or incomplete),**

a. In all cases where surgical resection is performed (see below § 6, surgery) before chemotherapy, the guidelines for pathology process are the same as those for LNESG1 / LNESG2 protocols (ref)
b. In these samples the tissue volume is sufficient to apply the morphological criteria of the INPC. It will therefore be possible to assign the tumor to one of the four PNTs categories, i.e.: neuroblastoma stroma poor, ganglioneuroblastoma stroma mixed nodular, ganglioneuroblastoma stroma rich intermixed, ganglioneuroma, and to determine histopronostic categories: Favorable or Unfavorable. Moreover, the differentiation (i.e.: undifferentiated, poorly differentiated and differentiating) and the MKI (low <2%, intermediate between 2 and 4%, and high >4%) will be determined in the neuroblastoma stroma poor component of the tumor for each individual tumor as developed in the INRG pre-treatment risk grouping scheme.
c. These data will be quoted in the "Pathology Form".

**3. In case of fine needle aspiration, or in case of core biopsy with less than 5000 tumor cells in the studied material, the INPC is not applicable.**

**4. Review process.**

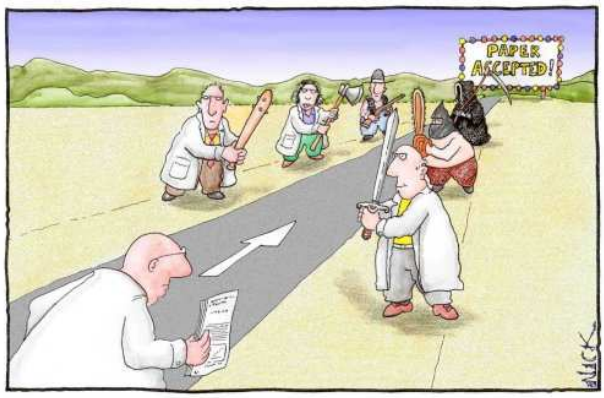
One HE-stained section from each paraffin block of the tumor must be sent to one of the reference pathologists in the SIOP European Neuroblastoma Pathology Review Panel (Gabriele Amann, Klaus Beiske, Catherine Cullinane, Emanuele S.G. d'Amore, Claudio Gambini, Samuel Navarro, Michel Peuchmaur).

The pathology subcommittee will organize this review process, design the form accompanying the slide(s) and inform the local pathologist about the result of the review. The time between finalization of local histopathological report and the review by a member of the SIOP European Neuroblastoma Pathology Review Panel have to be determined in the protocol.

In order to ease their review task, clinical information on catecholamines, site of tumour, MIBG data, or other relevant datas should be sent to the Pathology subcommittee members along with the slide(s). In case of undifferentiated tumors, tissue block(s) could be required to perform immunohistochemical studies.

The tumor cell content of the frozen specimen dedicated to biological studies will be analysed by the local pathologist.

Each member of the Panel will organize the review for her/his country and will review the cases from countries which are not represented in the panel (this point must be finalize in agreement with national coordinators).




Most scientists regarded the new streamlined peer-review process as 'quite an improvement.'

# Radiotherapy

Mark Gaze

The main work of the radiotherapy group has been a quality-control review of radiotherapy related imaging and data stored on the SIOPEN computer database relating to patients treated in the High-Risk study. This has not been an easy task as individual centres have not been good at ensuring all relevant data have been uploaded at the time of treatment. Partial data sets are not interpretable, and even complete radiotherapy data sets cannot be analysed if the corresponding radiology images are not also on the system. Karin Dieckmann, Tom Boterberg, Mark Gaze and Kevin Sullivan had a three day meeting in Vienna in December to do this, and were very fortunate to be joined by Marcus Hoermann who interpreted the radiology images for us. The task is not yet complete, and we shall be meeting again in May 2009 to do more of the same. It would greatly facilitate this project if national coordinators would work to ensure data completeness for both radiotherapy and radiology. Our other project has been to write the radiotherapy guidelines for the proposed Low- and Intermediate-risk Neuroblastoma European protocol. In this study, radiotherapy will be systematically administered to patients over 18 months of age with INRG L2 disease of poorly differentiated or undifferentiated histology.

# Biology

## Activities of the SIOPEN Biology Group

*Peter F. Ambros (Austria, Chairman), Inge Ambros (Austria), Frank Speleman, Nadine Van Roy (Belgium), Ales Vicha (Czech Republic), Jean Benard, Valérie Combaret, Jérome Couturier, Olivier Delattre, Gudrun Schleiermacher, Alexander Valent (France), Raymond L. Stallings (Ireland), Marta Jeison (Israel), Raffaella Defferrari, Katia Mazzocco, Gian Paolo Tonini (Italy), Klaus Beiske (Norway), Barbara Marques (Portugal), Nicole Gross (Switzerland), Rosa Noguera (Spain), Tommy Martinsson (Sweden), Nick Bown, John Lunec, Deb Tweddle (UK).*

Within the last year the group members have made substantial progress in concerted actions leading to a number of cooperative studies and publications on different aspects of neuroblastoma biology. In a bi-national study a DNA based array technique (array CGH) was applied to better define genetic risk groups in neuroblastic tumours re-emphasizing the biological impact of segmental chromosomal aberrations. In a large SIOPEN study the clinical impact of the expression of 59 genes was studied. The correlation of the expression profile with the clinical data from 579 patients revealed an excellent outcome prediction of this RNA based approach. The international neuroblastoma risk grouping biology (INRG Biology) Group could finish the standardization on handling and nomenclature of genomic information originally initiated by the SIOPEN Biology Group. INRG Biology Group reached consensus on methodology and interpretation of data on international level in order to improve future risk assignment algorithms applying genome-wide techniques. Furthermore, as already mentioned in the last report, over the last two years a robust and standardized technique (MLPA) for the detection of the most frequent genomic changes in neuroblastomas could be successfully implemented in most SIOPEN biology reference centres. This technical improvement already resulted in the first publication on this technique on neuroblastic tumours. Furthermore, two independent projects aim to answer the question on the impact of 2p gain on the outcome of neuroblastoma patients.

## Overall Genomic Pattern Is a Predictor of Outcome in Neuroblastoma

*Isabelle Janoueix-Lerosey, Gudrun Schleiermacher, Evi Michels, Veronique Mosseri, Agne`s Ribeiro, Delphine Lequin, Joelle Vermeulen, Jerome Couturier, Michel Peuchmaur, Alexander Valent, Dominique Plantaz, Herve Rubie, Dominique Valteau-Couanet, Caroline Thomas, Valerie Combaret, Raphael Rousseau, Angelika Eggert, Jean Michon, Frank Speleman, and Olivier Delattre*
J Clin Oncol 27 1-11 (2009)

For a comprehensive overview of the genetic alterations of neuroblastoma, their association and clinical significance, we conducted a whole-genome DNA copy number analysis. A series of 493 neuroblastoma (NB) samples was investigated by array-based comparative genomic hybridization in two consecutive steps (224, then 269 patients). Genomic analysis identified several types of profiles. Tumors presenting exclusively whole chromosome copy number variations were associated with excellent survival. No disease-related death was observed in this group. In contrast, tumors with any type of segmental chromosome alterations characterized patients with a high risk of relapse. Patients with both numerical and segmental abnormalities clearly shared the higher risk of relapse of segmental-only patients. In a multivariate analysis, taking into account the genomic profile, but also previously described individual genetic and clinical markers with prognostic significance, the presence of segmental alterations with (HR, 7.3; 95% CI, 3.7 to 14.5; P < .001) or without MYCN amplification (HR, 4.5; 95% CI, 2.4 to 8.4; P < .001) was the strongest predictor of relapse; the other significant variables were age older than 18 months (HR, 1.8; 95% CI, 1.2 to 2.8; P = .004) and stage 4 (HR, 1.8; 95% CI, 1.2 to 2.7; P = .005). Finally, within tumors showing segmental alterations, stage 4, age, MYCN amplification, 1p and 11q deletions, and 1q gain were independent predictors of decreased overall survival. The analysis of the overall genomic pattern, which probably unravels particular genomic instability mechanisms rather than the analysis of individual markers, is essential to predict relapse in NB patients. It adds critical prognostic information to conventional markers and should be included in future treatment stratification.

## Improved Outcome Prediction of Children with Neuroblastoma using a Multigene Expression Signature, a SIOPEN Study

*Joëlle Vermeulen, Katleen De Preter, Arlene Naranjo, Liesbeth Vercruysse, Nadine Van Roy, Jan Hellemans, Katrien Swerts, Sophie Bravo, Paola Scaruffi, Gian Paolo Tonini, Rosa Noguera, Marta Piqueras, Isabelle Janoueix-Lerosey, Olivier Delattre, Valérie Combaret, Matthias Fischer, André Oberthuer, Peter Ambros, Klaus Beiske, Jean Bénard, Barbara Marques, Jean Michon, Gudrun Schleiermacher, Bruno De Bernardi, Hervé Rubie, Adela Cañete, Victoria Castel, Janice Kohler, Ulrike Pötschger, Ruth Ladenstein, Michael D. Hogarty, Patrick McGrady, Wendy B. London, Geneviève Laureys, Frank Speleman, Jo Vandesompele*
*in submission*

"Fifty-nine genes were carefully selected based on an innovative data-mining strategy and profiled in the largest neuroblastoma patient series (n=579) to date using RT-qPCR starting from only 20ng of RNA. A multigene expression signature was built using 30 training samples, tested on 313 test samples and subsequently validated in a blind study on an independent set of 236 additional tumours. The signature classifies patients accurately with respect to overall and progression-free survival ($p<0.001$). The signature has an accuracy, sensitivity and specificity of 85%, 84% and 87% respectively to predict patient outcome. Multivariate analysis indicates that the signature is a significant independent predictor after controlling for currently used risk-factors. Patients with high molecular risk have a 19-fold higher risk to die from disease and a 4-fold higher risk for relapse/progression than patients with low molecular risk. Patients with increased risk for adverse outcome can also be identified within the current treatment groups demonstrating the potential of this signature for improved clinical management. These results were confirmed in the validation study. The high patient/gene ratio (579/59) underlies the observed statistical power and robustness."

## International consensus for neuroblastoma molecular diagnostics: report from the International Neuroblastoma Risk Group (INRG) Biology Committee

*PF Ambros, IM Ambros, GM Brodeur, M Haber, J Khan, A Nakagawara, G Schleiermacher, F Speleman, R Spitz, WB London, SL Cohn, ADJ Pearson and JM Maris*
*British Journal of Cancer, in press.*

Neuroblastoma serves as a paradigm for utilising tumour genomic data for determining patient prognosis and treatment allocation. However, before the establishment of the International Neuroblastoma Risk Group (INRG) Task Force in 2004, international consensus on markers, methodology, and data interpretation did not exist, compromising the reliability of decisive genetic markers and inhibiting translational research efforts. The objectives of the INRG Biology Committee were to identify highly prognostic genetic aberrations to be included in the new INRG risk classification schema and to develop precise definitions, decisive biomarkers, and technique standardisation. The review of the INRG database (n = 8800 patients) by the INRG Task Force finally enabled the identification of the most significant neuroblastoma biomarkers. In addition, the Biology Committee compared the standard operating procedures of different cooperative groups to arrive at international consensus for methodology, nomenclature, and future directions. Consensus was reached to include MYCN status, 11q23 allelic status, and ploidy in the INRG classification system on the basis of an evidence-based review of the INRG database. Standardised operating procedures for analysing these genetic factors were adopted, and criteria for proper nomenclature were developed. Neuroblastoma treatment planning is highly dependant on tumour cell genomic features, and it is likely that a comprehensive panel of DNA-based biomarkers will be used in future risk assignment algorithms applying genome-wide techniques. Consensus on methodology and interpretation is essential.

## Comparison of different techniques for the detection of genetic risk-identifying chromosomal gains and losses in neuroblastoma

*Eva Villamón, Marta Piqueras, Carlos Mackintosh, Javier Alonso, Enrique de Álava, Samuel Navarro and Rosa Noguera*
*Virchows Arch (2008) 453:47–55*
After the comparative analyses of the results obtained with different techniques for the detection of genomic data in 20 patients with NB we conclude that: 1) The multigenomic techniques showed a high degree of concordance; 2) NB consists of biologically distinct subgroups that differ by genetic characteristics of prognostic relevance; 3) FISH will be essential for the mandatory study of MYCN status; and 4) The use of MLPA as routine technique is an advantage procedure for detecting the implication of the common genetic alterations in NB.

**Ongoing projects:**

2p24 gain region harboring MYCN gene compared to MYCN amplified and non-amplified neuroblastoma: biological and clinical characteristics.

Marta Jeison, Shifra Ash, Gili Halevy-Berko, Jacques Mardoukh, Drorit Luria, Smadar Avigad, Galina Feinberg-Gorenshtein, Yacov Goshen, Joseph Kapelushnik, Ayelet Ben Barak, Dina Attias, Ran Steinberg, Jerry Stein, Batia Stark and Isaac Yaniv.

The aim of this study was to compare the cytogenetic and clinical features of MYCN/2p24 region gain group, to MYCN-amplified and MYCN-nonamplified neuroblastoma, and to determine its impact on disease outcome. 177 samples (49 from SIOPEN HRNBL1 study) from neuroblastoma patients (all stages) were enrolled in this study. FISH was performed including MYCN, 1p, 17q, and 11q regions. MYCN/2p24-gain was identified in 25 patients, MYCN amplification in 31, and MYCN nonamplification in 121. Enlarged gain of 2p region was confirmed by MLPA or ALK gene.

Patients with MYCN/2p24-gain had a significantly worse five-year event-free survival than patients in the MYCN-nonamplified group ($p<0.001$), and an intermediate five-year overall survival between the MYCN-amplified and MYCN-nonamplified groups ($p=0.018$ and $p=0.01$ respectively). All the MYCN/2p24-gain samples harbored in addition, at least one segmental alteration in the other tested parameters: 1p, 11q and 17q. This strongly suggests that these NB tumors may be classified into genomic type 2 associated with a poorer outcome.

MYCN test is still the gold standard for neuroblastoma characterization. The use of a simultaneous internal control as LAF gene on 2q11 by FISH in order to determine the presence of extra copies of MYCN gene, will give indirect information of other segmental aberrations, as demonstrated in the present study, and would help to improve risk stratification.

**2p gain in neuroblastoma tumours**

*Proposal from: Gian Paolo Tonini, Katia Mazzocco, Raffaella Defferrari.*
*Italian Neuroblastoma Foundation/Translational Paediatric Oncology, National Cancer Research Institute, Genoa, Italy.*
*Participating countries: Austria, Belgium, Czech Republic, France, Israel, Italy, Norway, Spain, United Kingdom.*

Aim: Molecular cytogenetic analyses have revealed that a relatively high number of neuroblastoma tumours, independent of stage, show an up to 4-fold excess of MYCN copies in relation to the reference probe on the long arm of chromosome 2. According to the ENQUA and INRG Group definitions this aberration has been defined as 2p24 gain. However, the prognostic significance of this segmental aberration is not clear so far.

Aims of this study are: 1) to determine the genetic characteristics of these tumours by using pan- or multigenomic techniques (MLPA and/or array-CGH analysis); 2) to evaluate the prognostic impact of 2p gain on outcome of 207 European NBs by comparing the clinical outcome of 2p gain patients with that of a non 2p gained control group (matched by age, stage and treatment protocol).

Status of the study: up to now pan- or multigenomic data are available for the majority of cases. In order to finalize this study, clinical data on the enrolled patients and the identification of the control group are urgently needed

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# Parents' corner

# CONE

**Susan Hay**

**SIOPEN Annual General Meeting**
**15-17 October 08, Lausanne**

A meeting of the charities, with members of SIOPEN Executive, was held on 15 October, in conjunction with the AGM. Those attending were from the UK and France only, and represented by Stephen Smith (Neuroblastoma Society UK), Susan Hay (Adam's Hats UK) and Anne Gouin (France).

The main outcomes of the discussion were:

• SIOPEN needs to have a clearer picture of the role that charities can play in support of its work, which includes bringing the parent perspective to SIOPEN projects; advocacy in home countries and at European level; and raising funds.

• SIOPEN's new Statutes enable charities to contribute through being represented on the SIOPEN Advisory Board, and provide a framework for charities to be developed into an effective group.

The Statutes, which were adopted at the AGM, state:

**1. Premises**
There already exist a limited number of nationally-based non-profit associations and charities which occasionally sponsor neuroblastoma research. With interaction and collaboration, these associations and charities could achieve greater impotance, and potentially influence the political factor and collect more funds.

**2. Objectives**
a. to assist collaboration of existing nationally-based no-profit associations and charities with a special interest in neuroblastoma
b. to stimulate the foundation of Neuroblastoma Support Groups (NSGs) in other countries participating in SIOPEN
c. to identify individuals responsible associations and charities of each NSG authorized to interact with SIOPEN
d. to allow these representatives to attend meetings of SIOPEN as Associate Members/Corporate Members.

**3. Representation on the Board**
One representative of the parents' associations and charities should be represented on the Board.
Subsequent to the meeting, Susan Hay has been asked by the SIOPEN Executive to take on this role, whilst the charities group is being formed within the provisions of the new Statutes.

• SIOPEN undertook to develop a template for project support, in discussion with charities, to cover SIOPEN's needs, as part of a broader review of its communication tools.

• A day's meeting devoted to developing the role of charities is to be planned for the Spring, which will hopefully re-engage the larger groups, as well as those representing childhood cancer on a wider frame. This meeting will be hosted in Genoa or London.

Susan Hay reported the main outcomes of the meeting to the AGM on 17 Oct, and asked all country representatives to encourage charities in their country to attend the proposed all-day meeting. The value of engaging in support for SIOPEN could be:

- the challenge of working at a European level
- recognising that research into neuroblastoma has the potential to be of value across childhood cancers
- the importance of parent support to targeting therapies, and the critical role charities can play as a conduit between SIOPEN and parents
- how the wider networks of charities can assist raising SIOPEN's profile and potential to be funded.

# Therapeutic Perspectives

## Allogeneic SCT

**by Isaac Yaniv**

During the AGM in Lausanne a session on allogeneic transplant in Neuroblastoma took place.
Following an introduction by Isaac Yaniv, Shifra Ash from Israel described the research demonstrating allo transplants to be superior to autologous transplants in a mouse model of Neuroblastoma.
NK and alloreactivity were discussed by Antonio Pérez-Martínez from Spain. He developed a detailed explanation of NK alloreactivity and presented the preliminary results on 3 Haplo transplants suggesting that alloreactive NK cells from a donor KIR-HLA receptor ligand mismatch enhance graft versus tumor effect in HSCT, and that donor activating KIRs influence outcome.
The Italian cooperative study on allo HSCT (MFD or URD) was presented by Arcangelo Prete suggesting that allogeneic transplants my be useful in responding patients if there is a suitable family donor and the patients develop acute GVHD grade 2-3. In their cohort 10 out of 19 patients were alive with a median follow up of 15 months and no transplant related mortality.
The French experience of RIC allo in relapsing or resistant Neuroblastoma presented by Catherine Paillard demonstrated the feasibility and low toxicity in heavily pre-treated children as well as clinical suggestion of Graft Versus Neuroblastoma effect.
The last presentation by Peter Lang described the German experience with HAPLO Transplants in 20 patients with metastatic relapse. Eleven patients responded and 7 are alive with a median of 1.8 years from transplant.

It was concluded that by the end of 2008 several crucial questions remain:
For which category of patients will this approach be indicated?
At what point in the disease course allo-HSCT should be incorporated?
The best conditioning regimen
Donor cell source and graft composition
How to enhance Graft versus NBL effect?

Further discussions within the SIOPEN group on this experimental approach are planed.

## Impact on therapy decisions

**Julie R. Park,** *Seattle Childrens Hospital and University of Washington, Seattle WA, USA;*

**Background**: There is lack of consensus on prognostic markers other than age and tumor MYCN amplification for children with INSS stage 3 neuroblastoma and on optimal therapy for children over 18 months of age with stage 3 MYCN non-amplified neuroblasotma.
**Methods:** The INRG database (n=8800 patients) was utilized to assess whether age, elevated ferritin (> 96 ng/ml), tumor histology or somatically acquired chromosomes (ch) 1p or 11q aberrations impact EFS and OS from INSS stage 3, MYCN not amplified (NA) neuroblastoma. CCG 3891 data was reviewed to assess impact of myeloablative chemotherapy on EFS and OS for patients with high risk Stage 3 neuroblastoma defined as greater than 1 year of age with amplified MYCN copy number (MYCN-A), unfavorable Shimada histopathology or elevated serum ferritin level. The INRG database was also used to assess impact of therapy on EFS and OS.
**Results:** Of 1,483 patients with INSS stage 3 tumors, 1,013 had MYCN-NA tumors. Among these, 654 (64.6%) patients were age <547 days (<18 months) at diagnosis. The 5-year EFS and OS rates for patients with INSS stage 3 MYCN-NA tumors were 81%±2% and 89%±1%, respectively. Age >18 months at diagnosis was associated with significantly decreased EFS and OS compared to age <18 months (EFS 64%±3% vs. 90%±2% p<0.0001; OS 76%±3% vs. 95%±1%, p<0.0001). For patients <18 months of age, tumor ch11q aberrations had a significantly inferior EFS (64%±22%) that remained independently prognostic in multivariate analysis (HR 5.3, p=0.0351). The presence of tumor ch11q aberrations or elevated serum ferritin was associated with a significantly worse OS (73%±22% and 88%±4%, respectively) but neither was independently prognostic in the multivariate model. For patients age >18 months, poorly/undifferentiated histology and elevated serum ferritin led to a significantly inferior EFS and OS but only ferritin was independently prognostic in multivariate analyses (EFS: HR 2.5, p=0.0319; OS: HR 2.7 p=0.0052). The 5-year event-free survival (EFS) and overall survival (OS) rates for patients with high risk stage 3 neuroblastoma enrolled onto the CCG 3891 randomized clinical trial were 55 +/- 6% and 59% +/- 6%, respectively (n=72). Patients with high risk stage 3 neuroblastoma randomized to autologous bone marrow transplant (n=20) had 5-year EFS of 65% +/- 11% and OS of 65% +/- 11% compared to 41% +/- 11 (p=0.21) and 46% +/- 11% (p=0.23) for patients randomized to continuation chemothearpy (n=23), respectively. The 5-year OS for patients < 18 months of age with non-amplified MYCN tumors (n= 7) was 100% as compared to a 5-year OS of 74% + 9% for patients > 18 months with non-amplified MYCN tumors (n= 30, p=0.1545), respectively. There was a trend toward improved EFS following use of myeloablative consolidation therapy for those patients greater than 18 months of age with stage 3 neuroblastoma, MYCN nonamplified with either elevated serum ferritin or undifferentiated histology analyzed through the INRG database (p= 0.084).
**Conclusions:** Patients <18 months of age at diagnosis of INSS stage 3 MYCN-NA neuroblastoma with somatically acquired ch11q aberrations are at significantly higher risk for relapse. In older patients, elevated serum ferritin or poorly/undifferentiated tumor histology significantly increase risk for recurrence. These biomarkers should be considered in treatment algorithms. Further studies are warranted to determine if myeloablative consolidation statistically significantly improves outcome.

## ALK mutations and overexpression in advanced/metastatic neuroblastoma patients: a potential therapeutic target

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Anaplastic Lymphoma Kinase (ALK) is a transmembrane tyrosine kinase (TK) receptor originally identified as the C-terminal part of the transforming fusion protein NPM (Nucleophosmin)-ALK in Anaplastic Large Cell Lymphoma. During embryonic development native full-length ALK receptor and its putative ligand pleiotrophin (PTN) work as a receptor/neurotrophic factor pair in neuronal autocrine differentiation and physiology. In vitro studies indicated ALK expression in different solid tumor derived cell lines such as neuroblastoma (NB), melanoma, glioblastoma, breast carcinoma and rhabdomyosarcoma. Recent evidence indicates a pivotal role for the ALK receptor in both familial and sporadic NB pathogenesis.
We performed a mutational analysis of the TK domain in 115 sporadic cases and in 15 human NB cell lines. We identified already known missense mutations and one new mutation (I1170S) in both tumor samples and cell lines. Interestingly, we found ALK mutations also in localized NBs, which had a poor outcome. To gain more insights into the ALK oncogenic and clinical relevance in NB, we investigated the expression level and activation of ALK protein in 82 NB specimens and in a panel of NB-derived cell lines.
Our results show that ALK expression was significantly upregulated in advanced/metastatic (stage 3-4) compared to localized (stage1-2) NBs. Our findings indicate that ALK constitutive phosphorylation/activation occurs only in ALK highly-expressing NB cells with either a wild-type or mutated ALK receptor. Accordingly, ALK inhibition, either by siRNA or small molecule inhibitors, hampers cell proliferation and induces cell death of NB cells expressing high levels of constitutively active native or mutated ALK. Therefore, it is conceivable to hypothesize that ALK targeting by small molecule inhibitors may be a valid therapeutic strategy for NB patients with high levels of expression of ALK tyrosine kinase receptor, regardless of the mutational status of the gene.
Conclusively, we propose that despite activating mutations ALK overexpression is a feature of NB tumorigenesis that defines NB patients with a poor prognosis, and it represents a novel potential therapeutic target.

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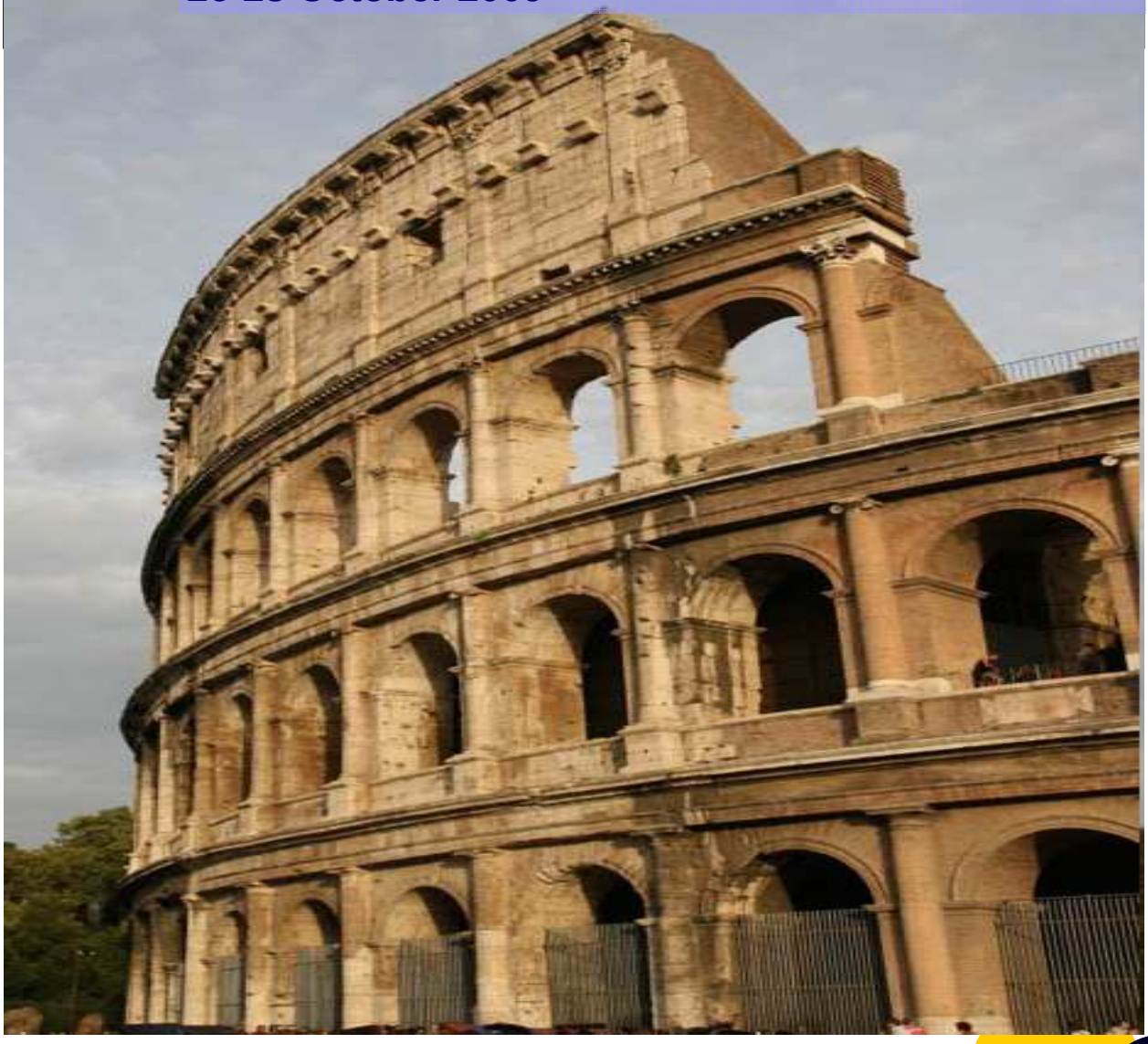
# Meeting Planner

## 2009
## S I O P E N Annual Meeting

**Bambino Gesù Children's Hospital, Roma, Italy**
**26-28 October 2009**



# All over the world …

## EBMT 2009,
29/03 – 01/04,
Göteborg (Sweden)
http://www.congrex.ch/ebmt2009/

## AACR 2009,
18/04 – 22/04,
Denver (USA)
http://www.aacr.org/home/scientists/meetings--workshops/aacr-100th-annual-meeting-2009.aspx

## ASCO 2009,
29/05 – 02/06,
Orlando (USA)
http://www.asco.org/ASCO/Meetings/ASCO+Annual+Meeting/

## ECCO 15 – 34th ESMO,
20-24/09/2009,
Berlin, Germany
http://www.ecco-org.eu/Conferences-and-Events/ECCO-15/page.aspx/216

## SIOP 2009,
5-9/09,
Sao Paolo (Brezil)
http://www.asco.org/ASCO/Meetings/ASCO+Annual+Meeting/