E-SIOP Neuroblastoma

NEWSLETTER

#5 May 2004

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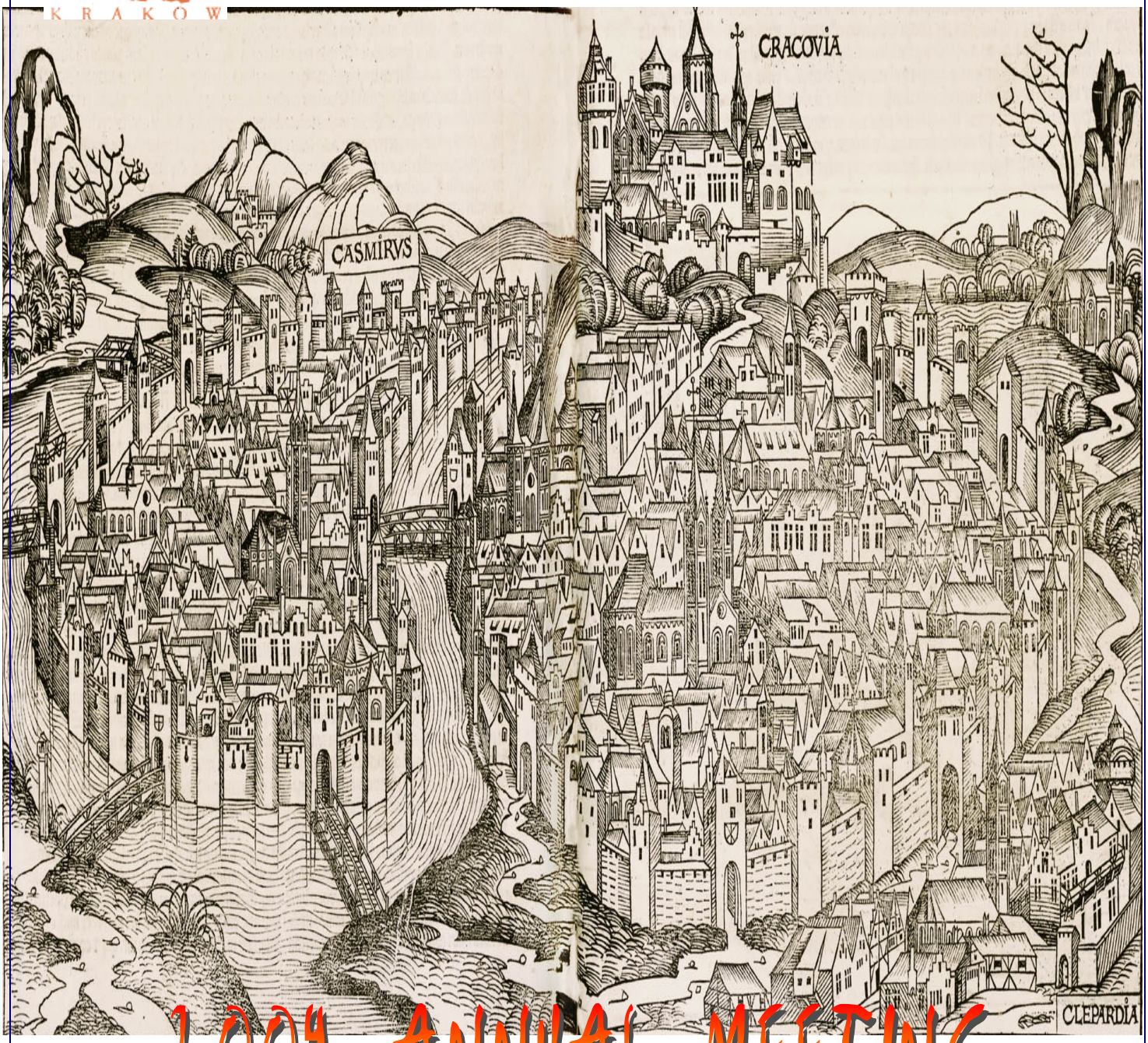


2004 ANNUAL MEETING

Krakow, Poland, October 22-23, 2004

E-SIOP Neuroblastoma Annual Meeting Krakow, 22-23 October 2004

REGISTRATION FORM

Surname _____

First name _____

Address _____

_____

Zip Code _____ **City** _____

Country _____

Telephone _____ **Fax** _____

E-mail _____

I plan to attend the Meeting:

22 October **Yes** ☐ **No** ☐

23 October **Yes** ☐ **No** ☐

I plan to attend the Social Dinner:

22 October **Yes** ☐ **No** ☐

I'd like to organise a Sub-Committee Meeting and I need a room for ….. persons:

Name of the Sub-Committee:……

Time of the Meeting:……

Please send a Fax to Dr WALENTYNA BALWIERZ
+48-12-658-02-61

ANR 2004

Advances in Neuroblastoma Research 2004

Genova - June 16-19, 2004

Programme

THURSDAY, JUNE 17, 2004

8:15 AM	**Welcome by the Local Organising Committee** **Introductory Remarks, *by Audrey Evans***
8:30 – 10:30 AM	**PLENARY SESSION A: Genetics** Chairmen: Akira Nakagawara (J) - Manfred Schwab (D)
10:30 – 11:00 AM	**Break**
11:00 AM – 01:00 PM	**PLENARY SESSION B: Biology** Chairmen: Nai-Kong Cheung (US) - Jerry Melino (I)
1:00 – 2:00 PM	**Lunch**
2:00 – 3: 30 PM	**PARALLEL SESSION A: Translational** Chairmen: Michelle Haber (AUS) – Carol J Thiele (US)
	PARALLEL SESSION B: Clinical Chairmen: Frank Berthold (D) – Robert Castleberry (US)
3:30 – 4:00 PM	**Break**
4:00 – 5:00 PM	**POSTER DISPLAY** **ROOM A Biology – Clinical – Genetics** **ROOM B Genetics - Molecular Biology - Translational**
5:00 – 7:00 PM	**SELECTED POSTERS PRESENTATION** Chairmen: Kate K Matthay (US) – Gian Paolo Tonini (I)
7:00 – 9:00 PM	**Opening Ceremony & Welcome Reception**

FRIDAY, JUNE 18, 2004

08:30 – 10:30 AM	**PLENARY SESSION C: Translational** Chairmen: Jean Bénard (F) - Mirco Ponzoni (I)
10:30 – 11:00	**Break**
11:00 AM – 1:00 PM	**PLENARY SESSION D: Molecular Biology** Chairmen: C Patrick Reynolds (US) - Massimo Romani (I)
1:00 – 2:00	**Lunch**
2:00 – 3:30	**PARALLEL SESSION C: Biology** and **Genetics** Chairmen: Peter Ambros (A) - Rogier Versteeg (NL)
	PARALLEL SESSION D: Clinical Chairmen: Ruth Ladenstein (A) – Michio Kaneko (J)
3:30 – 4:00 PM	**Break**
4:00 – 5:00 PM	**POSTER DISPLAY** **ROOM A** **Biology – Clinical – Genetics** **ROOM B** **Genetics - Molecular Biology - Translational**
05:00 – 07:00	**SELECTED POSTERS PRESENTATIONS** Chairmen: Vito Pistoia (I) - Robert C Seeger (US)
8:30 – 11:00 PM	**Gala Dinner**

SATURDAY, JUNE 19, 2004

08:20 AM	**Programme of the day**
08:30 – 10: 30 AM	**PARALLEL SESSION E: Molecular Biology – Translational** Chairmen: Garrett M Brodeur (US) - Giuseppe Raschellà (I)
	PARALLEL SESSION F: Clinical Chairmen: Adela Cañete (E) - Hervé Rubie (F)
10:30 – 11:00 AM	**Break**
11:00 – 12:30 AM	**PLENARY SESSION E: Translational – Clinical** Chairmen: Audrey Evans (US) – Andrew DJ Pearson (UK)
12:30 AM	**Assignment of ANR 2004 Awards** **Conclusive Remarks, by *Akira Nakagawara***

programme

Application of Microarray-Based Technology to Neuroblastoma

Wednesday June 16, 2004

Workshop

3:00 – 7:00 PM **Hall "Scirocco & Libeccio"**

The completion of the human genome sequence has provided a framework for the molecular analysis of cancer. Microarray technology allows for simultaneous analysis of thousands of genomic and/or transcriptomic alterations within tumor cells. In the most recent Advances in Neuroblastoma Research meeting, many groups presented papers utilizing these evolving tools. The aim of the Workshop is intended to focus on the great potential, but also the possible pitfalls of this promising technology, on how it can be applied to the molecular analysis of neuroblastoma, and on its integration with other technologies, with the aim to develop a better understanding of the alterations of this paediatric tumour, and to identify novel diagnostic markers and therapeutic targets.

10' **Introduction to microarray technology: Basic principle and pre-clinical approach**
Gian Paolo Tonini, *National Institute for Cancer Research, Genoa, Italy*

Gene expression - Session chaired by Rogier Versteeg (NL)

30' **Global gene expression analyses in neuroblastoma: What do we learn from different techniques?**
Frank Westermann, *German Cancer Research Centre, Heidelberg, Germany*

15' **Quantitative profiling.**
Rogier Versteeg, *University of Amsterdam, Amsterdam, Netherlands*

15' **Use of oligonucleotide microarrays versus SAGE versus proteomics in neuroblastoma cell culture models: Can we compare the data and what do we learn from them?**
Angelika Eggert, *University Children's Hospital of Essen, Essen, Germany*

15' **Designing a specific Neuroblastoma Oligonucleotide Microarray**
Matthias Fischer, *University Children's Hospital, Cologne, Germany.*

15' **Identifying subsets of metastatic neuroblastomas and cell lines with oligonucleotide microarray expression profiling.**
Robert C Seeger, *Childrens Hospital Los Angeles Research Institute, Los Angeles, USA*

15' **Response of neuroblastoma to hypoxia: An approach with microarray technology**
Luigi Varesio and Gian Paolo Tonini, *Giannina Gaslini Children's Hospital and National Institute for Cancer Research, Genova, Italy*

15' **Coffee Break**

DNA gain and loss - Session chaired by John M Maris (USA)

30' **The impact of integrated analysis of genetic and genomic alterations using microarrays in neuroblastoma**
Akira Nakagawara, *Chiba Cancer Center Research Institute, Chiba, Japan.*

30' **Comparison of arrayCGH versus oligonucleotide microarrays (Affymetrix GeneChip® Mapping 10K Array and Assay Set) for high-resolution analysis of DNA copy number in neuroblastoma**
Frank Speleman, *Gent University Hospital, Gent, Belgium*

15' **Dissecting the genome of human neuroblastomas with array CGH**
Yael Mosse, Philadelphia Childrens Hospital, Philadelphia, USA

15' **Use of CGHarray to identify DNA gain and loss in neuroblastoma.**
Paola Scaruffi and Stefano Moretti, *National Institute for Cancer Research, Italian Neuroblastoma Foundation, Genova, Italy*

15' **Study of genetic rearrangements in neuroblastoma tumors using commercial pangenomic CGH arrays and dedicated laboratory-made BACs arrays.**
Alexander Valent, *Institut Gustave Roussy, Paris, France*

15' **Conclusion**
John M. Maris, *The Children's Hospital of Philadelphia, Philadelphia, USA*

Workshop

Spinal Cord Compression

Saturday June 19, 2004

2:00 - 5:00 PM **Hall "Levante"**

A minority of children with neuroblastoma (mostly localised) present with symptomatic spinal cord compression. Although this complication does not influence the eventual outcome, it may heavily compromise the quality of life, if not timely recognised and promptly treated. However, there is little agreement on the optimal way to treat this condition. Neurosurgeons, oncologists and sometimes radiotherapists often compete on who should act first. There is also little information on the long-term side results of these treatments.
This Workshop intends to focus on the unclarified issues of this complication and lay the foundations of a more exhaustive meeting that might take place in one year.

Chairpersons	**Bruno De Bernardi, Dominique Plantaz**	
2:00 PM	***Introduction***: Audrey Evans	*(Philadelphia, USA)*
2:10	***Literature Review***: Dominique Plantaz	*(Grenoble, France)*
2:25	***Pathophysiology***: Jean-Guy Passagia	*(Grenoble, France)*
2:40	***Neuroradiology***: Paolo Tortori Donati	*(Genova, Italy)*
2:50	***Case series with comments by neurosurgeons and radiotherapists***	
	Walentyna Balwierz	*(Warsaw, Poland)*
	Joanna Begent	*(London, UK)*
	Frank Berthold	*(Koeln, Germany)*
	Sue L Cohn	*(Chicago, USA)*
	Kim Kramer	*(New York, USA)*
	Dominique Plantaz	*(Grenoble, France)*
	Maria Luisa Garré	*(Genova, Italy)*
	Neurosurgeons	
	Armando Cama	*(Genova, Italy)*
	Jean-Guy Passagia	*(Grenoble, France)*
	Radiotherapist	
	Guido Sotti	*(Padova, Italy)*
4:50	***Late Effects***: Paola Angelini	*(Genova, Italy)*
5:00	**Conclusion**	

Opsoclonus Myoclonus

Saturday June 19, 2004

Workshop

2:00 - 5:00 PM **Hall "Ponente"**

This Workshop intends to focus on the pathogenesis, clinical features and therapeutic approach of opsoclonus-myoclonus syndrome (OMS), with emphasis on the cases occurring in association with neuroblastoma. Diagnosis and follow-up of neurological manifestations will be covered in detail in view of the novel therapeutic protocol recently proposed by the COG and based upon the use of the immunosuppressive and cytotoxic agent cyclophosphamide. This topic will be the matter of a specific presentation pointing to the feasibility of cyclophosphamide treatment in neuroblastoma-associated OMS. Hopefully, the Workshop will allow to reach a consensus on different aspects of this severe and disabling syndrome, for which no efficacious treatment has been identified so far. International collaboration on such a rare disorder is warranted to radically change the prognosis of OMS.

2:00 ***K.K. Matthay***: Overview of NB-associated OMS: diagnosis, prognosis, immunological aspects and long term outcome

2:20 ***M. Pike***: Diagnostic criteria and neurological evaluation of OMS patients

2:40 ***W. Mitchell***: Follow up, late complications and treatment of OMS patients

3:00 ***V. Pistoia***: Lymphoid infiltration in OMS-associated neuroblastoma

3:15 ***F. Blaes***: Immunopathogenesis of OMS

3:30 ***P. De Alarcon***: US COG-protocol for NB-associated OMS

3:45 ***B. Hero***: Use of cyclophosphamide in the treatment of OMS

4:00 ***M. Pike and W. Mitchell***: The neurologists' opinion on COG protocol

4:20 **General discussion**

5:00 **Conclusion**

INTERNATIONAL NEUROBLASTOMA PATHOLOGY COMMITTEE MEETING

(by invitation)

Giannina Gaslini Children's Hospital
Genova,
June 20-22, 2004

AGENDA

Sunday, June 20

9:00 - 10:00 AM	Welcoming & Activity report
10:00 - 10:30	*Coffee Break*
10:30 - 12:30	Slide Review
12:30 – 1:30 PM	*Lunch*
1:30 - 3:30	Slide Review
3:30 – 4:00	*Coffee Break*
4:00 – 6:00	Slide review

Monday, June 21

9:00 - 10:00	Slide Review
10:00 - 10:30	*Coffee Break*
10:30 - 12:30	Slide Review
12:30 – 1:30 PM	*Lunch*
1:30 - 3:30	Open Seminar
3.30 – 4:00	*Coffee Break*
4:00 – 6:00	Slide review

Tuesday, June 22

9:00 - 10:00	Slide Review
10:00 - 10:30	*Coffee Break*
10:30 - 12:30	Slide Review
12:30 – 1:30 PM	*Lunch*
1:30 - 3:30	Open Seminar
3:30 – 4:00	*Coffee Break*
4:00 – 5:00	Slide review
5:00 - 6:00	Business Meeting

Open Seminars

Aula Magna – Giannina Gaslini Children's Hospital

June 21
2:30-3:30 PM **Vijay V Joshi**: Non-Wilms' Tumors in Children

June 22
1:30-2:10 PM **Jun-ichi Hata:** Molecular Pathology of Wilms' Tumors
2:10-2:50 **Michel Peuchmaur:** Advances in Berger's disease
2:50-3:30 **Hiroyuki Shimada:** Pathology Review for the Children's Oncology Group Neuroblastoma Study

MEETING TO DISCUSS INTERNATIONAL NEUROBLASTOMA RISK GROUPS

JUNE 16, 2004 – 7:30-10:30 PM (GENOVA, JOLLY MARINA HOTEL)

Victoria Castel, Bruno De Bernardi, Frank Berthold and Andy Pearson believed that there was a need to reach an international consensus regarding International Neuroblastoma Risk Groups, particularly the definition of stage 3/unresectable neuroblastoma.

The major paediatric oncology co-operative groups (Children's Oncology Group of North America, SIOP Europe Neuroblastoma, German Paediatric Oncology Group, Japanese Paediatric Oncology Group and Australian Paediatric Oncology Group) all agreed that this would be a very useful initiative. The Forbeck Foundation very generously agreed to fund this. It was felt that, to be maximally effective, a small group should meet and therefore a limited number of representatives from the major international groups are participating. From SIOP Europe Neuroblastoma this includes the Board, Keith Holmes and Tom Monclair representing Surgery, Peter Ambros representing Biology Klaus Beiske representing Bone Marrow assessment and Michel Peuchmaur representing Pathology. Each of the major international groups will present their current risk groups, i.e. the eligibility criteria for the different protocols. It is hoped and expected that, following discussion, a consensus may be reached whereby the different international groups can agree on common risk groups. This would allow a much more meaningful comparison of the results of treatment. The session will be chaired by Sue Cohn and Andy Pearson and Jean Michon will present the information from SIOP Europe Neuroblastoma. The conclusions of the meeting will be presented in the next edition of the SIOP Europe Neuroblastoma Newsletter.

Fourth RT-PCR Working Group Meeting

Tuesday 15th June 2004
14.00-18.00
Building N12, Gaslini Institute, Genoa

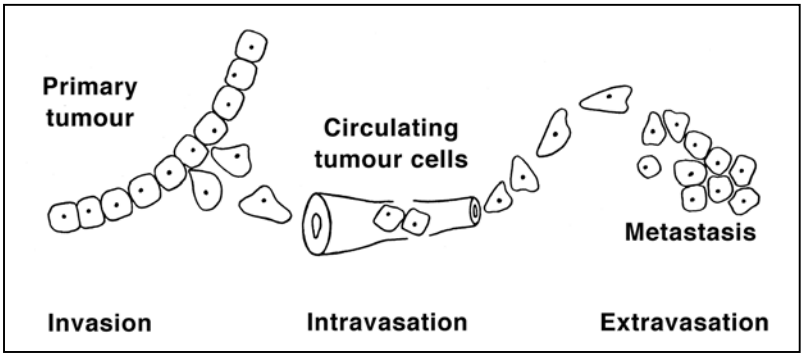
The primary objectives of this meeting are to review

1. *Standard methods for collection, transportation, storage and extraction of RNA from clinical samples across participating countries.*
2. *Quality control series*
3. *Recruitment of patients into RT-PCR biological study*

Detection of neuroblastoma cells by RT-PCR for tyrosine hydroxylase mRNA

***Sue Burchill, +Annelise Bennaceur, #Maria V Corrias, ~Bertil Kagedal, ^ Silvestre Oltra, "Katrien Swerts, 'Ales Vicha,.** *Leeds, UK; +Villejuif, France; #Genova, Italy; ~Linköping, Sweden; ^Valencia, Spain; "Gent, Belgium; 'Prague, Czech Republic.

This biological study is in conjunction with the clinical trial HR-NBL1/ESIOP, a multi-centre international randomised phase III study in children with high-risk neuroblastoma (NBL).



The biological study is observational and will not impact on clinical management of patients in this trial. Whether the eradication of minimal residual disease by treatment with 13-cis retinoic acid (Matthay et al N Engl J Med: 341; 1165-1173. 1999; Villablanca et al. J Clin Oncol: 13; 894-901.1995) with or without ch14.18 anti-GD2 monoclonal antibody (Manzke et al. Med Pediatr Oncol: 36; 185-189.2001; Fukuda et al. Int J Mol Med: 2; 471-475.1998; Frost et al. Cancer: 1580 ; 317-333.1997) therapy offers a survival advantage for children with high risk NBL is a critical randomisation question in the current european clinical trial. Since outcome for many of these children is dependent on the presence of metastatic disease, accurate and sensitive assessment of disease status is essential. In this biological study, reverse transcriptase polymerase chain reaction (RT-PCR) for tyrosine hydroxylase (TH) mRNA is used to detect NBL cells in bone marrow aspirates (BM), peripheral blood (PB) and peripheral blood stem cell harvests (PBSC) collected from children through out the treatment protocol. This will be particularly powerful to assess the eradication of minimal residual disease after the final randomisation (R2) for treatment of minimal residual disease in HR-NBL1/ESIOP.

The detection of clinically significant low volume disease by RT-PCR may provide risk information on patients at diagnosis and through out disease course, providing a method to assess response to therapy and monitor disease course during and after therapy. This is likely to improve clinical management of NBL, with the aim of increasing survival from this disease.

To ensure robust and reliable information is acquired from this multi-centre, prospective clinical outcome study the priority for the RT-PCR task force has been to develop standard operating procedures for optimal clinical sample collection, storage and processing. PAX gene blood RNA tubes are practical for collection, storage and transportation of clinical samples across multiple centres. Samples are stable when stored in PAX gene tubes at -80ºC. To overcome variability in sample volumes, each sample is to be weighed before RNA is extracted; this will allow an assessment of tumour contamination in a fixed volume of sample.
The PAX gene blood RNA kit is useful for the isolation of RNA from 2ml blood samples, however the capacity of the column used in this kit is insufficient to isolate all the RNA from 0.5ml of diagnostic bone marrow. However, this can be overcome by isolating RNA using a Midi RNA column and treating samples with DNAse 1 whilst on the column to reduce the amount of contaminating DNA in the isolated RNA, with no loss of RNA yield.

Through quality control rounds it is clear that analysis of cDNA across all laboratories is sensitive and specific, confirming that the PCR reaction is robust in all participating laboratories. Although the specificity of RNA analyses was good, there was unacceptable variability in sensitivity. Standardisation of the amount of RNA, RT conditions and source of RT enzyme increased the sensitivity of tyrosine hydroxylase mRNA detection to 10pg or 1 cell. Such international quality assurance programmes are important to ensure accuracy of molecular assays and optimal information from this clinical trial.

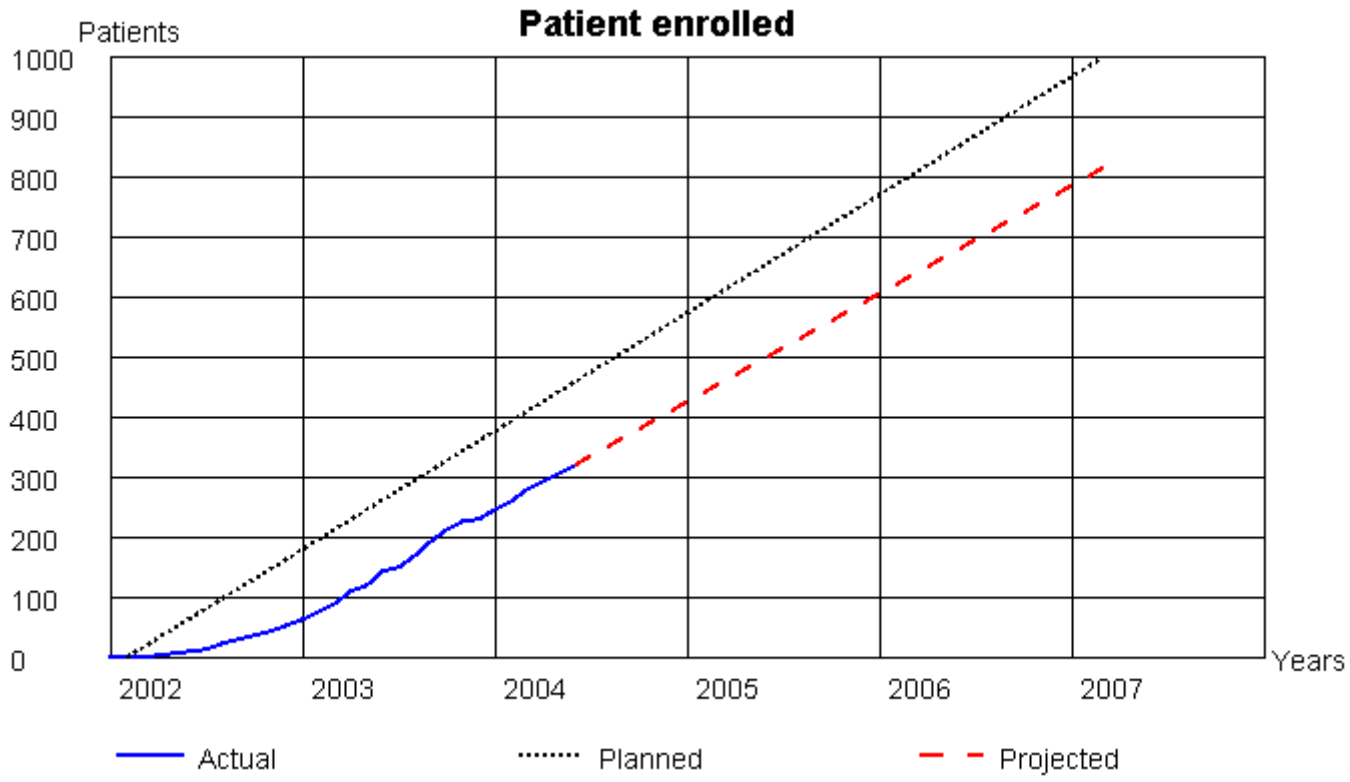
☺Thank you for your help collecting samples for this important biological study☺

SA Burchill
Cancer Research UK Clinical Centre,
St James's University Hospital,
Beckett Street, Leeds LS9 7TF.

NEWS FROM THE HR-NBL-1/ESIOP STUDY
on behalf of the Study Steering Committee
from Ruth Ladenstein (Study Coordinator)

ADMINISTRATIVE INFORMATION

Study Status		2004-06-03		
Cornerstones				
	Date of study start	2002-02-02		
	Date of study end (planned)	2007-02-02		
	Total study time in years // months // days	5 // 60 // 1826		
	Patients to be enrolled	1000		
Current Status		Achieved	[%]	Left
	Days Running	852	46.6	974
	Patients enrolled	319	31.9	681
Statistics		Monthly	Annually	
	Planned accrual rate	16	200	
	Current accrual rate (3 months average)	15.3	183	
	Projected final accrual (projected)	815		
	Required accrual rate (for planned total)	20	240	



HR-NBL 1/ESIOP STUDY STEERING COMMITTEE MEETING

Vienna 30. April 2004 – 1. May 2004

Participants:
Peter Ambros, Bruno de Bernardi, Walentyna Balwierz, Klaus Beiske, Victoria Castel, Sandro Dallorso, Alberto Garaventa, Ruth Ladenstein, Genevieve Laureys, Holger Lode, Roberto Luksch, Jean Michon, Maya Nenadov-Beck, Vassilios Papadakis, Andy Pearson, Frida Ponthan, Ulrike Pötschger, Günther Schreier, Dominique Valteau, Isaac Yaniv.

The aims of this meeting were to review accrual, randomisations rates, SAE reporting, toxicities and the current status of the ch14.18 AntiGD2 antibody production. This was a very fruitful meeting with lively discussions over two days and major achievements for the integrity of our group.

Two major contribution prior to the steering committee meeting:

- **The Statistical Subcommittee have reviewed the open data and helped to prepared the DMC report. The conclusion given by the SC were the following statements:**
  1. The overall response-rate following COJEC induction is very much in line with the results previously published (De Bernardi *et al*. 2003 *Journal of Clinical Oncology*, **21**,1592-1601).
  2. The type and level of toxicities are as anticipated from those reported for the ENSG5 trial.
  3. There is no evidence to suggest that G-CSF has any adverse effect on the ability to complete stem cell harvest.
  4. At the current rate of recruitment, there will be sufficient patients to answer the G-CSF-question (R0) by the end of 2004. As a consequence, first steps in the preparation of the publication should be instituted.
  5. Although the randomisation rate to R1 is somewhat lower than had been specified in the protocol, at 50% it is broadly in line with what might be expected. It is likely to increase as the window for randomisation has been extended to 150 days.
  6. The prospect of the R0 trial completing within 2004, provides an opportunity to review the choice of induction regimen for the R1 trial as from 01 January 2005.
  7. A strong recommendation was made that other centres should be encouraged to join the R0 trial so as to complete it with minimum delay. Likewise an active search for new centres to join R1 should be instituted.
  8. A DMC report shall be submitted by mid-March.

- **Second DMC Report (April 13th, 2004)**

Members of the DMC: Dr Kate Matthay; Dr Leslie Robison; Dr David Baker

The DMC had reviewed an extensive report on the study progress in February 2004 and recommended the study to continue to accrue patients but to address the following issues:

(1) Incidence of fungal infections in induction.
(2) Feasibility of conducting the R2 randomisation.
(3) Re-evaluate accruals and closure time for the R1 randomisation.
(4) Consider adopting INRC guidelines for disease response evaluations in induction, MAT and biotherapy phases.
(5) Clarify what the study committee will recommend for induction at the closure of the R0 randomisation at the end of 2004.

An adopted report on HR-NBL-1/ESIOP study results (excluding randomisation data and study endpoints) was distributed to the Board, all National Coordinators of the HR-NBl-1/ESIOP Study and the Subcommittee Chairs. Patients on study in February 2004 and referred to in this report were 264 patients. Current recruitment as of June 3rd is 319 patients. We wish to thank all of you for the enormous input in the study and delivering the necessary data! As a study coordinator I wish to take the opportunity here to express my gratefulness and thanks for the excellent support in the study management by Ditha Modritz and Ulrike Pötschger at the Vienna international data centre.

Major Topics during the study Committee meeting:

- **ch14.18 AntiGD2 antibody production**

Holger Lode reviewed the current status of the ch14.18 AntiGD2 antibody production on behalf of the Immunotherapy Subcommittee Task Group. Details are reported separately in this Newsletter. The group had a chance to encounter Prof. Hermann Katinger from Polymun Company having supported the ESIOP Neuroblastoma Group tremendously. After successful recloning the product is ready now to go into production.

- **Guidelines and Definition for SAE reporting in the HR-NBL1/ESIOP protocol**

Time was devoted to carefully review the SAE section in the report and to discuss more homogenous common definitions on SAEs on how to define expected and unexpected events within this protocol:

The following definitions are standard ICH GCP definitions and are to be included in all protocols.
Any untoward medical occurrence that at any dose:

- results in death;
- is life threatening;
- requires in-patient hospitalisation or prolongation of existing hospitalisation;
- results in persistent or significant disability/incapacity; or
- is a congenital anomaly/birth defect.

Exceptions may be defined to avoid over reporting of expected toxicities within intense oncological treatment protocols, as long as these are clearly stated in the protocol. Truly unexpected SAEs need immediate reporting and need to trigger a high awareness level and rapid review in view of patient safety (for reference see also EC directive April 2003: Detailed guidance on the collection, verification and presentation of adverse reaction reports arising from clinical trials on medicinal products for human use).

Suggested Rating of SAEs in the HR-NBL 1 ESIOP Study:

- **Progression of disease**: expected SAE within a high risk population
- **Death due to tumour progression**: expected SAE within a high risk population

CTC Toxicity Score

- **Haematological toxicity grade 4:** expected SAE
- **Infections**:
  - Life threatening with hypotension: unexpected SAE
  - Major infections:
    a. Expected SAEs
       - septicaemia alone (controlled infection)
       - pneumonia alone
       - urinary infection alone

b. Unexpected SAEs
   - septic shock
   - fungal infection (for prevention and treatment of fungal infections please adhere to UK guidelines)
   - severe soft tissue infection

- **Renal toxicity**:
  - all GFR rates ≥ Grade 2: unexpected SAE

    all GFR rates ≥ Grade 2 ➔ patient is ineligible for R1

  Since recovery of GFR rate is observed the evaluation of GFR should be repeated after 2 to 4 weeks.
- **Ototoxicity**:
  - Brock grade 3: unexpected SAE

    Brock grade 3 ➔ patient is ineligible for R1

    Evaluation of Ototoxicity has to be performed before and after MAT; follow up of ototoxicity is required

- **VOD after MAT/SCR:** expected SAE if not life threatening

● **Accrual on study**

Taking into account the official study start in the individual countries the recruitment is in line with previous expectations. Accrual was also affected by the decision of France to put study recruitment on hold for toxicity concerns since October 2003. Following intense review of data and discussions along with more precise supportive guidelines for fungal infections (see below), the French Group has come to common agreement after the study committee meeting of April 30th to restart accrual on the HR-NBL-1/ESIOP. The loss of patient numbers caused by suspension of French accrual was compensated by additional patients from our newer partners. In case France will restart we might end up with an annual patient recruitment exceeding initial expectations which will influence positively the number of patients for R0, R1 and R2 on trial. In March Greece has started accrual and Switzerland is about to be ready too by now. Contacts with further new partners are underway and integration may be possible in the near future.

● **R0 Randomisation**

Conclusion of GCSF question and subsequent publication of results was judged to be of vital importance and all participants confirmed to aim at completion of R0 by the end of 2004 and reassured to support this goal. Italy will try to convince at least some Italian sub-centres to participate in R0.

Potential impact of conclusion of R0:
- On administration of chemotherapy
- Might potentially increase R1 rates
- Might lower toxicities

The overall Incidence of fungal infections during rapid COJEC is currently 11% (24 cases). The international data centre will continue to chase the data on the type of fungal infections reported (local or systemic, proven or suspected) and their severity. Right now there are 11 cases of confirmed systemic fungal infections reported in the SAE section.

● **Response after end of induction**

Skeletal response on MIBG in patients with skeletal metastases at diagnosis having completed all 8 rapid COJEC cycles is as given:

52% had CR and 31% had PR ➔ 83% MIBG response rate

This is equivalent to results of previous reported studies, but achieved in a shorter time.

The DMC committee recommended adopting the INRC guidelines for response which could provide at least two benefits:

(a) Increase R1 randomisation rates due to the dropping of the 3 spot rule for MIBG response.

(b) Allow more valid comparisons of induction response rates between different international clinical trials, viz. COG A3973 for high risk NBL.

Response evaluation (and stratification for R1) according to INRC guidelines as also proposed by DMC:
- INRC criteria: PR at metastatic sites: all measurable sites decreased by > 50%. Bones and bone marrow: number of positive bone sites decreased by > 50%; no more than 1 positive bone marrow site allowed (one positive marrow aspirate or biopsy allowed for PR if this represents a decrease from the number of positive sites at diagnosis).
- The HR-NBL 1 ESIOP Study required cytomorphological CR in 2/2 BM aspirates for R1 eligibility (only aspirates and not trephine biopsy results considered). The committee decided to consider BM biopsy within the R1 eligibility criteria with effect of 1.May 2004 and to adhere to INRC criteria instead of considering aspirates only.
- However, it was felt that currently we will not change the mIBG 3 spot rule apart from the 50% response cut off in mIBG response since for this the pre MAT review process should be in place for the critical cut off. The online system is currently under development and to be tested by the NucMed & P SC members in the next months. Thus this possibility should be discussed again at the next study committee meeting and may potentially be seen within the frame of revised INRC criteria for mIBG skeletal response evaluation.

● **Improvement of response and R1 rates**

Until R0 is concluded all National Coordinators agreed that the induction treatment will definitely not be modified; potential future prolongation of induction chemotherapy for all patients or patients only who did not achieve response eligibility criteria for R1 may consist of 2 cycles TVD. The response rates of TVD and CADO will be evaluated before further discussion based on currently treated patients in the study population who went off protocol. Since Rapid Cojec induction is short we may consider to improve the response rate further with the introduction of an doxorubicin containing regimen at least in view of an eligible R2 population.

The aim is to achieve an R1 rate of at least 50-55% which has been attained with the ongoing rapid COJEC regimen by France, Israel, Austria, Denmark and Czech Republic.

● **Integration and feasibility of R2**

The R2 question could be answered in a reasonable time frame by increase of number of eligible patients.

Patients for R2 antibody question can be recruited of different cohorts

a. Patients with rapid COJEC induction, followed by R1 – MAT and radiotherapy (regular treatment according to HR-NBL 1 ESIOP protocol). Currently patient recruitment is within the expected recruitment rates.

b. Patients with rapid COJEC induction, insufficient response after end COJEC, additional chemotherapy (i.e.TVD or CADO) followed by elective MAT.

c. Patients with elective MAT for other reasons after Rapid Cojec.

Revised Supportive Care Guidelines for the Therapy for Febrile Neutropenia for the Rapid Cojec Regimen (by Andy Pearson et al)

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

1. If there is fever (>38^{O}C) and the neutrophil count is less than 1.0 X 109/L, then the centre's usual combination of broad-spectrum antibiotics should be commenced.

2. If fever persists (>38^{O}C) for 48 hours despite broad-spectrum antibiotics, then antifungal therapy should be started, regardless of the clinical condition of the patient. The preferred antifungal therapy is liposomal amphotericin (ambisome) at a dose of 1mg/kg/day. However, it this is not available then amphotericin B 0.5 mg/kg for the first dose then increased to 1.0mg/kg after 24 hours should be given. If impaired renal function liposomal amphotericin is recommended. In addition a CXR should be carried out.

Other antifungal therapy e.g. fluconazole is not permitted in view of the substantial risk that the underlying fungal infection is aspergillosis and fluconazole will not be active.

3. If fever persists for a further 48 hours (i.e. a total of 96 hours), without another identified cause then:
    - The dose of ambisome should be escalated to 3mg/kg/day;
    - If the patient is receiving amphotericin B, then very careful consideration should be given to substituting ambisome for amphotericin B;
    - Careful consideration should be given to carrying out a CT scan of the chest,

    If there are any abnormalities on the CT scan then:
    - GSCF should be commenced at 5ug/kg/day;
    - Consideration should be given to the introduction of voriconazole or another antifungal agent;
    - Consideration should be given to other specific, appropriate investigations e.g. imaging, biopsies and broncho-alveolar lavarge.

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

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

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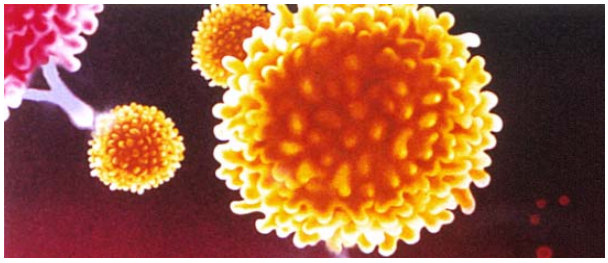
Status Report on Production of ch14.18 at Polymun for the High Risk Study

Antibody Task Force
Ruth Ladenstein
Jean Michon
Holger Lode
Vito Pistoia
Gillan Lewis



Summary

Since the last report in Toulouse, two meetings were held in Vienna (March 22nd and April 29th) together with Polymun (D. Katinger, H Katinger, Renate Kunert, Vito Pistoia, Ruth Ladenstein and Holger Lode). The purpose of these meetings was to define a cell clone useful for the production of 200 g of ch14.18 antibody.

The decision process involved detailed testing of the new clone concerning stability and productivity in serum free conditions and in the presence and absence of MTX. Furthermore, functional data were generated using purified recloned antibody including binding to GD2 and killing of neuroblastoma cells by antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC).

This testing resulted in the identification of clone ch14.18/23B11/20D2/6A8 which is a stable producer of ch14.18 antibody to a more than sufficient amount to generate the 200 g needed. The productivity is stable under serum free conditions and it is stable long enough in the absence of MTX.

Functional testing of the newly purified ch14.18 antibody from CHO cells demonstrated identical binding toGD2 compared with ch14.18 antibody preparations from NS0 cells and SP2/0 cells. This aplies also to CDC and ADCC reactions against neuroblastoma cells in vitro. There was no difference between ch14.18 produced either from CHO, NS0 or SP2/0 cells.

Based on these findings, we decided to produce a master cell bank (MCB) using clone ch14.18/23B11/20D2/6A8. The estimated End of Production remains September 2004. The release of antibody for the use in patients requires further testing and filling. Thus the availability of the product for clinical use is February 2005.

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ESIOP (neuroblastoma) Nuclear Medicine & Physics sub-committee report

Diagnostic

Within the SIOPEN-R-NET grant the remit of the Nuclear Medicine and Physics (NMPSC) is to determine response to induction chemotherapy using a semi-quantitative mIBG scan scoring system. At the first central review meeting in London last year 20 members and associates were involved in scoring 156 sets of scan data belonging to 76 patients. Two sets of patient data were received in 52% of eligible cases. This was not thought to be a bad response for a first meeting, although it highlights the difficulty of obtaining scan data from local centres. This is an important issue to address, and will hopefully be made easier as electronic image archiving becomes available. Of the scan sets received, half were considered incomplete, and 20% were considered to be of 'poor' quality. In some cases poor quality scans led to discrepancies in reporting, showing the difficulties faced by many reporters. In 10% of cases, response was determined to be between 40 – 60%, and in these cases further review is essential. This invaluable meeting has led to a support network to provide second (or even third, fourth, or tenth!) opinions on the reporting of scans that prove difficult to interpret. Results will be presented at this year's ANR conference and at the EANM congress in September.

Therapy

I-131 mIBG therapy has been used to treat neuroblastoma for 20 years. In April a meeting was hosted by Ghent University Hospital for those with an interest in dosimetry-based therapy, and in particular the UKCCSG 4Gy whole-body dose protocol which has been piloted at the Middlesex hospital. This meeting was held in two parts – in the morning Hubert Thierens (Ghent) hosted a meeting of a group of physicists and nuclear medicine physicians who discussed dosimetry and image quantification. It was pointed out by Fred Courbon (Toulouse) that the methodology discussed would be applicable to other therapies which would facilitate standardisation. In the afternoon a meeting was held that was more concerned with clinical issues. Mark Gaze (Middlesex) showed that it is possible to accurately administer a double fraction according to a 4 Gy whole-body absorbed dose. Ruth Ladenstein (Vienna) showed impressive results and presented response and toxicity data, and Rita Castellani and Carlo Chiesa (Milan) showed interesting results from tumour dosimetry. Bieke Lambert and Myriam Monsieurs (Ghent) presented the practicalities of administration. Simon Meller discussed the rationale behind the 4 Gy approach. The next meeting will be held in Genoa prior to the ANR meeting, and it is envisaged that more regular meetings will be held and funding sought as dosimetry-based treatment becomes more prevalent. Please contact me if you would like your name added to the mail list.

Glenn Flux (glenn.flux@icr.ac.uk)
May 10th 2004

overview on siopen-r-net activities

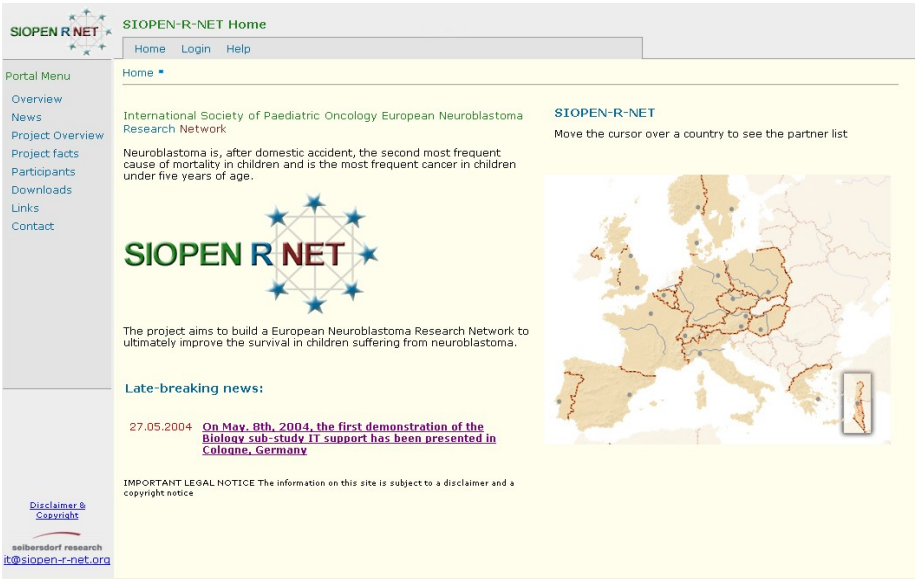
Dear members of the E-SIOP Neuroblastoma Group!
As the IT partner of the SIOPEN-R-NET we strive to provide a specifically tailored electronic home for all of your various research activities. This report intends to give a brief overview on our activities and achievements during the first SIOPEN-R-NET project year and an outlook to the upcoming project activities.

During the first project year we focused on the basic IT infrastructure of the SIOPEN-R-NET and the web-based system for the HR-NBL-1 clinical trial.
As of May 27th 2003, statistics on the web-based activity related to this trial indicate a huge level of activity and amount to the entry of more than 220.000 data items on 318 patients by 151 active users (registered users: 304) in 112 active institutions (registered institutions: 178) from 15 countries.

An important milestone has also been the launch of the web portal www.siopen-r-net.org (see figure).The SIOPEN-R-NET homepage, the central point of access to the SIOPEN-R-NET.

In the future, this web portal will serve as the central access point for all electronic activities of the group. Each member will receive a personal account consisting of username and password so as to login to the members area and to access the clinical trials and sub-studies to which the respective user is authorised.

In the ongoing second project year our efforts will be devoted to the following main topics:
- providing the subcommittees with IT structures to have their sub-studies electronically supported and linked to the HR-NBL-1/ESIOP clinical trial,
- developing the patient registry,
- providing different levels of image management, and
- designing the "Virtual Biomaterial Databanks".



Sub-studies
Test versions of the Nuclear Medicine & Physics sub-study as well as the Biology sub-study are already available. This means that a few subcommittee members already have access to those studies. The Bone Marrow and the Pathology sub-studies are currently in the definition phase which means that we need some additional input before we start to implement the sub-studies.

Patient registry
The patient registry is intended as a central structure to register patients to the database in the same way for all studies. This will facilitate uniqueness and overview and will allow to quickly assess the actual status and the "history" of patients with respect to their participation in trials. The patient registry will also link the clinical trials with the sub-studies and provide the possibility for pre-study evaluation procedures, drawing on the Europe-wide review-network provided by the various subcommittees. This gives you the means to check patient eligibility on the level of experts. Eventually, it would even be possible to "drag and drop" patients from one trial to a subsequent trial, i.e. from any first line concept to any phase II concept.

Image management
The so called simple image management (SIM), allowing basic file up and download is already available in the framework of the sub-studies mentioned above. The next step will be basic image management (BIM), which will provide for convenient handling of more complex image data and formats like DICOM. The respective software module is under construction and will be provided by our image management partner icoserve at the end of June. Advanced image management (AIM), finally, will bring direct interfacing to PACS and DICOM modalities as well as features necessary to effectively deal with high volume data like CT or MRI studies.

Virtual Biomaterial Databanks
Recently, we started to design a biomaterial specific view to the SIOPEN-R-NET database. This development intends to provide IT support for the establishment of the virtual tumour and serum databanks, which we subsume under the term "virtual biomaterial databank". Within the next weeks, a specific questionnaire will be sent out to all those of you who deal with various kinds of biomaterials. So be prepared!

Once we have managed to integrate all those components and activities, the SIOPEN-R-NET will be more than just the sum of its parts - a uniquely versatile research network with the potential to increase both, the speed and the quality of your research. But – of course – we cannot do this development all alone! We need your feedback constantly so as to get an idea on what is really important to you. And we also need a little bit of your patients, since - with limited resources - we can do all this only step by step.

Best regards,

Günter Schreier, Seibersdorf research

seibersdorf research

An infant with stage 4 neuroblastoma

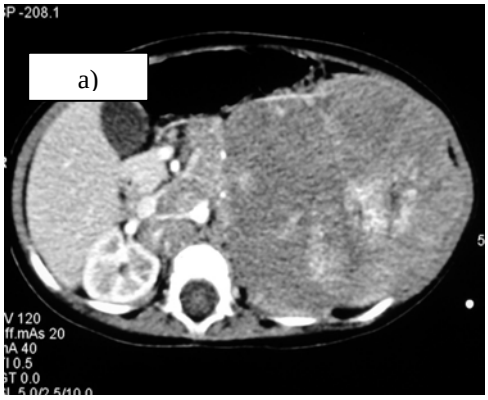
GA, born on May 28, 2002.

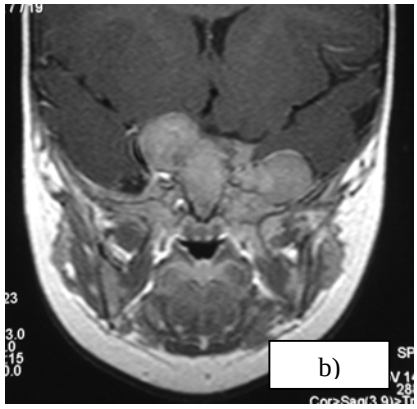
Diagnosis on April 2003 (eleven months). Enormous abdominal mass (a) plus multiple bone lesions plus massive disease at skull basis (b), plus positive bone marrow.
Treatment according to 99.3 protocol with slow disease response, making necessary additional therapy with four TVD cycles,

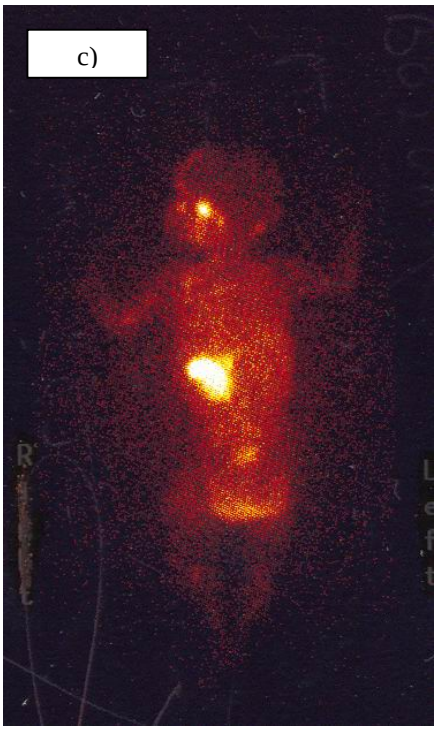
On April 2004, the skull lesion is still present (but much reduced) and uptakes mIBG (c). At this time, the abdominal mass is also much reduced (d), allowing its removal (e). No further therapy is given from time of surgery.

Questions
- *is any therapy advisable ?*
- *what is the prognosis of this case ?*

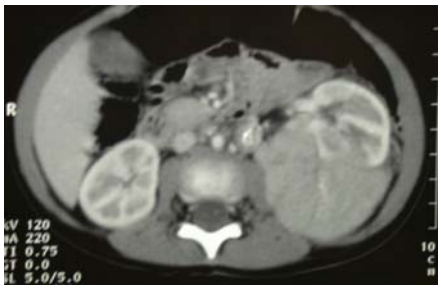
Pier Luigi Marradi and Rita Balter
Paediatric Oncology, Verona – Italy

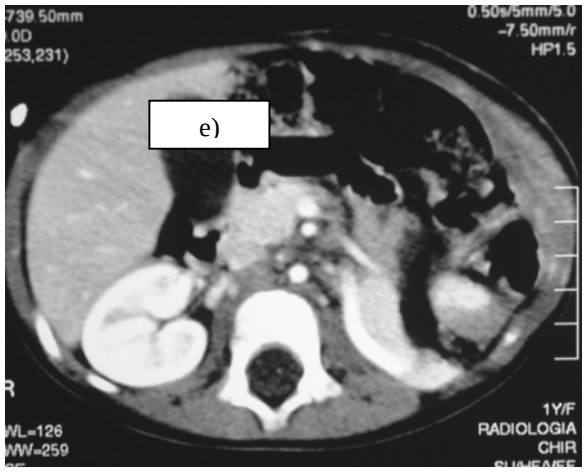






d)





Oncogene (2004) 00, 1–11

www.nature.com/onc

ORIGINAL PAPER

Drug-mediated sensitization to TRAIL-induced apoptosis in caspase-8-complemented neuroblastoma cells proceeds via activation of intrinsic and extrinsic pathways and caspase-dependent cleavage of XIAP, Bcl-x_L and RIP

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Neuroblastoma (NB) is a childhood neoplasm which heterogeneous behavior can be explained by differential regulation of apoptosis. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) selectively induces rapid apoptosis in most tumor cells and thus represents a promising anticancer agent. We have reported silencing of caspase-8 expression in highly malignant NB cells as a possible mechanism of resistance to TRAIL-induced apoptosis. To explore the particular contribution of caspase-8 in such resistance, retroviral-mediated stable caspase-8 expression was induced in the IGR-N91 cells. As a result, sensitivity to TRAIL was fully restored in the caspase-8-complemented cells. TRAIL-induced cell death could be further enhanced by cotreatment of IGR-N91-C8 and SH-EP cells with cycloheximide or subtoxic concentrations of chemotherapeutic drugs in a caspase-dependent manner. Sensitization to TRAIL involved enhanced death receptor DR5 expression, activation of Bid and the complete caspases cascade. Interestingly, combined treatments also enhanced the cleavage-mediated inactivation of antiapoptotic molecules, XIAP, Bcl-x_L and RIP.
Our results show that restoration of active caspase-8 expression in a caspase-8-deficient NB cell line is necessary and sufficient to fully restore TRAIL sensitivity. Moreover, the synergistic effect of drugs and TRAIL results from activation of the caspase cascade via a mitochondrial pathway-mediated amplification loop and from the inactivation of apoptosis inhibitors.
Oncogene (2004) **0**, 000–000. doi:10.1038/sj.onc.1207704

Keywords: apoptosis; TRAIL; caspase-8; neuroblastoma; chemotherapeutic drugs; cycloheximide

I would say that we show here, in contrast to what we had postulated, that caspase-8 can be sufficient to mediate full TRAIL-sensitivity in N-type NB cells. The second point is that, as already shown, TRAIL-sensitivity can be enhanced by combination of TRAIL with by sub-toxic concentrations of doxorubicin (DOX) or cycloheximide (CHX). But we provide here several explanations for such synergic action: non-toxic doses of DOX or CHX enhance the expression of TRAIL receptors R2, activate the overall caspases cascade, increasing the cross-talk between both death pathways. Finally, the most interesting and original point is that the enhanced apoptotic activity also results from an activated caspase-dependent cleavage and inactivation of inhibitors of apoptosis such as BclxL, XIAP and RIP. Such findings strengthen the interest of developing pro-apoptotic approaches that combine low doses of several drugs in addition to caspase-8-inducing treatments.

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Low-dose interferon-gamma-producing human neuroblastoma cells show reduced proliferation and delayed tumorigenicity.

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Interferon-gamma (IFN-gamma) directs T helper-1 cell differentiation and mediates antitumour effects in preclinical models. However, high-dose IFN-gamma is toxic in vivo, and IFN-gamma-transfected neuroblastoma (NB) cells secreting high amounts of the cytokine may be lost due to cell apoptosis or differentiation. Two human NB cell lines (ACN and SK-N-BE2(c)) differing as to genetic and phenotypic features were transfected with the human IFN-gamma gene and selected on the grounds of the low concentrations of IFN-gamma produced. In both IFN-gamma-transfected cell lines, autocrine and paracrine activation of IFN-gamma-mediated pathways occurred, leading to markedly reduced proliferation rate, to increased expression of surface HLA and CD40 molecules and of functional TNF binding sites. ACN/IFN-gamma cells showed a significantly delayed tumorigenicity in nude mice as compared to parental cells. ACN/IFN-gamma tumours were smaller, with extensive necrotic area as a result of a damaged and defective microvascular network. In addition, a significant reduction in the proliferation index was observed. This is the first demonstration that IFN-gamma inhibits in vivo proliferation of NB cell by acting on the tumour cell itself. This effect adds to the immunoregulatory and antiangiogenic activities operated by IFN-gamma in syngeneic tumour-bearing hosts.British Journal of Cancer (2004) 90, 2210-2218. doi:10.1038/sj.bjc.6601842 www.bjcancer.com Published online 4 May 2004