E-SIOP NEUROBLASTOMA

a quarterly NEWSLETTER

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Advances in Neuroblastoma Research 2002
Paris, June 17-19


SIOP 2002
Porto, September 18-21


E-SIOP NB Annual Meeting
Prague, November 8-9


Advances in Neuroblastoma Research 2004
Genova, June 16-19

Editorial

It has been now 7 years since the activation of the first SIOP Europe Neuroblastoma (E-SIOP NB) protocol, labelled LNESG1. With approximately 1,000 cases of newly diagnosed localised neuroblastoma enrolled in a 5-year period by 12 national groups E-SIOP NB has clearly proposed itself as an important instrument to design relevant clinical trials and biological research. The major clinical conclusions of LNESG Trial are presented herewith by Jean Michon, actual E-SIOP NB Chairman, while Maja Nenadov Beck reports on the LNESG 2, the second study on localised resectable neuroblastoma, which is now in the final preparatory steps, due to be launched in Prague.

Three studies are currently open:

(a) **Unresectable Neuroblastoma Protocol**, chaired by Jan Kohler, whose progression is slower than expected, mainly for the delayed adhesion of some major national groups,

(b) **Infant Neuroblastoma Protocol**, conducted by Mary Gerrard, made of four Trials, of which Herve' Rubie, Mary herself, Bruno De Bernardi, and Adele Cañete are the persons in charge,

(c) **High-Risk Protocol (HR-NBL-1)**, officially opened on February 2, 2002, headed by Ruth Ladenstein. Possibly, new groups from Eastern Europe and Israel will join this exciting study.

The E-SIOP NB counts on the indispensable activity of several Sub-Committees. While in this issue space is given only to two of them, (a) Bone Marrow Studies, and (b) Pathology, the following issues will be open to the reporting of the remaining ones.

For special clinical problems, such as *Opsoclonus Myoclonus Syndrome*, world-wide cooperation is essential. Andy Pearson reports here on a forthcoming protocol on a project which sees the two sites of the Atlantic working closely together.

Finally, I remind you a couple of important meetings:

- **Advances in Neuroblastoma Research #9, Paris, June 16-18, 2002**
- **E-SIOP NB Annual Meeting, Prague, November 8-9, 2002.**

Do not miss them !

Please note that E-SIOP NB Newsletter is committed to keep you informed regularly from now on !!!

Bruno De Bernardi, for the E-SIOP NB Board

Report on Protocols

Localised Neuroblastoma (LNESG 1)

Opened 1.11.1995
Closed 11.1999. Up-date 5.2002

Objective of the LNESG 1 trial was to evaluate safety and efficacy of surgery as the only treatment in the management of INSS Stage 2 neuroblastoma without *MYCN* amplification, and to describe prospectively risk factors predicting relapse. Patients suspected to have a localised neuroblastoma were registered before surgery. Tumour resectability was evaluated following established radiological guidelines. *MYCN* copy number as well as disease extension evaluation, using INSS criteria, were mandatory. Patients were eligible either in the **Trial** (if stage 2 without *MYCN* amplification), or in the **Study** (all other pts with localised NB). Prospective evaluation of histopathological features (Shimada and/or INPC), serum LDH level, ploidy and 1p deletion were recommended. Between 01.95 and 10.99, 937 pts were registered from 12 countries. In 12.2001, the last analysis was performed on 741 eligible pts (including 616 in the Study : 333 stage 1 , 42 stage 2 and 241 stage 3): 125 stage 2 pts were eligible in the Trial (55 stage 2A, 70 stage 2B) with a median follow-up of 44 months (0-76). 21 relapses occurred 1 to 33 months after surgery: 19 in the primary tumour site or regional lymph nodes and 8 in distant sites. 7 pts died of disease progression 10 to 56 months after surgery. Overall survival at 3 year is 94.4 [95% CI : 89.9-98.8]. The 3-year relapse rate (RR) is 18.6 % [95% CI : 11.4-25.9]. Shimada unfavourable histology, both locally evaluated and centrally reviewed by using INPC criteria, abnormal LDH plasma level, and unequivocal 1p deletion correlated with relapse. Neither age, nor ploidy did influence outcome. In conclusion, surgery alone is a safe approach for most pts with stage 2 neuroblastoma. The present study has identified biological characteristics associated with a higher probability of relapse.
Data on follow-up have been updated in May this year and results will be presented during the ANR 2002 meeting in Paris. This first step toward common evaluation and treatment of neuroblastoma patients in Europe has taught evaluation (histology and genotyping) without central tumor banking.

Jean Michon

Infant Neuroblastoma

Opened 1.11.1999
Up-date 30.5.2002

The Trial co-ordinators recently reviewed the latest report from the data centre produced from data submitted in December 2001.
We are concerned there continue to be problems with missing data. This has made proper assessment of the Trials extremely difficult at the present time. At the time the report was produced almost 500 queries had been sent out from the data centre, and only 25% had been returned. This has led to the situation where it is impossible to report an analysis of the data with any confidence.

Additional concerns relate to the number of patients who are ineligible for entry onto the trial for reasons such as late registration, or failure to perform the investigations as per the protocol.
We want to remind that if you register a child with this study it is essential that data are registered within 6 weeks of diagnosis. To be included in the Trial the results of the *MYCN* studies done in one of the accredited laboratories must be available by this time, otherwise the child can only be registered with the study rather than with any of the Trials. In addition the metastatic work up should be carried out in line with the recommendations in the protocol.

We apologise for the necessity of having to draw your attention to these problems, particularly if your data return has been amongst the "good" 25% but hope you agree that if children are registered and treated according to this Trial protocol, we all have a responsibility to ensure that accurate data are submitted on time.

Infant Neuroblastoma Protocol. Enrollment

	99.1	99.2	99.3	99.4	All Trials	Study	TOTAL	
	No.	No.	No.	No.	No.	No.	No.	(%)
Austria	3	5	1	3	12	17	29	(10.1)
Belgium	4	3	1	0	8	12	20	(6.9)
Eire	0	0	0	1	1	0	1	(0.3)
France	17	10	8	2	39	39	76	(26.6)
Italy	23	15	1	4	43	40	83	(29)
Norway	0	2	0	0	2	0	2	(0.6)
Spain	5	5	1	1	12	27	39	(13.6)
UK	5	8	4	3	20	16	36	(12.5)
Total	57	48	16	14	135	151	286	(100)

Among the 151 patients included in the trial 68% were not eligible in any trial since they had a Stage 1, 2 or 3 resected. 32% were not included for various reasons: no parental consent, no *MYCN* study, delay in eligibility decision, treatment performed not in accordance with protocol.

Mary Gerrard and Jean Michon

Unresectable Neuroblastoma

Opened 1.1.2001
Up-date 30.5.2002

Accrual

Country	Prereg	Registered	*MYCN* not amp	Baseline forms for eligible patients	Treatment completed
Belgium	3	0			
Italy	11	11	8	8	4
Portugal	2	0			
Spain	6	6	4	3	1
UK	6	5*	5	1	1
TOTAL	28	22	17	12	6

*** including one patient 2 days before first birthday**

Toxicity: No deaths/relapses reported. One grade 4 toxicity reported.

Comments:

- Accrual has been slow
- Not all pre-registered patients are being registered
- There are too many missing forms – please send them as soon as possible!

Jan Kohler

Localised Neuroblastoma #2 (LNESG 2) (in preparation)

Myself, Alessandro Jenkner, Annabel Foot, Nicole Gross, Paolo Bruzzi and Keith Holmes are working since one year to design the second study on localised newly diagnosed neuroblastoma without *MYCN* amplification. The LNESG 1 data have indicated that few of such patients are candidate to relapse, the chance being significantly increased if pathology has unfavourable INPC features. We are then considering to give these patients a short period of chemotherapy with the aim to reduce the risk of relapse.
Logistically, the challenge will be in the necessity of having the histology status validated by the National Pathology Panel within 6 weeks from surgery. We plan to be able to distribute the protocol in Prague. Its data base will be located in Genova.

Maja Nenadov Beck

High-Risk Neuroblastoma (HR-NBL-1)

After two years of strenuous efforts E-SIOP NB has been granted the EC money to build up an **European Neuroblastoma Network**. We have now entered the contract negotiations phase. Each National Co-ordinator and Sub-Committee Chair Responsible have defined their aims within the EC project. Nevertheless the complete network and the individual requirements and tasks that need to be fulfilled, as well as dead-lines that need to be met for the EC grant still need to be communicated to all the involved persons. A specific meeting to provide you with this important information is scheduled for June 16th, the day before the ANR meeting opens. We will convene from 9.00 AM to 5.00 PM at the Institute Curie, 26 rue d'Ulm, room Lacassagne in the Research Facility, with the following agenda:

1) general aims and goals of the EC grant
2) rules of the EC
3) structure of consortium and working group
4) IT project
5) aims, duties, and milestones of the individual partners (National Coordinators, IT Partner, Subcommittees (Biology, Bone Marrow, Immunotherapy, Nuclear Medicine and Physics, Pathology, Pharmacology, Radiology, Radiotherapy, Stem Cell, Surgery)
6) HR-NBL1 Study-Database and Website: Presentation of new achievements and completion of the full documentation by Guenther Schreier
7) room for discussion (problems, practical concerns, etc.).

I look very much to meet you on June 16th for this important event !!!

Ruth Ladenstein

Therapy for Resistant-Relapsing High Risk Neuroblastoma

Patients who fail to achieve an adequate remission status, as defined in high-risk neuroblastoma, are not eligible to proceed to myeloablative therapy. E-SIOP NB strongly aims to develop a series of protocols that will be open to such patients, thereby advancing knowledge of the therapy of neuroblastoma.
Dr Simon Meller and Dr Mark Gaze are currently co-chairing a small core-working group of Huib Caron, Genevieve Laureys, Thomas Klingebiel and Riccardo Riccardi to develop a phase II mIBG/ topotecan protocol for patients who are resistant to therapy.
A regimen developed in Italy of topotecan and doxorubicin is currently being considered as an alternative phase II therapeutic approach. For members of Euro-NAG, the option of treating the patients with the phase II study of temozolomide is in the near future a possibility. During the period that these studies are being developed, E-SIOP NB advises that patients, who are not eligible to progress to myeloablative therapy, receive two courses of CADO and are then re-evaluated to a full response.

Andy Pearson

Sub-Committees Reports

1. Bone Marrow Studies

After meeting #4

The Subcommittee in activity concerns the immunocytochemical evaluation of minimal residual disease by means of the anti-G_{D2} antibody on bone-marrow and peripheral blood samples from neuroblastoma patients.
The aim of the meeting was the microscopic evaluation of the Quality Control slides, previously circulated among the SC members, in order to define clear and reproducible criteria by which to analyse the samples actually examined in the various national laboratories. Every European country currently participating in the SC was represented (namely Austria, Belgium, France, Germany, Great Britain, Italy, Norway, Spain and Switzerland).
The meeting was carried out in a very practical manner: samples previously chosen by each member were jointly reviewed under a multi (13) headed-microscope. Each member proposed one case, showed his/her own results and started the discussion, which was aimed at reaching a consensus on any discrepancy which might have arisen during the evaluation.
The whole of Friday was devoted to this long, constructive and sometimes controversial debate. During the many hours spent at the microscope – in a typically histopathologist-like manner – we all learned from each other's experience, sharing opinions, doubts, problems and possible solutions. In the end, a final consensus was reached, which in turn gave rise – on the following day – to the detailed listing of collegially accepted criteria of evaluation, together with agreed upon methods of handling the specimens and carrying out the immunocytological staining procedures.
The listed criteria include data related to the main characteristics of the immunohistochemical staining, in terms of intensity, quality and cellular compartment localization of the staining itself. Also taken into account were the patterns based on the morphological features that the investigated cells commonly show on routine cytological examination.
The issue of the possibility of further studying individual cases by means of molecular biology or cytogenetic methods was also addressed, thus opening the door to possible future lines of research and co-operation with the RT-PCR study group.
Finally, the group agreed upon the format to be used in reporting the results of the evaluation, in order to communicate with the clinicians in a standardised way. Unequivocal expression of the results of the G_{D2} immunocytochemical investigation will be the basis of any future correlation with data from the clinical follow-up.
The Group worked hard and fruitfully; our joint efforts both increased the concordance of the results obtained and strengthened friendship among the members of the SC, which is no less important if we are to obtain a result that is meaningful for both our clinician colleagues and the patients.

Angela Sementa

2. Pathology

After meeting #5

The pathology subcommittee (Gabriele Amman, Klaus Beiske, Catherine Cullinane, Emanuele S.G. D'amore, Claudio Gambini, Samuel Navarro, Michel Peuchmaur) was created to assure a quality control on both the diagnosis of neuroblastoma and its subclassification. The age-linked INPC scheme ("Shimada system") provides important prognostic information in some stages of this pediatric neoplasm but it requires great experience and skill to achieve good reproducibility, especially for the evaluation of the MKI and of the different stroma poor and ganglioneuroblastoma subtypes.

The pathology sub-committee focused its attention on the 125 neuroblastoma stages 2A and 2B enrolled in a clinical trial of the SIOP Protocol LNESG1. During 5 meetings held in different European Countries between January 2000 and June 2002, 113 out of 125 cases were reviewed by the pathology panel using a

multihead microscope and a procedure that includes the following steps:

- initial review of all the available slides without knowledge of the clinical information; each participant makes a 'blind' independent diagnosis and MKI calculation ;
- discordant cases are reviewed again and discussed thoroughly to clarify the reasons of the discrepant diagnoses.
- if possible a consensus diagnosis is generated
- a pathology form is filled to finalize the review.

Preliminary statistical analysis has demonstrated that LDH, INPC classification and 1p status are important prognostic factors in stage 2 neurablastoma treated only by surgery. For this reason the E-SIOP NB group is discussing strategies to assure a rapid (possible on-line) revision of all the cases enrolled in next LNESG2 protocol.

In addition during next meeting in Paris, May 14-17, 2002 the Pathology panel will decide how to proceed for the revision of the neuroblastoma cases enrolled in all the other clinical protocols.

Finally, the ESIOP Pathology subcommittee was also contacted by the International Neuroblastoma Pathology Committee (INPC), lead by Dr. Hiro Shimada, and 2 of us (the writers of this report) have been invited to enter this intercontinental panel. In a recent meeting in Tokyo (May 2002) 70 cases of nodular ganglioneuroblastomas have been restudied in order to refine the criteria for the classification of this subtype. In addition an atlas (both printed and on CD-ROM) illustrating the main histopathologic criteria for the diagnosis of neuroblastoma is going to be prepared by the end of the current year.

Emanuele S.G. d'Amore and Michel Peuchmaur

Forthcoming Studies

Combined E-SIOP NB – Children's Oncology Group Study of Opsoclonus-Myoclonus

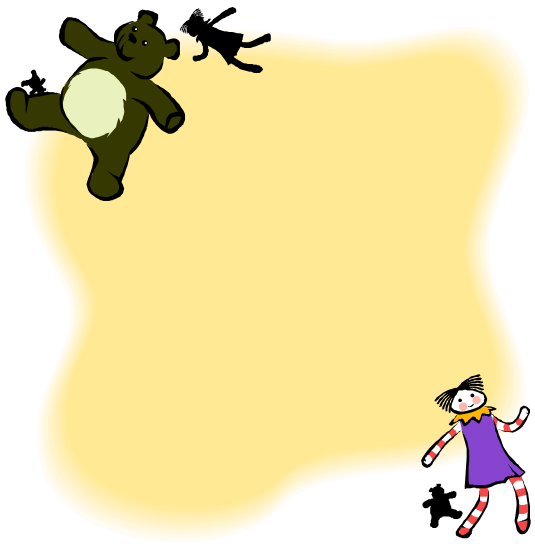
Following very enthusiastic support for the concept of a joint E-SIOP Neuroblastoma and Children's Oncology Group of North America study of opsoclonus myoclonus at the June meeting in Vienna, discussions have been held throughout the last year, both initially within Europe and then subsequently with the Children's Oncology Group. The E-SIOP NB Neuroblastoma Group is led by Dominique Plantaz and the first meeting was held in January 2002 in London. The Writing Committee for the study (Dominique Plantaz, Franz Blaes, Peppy Brock, Barbara Hero, Genevieve Laureys, Ingrid Ora, Ingebjorg Storm-Mathisen, Peter Beverley, Bethan Lang, Vito Pistoia, Antoine Carpentier, Carlos De Sousa, Mike Pike, Frank Berthold, Hugh Perry, Jon Pritchard and Kate Wheeler) included paediatric oncologists, paediatric neurologists and basic scientists with an interest in the disease. Discussion with representatives of the Children's Oncology Group – Pedro De Alarcon and Kate Matthay - confirmed that there was great interest in carrying out a collaborative study. The importance of the study in treating all patients with the rare complication of opsoclonus-myoclonus in the same manner has been widely agreed. In addition, there is a major opportunity to carry out vital biological studies to aid the understanding of the cause of the condition.
The paediatric neurologists, in Europe, are very keen that patients with opsoclonus-myoclonus, in whom no neuroblastoma can be detected, are also included in the study.

The mainstay of treatment has been agreed to be prednisone (although ACTH has been used very widely in North America). Two additional modes of therapy – immunoglobulin and immunosuppression with cyclophosphamide – have, in a number of studies, been suggested to be of therapeutic benefit. Discussions are at present ongoing as to which of these modes of therapy should be randomised to these patients.

An additional challenge to the design of the protocol is that the important endpoint of the study is long term, neurological and intellectual functioning.

This study has great potential in being the first E-SIOP NB and Children's Oncology Group joint study.

Andy Pearson



Important publications on neuroblastoma,
suggested by Peter Ambros

Pathology
Ambros IM, et al. Morphologic features of neuroblastoma (Schwannian stroma-poor tumors) in clinically favorable and unfavorable groups. Cancer 2002; 94(5):1574-83

Ikeda H, et al. Experience with International Neuroblastoma Staging System and Pathology Classification. Br J Cancer 2002 Apr 8;86(7):1110-6

Neuroblastoma Screening
Schilling FH, et al. Neuroblastoma screening at one year of age. N Engl J Med 2002 Apr 4;346(14):1047 53

Woods WG, et al. Screening of infants and mortality due to neuroblastoma.
N Engl J Med 2002 Apr 4;346(14):1041-6

Basic Science
Beltinger C, et al. TRAIL enhances thymidine kinase/ganciclovir gene therapy of neuroblastoma cells. Cancer Gene Ther 2002; 9(4):372-81

Fulda S, et al. IFNgamma sensitizes for apoptosis by upregulating caspase-8 expression through the Stat1 pathway. Oncogene 2002; 21(15):2295-308

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Genova, May 2002