Advances in Research Neuroblastoma

Eleventh Conference

June 16-19, 2004

Genova, Italy

Programme and Abstracts
Welcome to Genova

It is a pleasure for the local Organizing Committee and for myself to welcome all of you to Genova, especially those who have never been here before. This meeting is located in the Congress Center, once the cotton warehouses, in the heart of our busy harbor.

For us 2004 is both a very important year and a task, because Genova, together with Lille in France, is one of the two European Capitals of Culture.

I wish to thank all of you. I hope that everybody enjoys both the scientific sessions and the social events that have been arranged to provide you with some joyful intervals during these busy days.

I think that most of you do not know the past of our town and Region, so I wish to tell you a few things about the very peculiar history of our Region, a State without land. Since the old ages, Ligurians have been engaged in overseas trade because their narrow strip of land, lying between the sea and the mountains, could not provide them with a living. In the 13th century the Genoese founded the “Banco di San Giorgio” (St. George’s Bank, 1407), which minted the new coins Lira, soldo, denaro: one Lira was equal to 20 soldos and one soldo to 12 denarii. The same system was immediately adopted in England by the British Banks, where the Lira symbol “£” means pound-sterling, the soldo symbol “s” means shilling and the denaro symbol “d” means penny. Genoese people became very rich but they were jealous of their wealth, though they were generous with their guests. They kept on living in narrow lanes but their houses were luxuriously decorated and furnished, even equipped with warm-air, an old system of central heating. Rubens, who worked in Genova for several years in the so called “gold street” (Via Aurea, now Via Garibaldi), published a book so that the architecture of the town could become known in the northern countries.

Many other important painters and artists from other countries worked in Genova in the past, and among them I wish to remind you of Van Dyck. You may visit the exhibit called “Rubens’ age”, witness of our past, in the Ducal Palace, in the heart of our cultural town. But how could these people make so much money? Thanks to their shrewdness and great business and trade ability. For example, in the 15th century the Pope granted them the exclusive privilege of collecting his tithes all over Great Britain because the Genoese bankers renounced to all interest payments. They earned an enormous amount of money from the tax collection since they would buy wholesale goods from England and Holland and sell them retail on the Mediterranean markets. In the 16th and 17th centuries Genoese bankers granted big loans to several countries and even to kings. The King of Spain, who was unable to pay the agreed interest, gave them the privilege of collecting taxes throughout the whole country. Most of the money used by the Kings of Spain to finance Christopher Columbus’s enterprise to discover the new continent of America came from the Genoese banks. The Genoese soul was a merchant’s soul: they even sold to the City of London their own flag (a red cross on a white background) and their Patron, Saint George. This is why they had to add a new patron, St. John the Baptist. Even in these early centuries insuring property, goods, ships was common in Genova in order to avoid any risks. It is interesting to know that the sailors’ trousers were blue jeans, because they discovered that denim lasted longer than the usual material, it was cheap and was produced in small factories in the Ligurian country-side. “Jean” meant “Genoese” in sailors’ language all over the world.

With these few words I wanted to give you an outline of the typical characteristics and skills of Genoese people who differed from the inhabitants of all other Italian regions. While the latter aimed at extending their possessions and building large, luxurious towns, the Genoese only aimed at increasing their financial power and their riches.

Now, I wish to come back to this important international Congress. Neuroblastoma is the most fascinating and enigmatic of childhood malignancies from both the clinical and biological viewpoints. We clinicians must look at our sick children carefully and systematically, and discuss any peculiar characteristics among our staff members, while bearing ever in mind the statement “observation and experimental research”. We must likewise learn how to re-educate ourselves following this renewed marriage of clinical pediatrics and biological research, availing ourselves of and exploiting the rich resources that are available to us, so as to play an important role in building the necessary bridges between biology and clinical care. Nevertheless, our chances of success in this wonderful profession and in this field of biomedicine remain unchanged, and are founded on our conviction in what we are called on to do, on our curiosity, and on our creativeness.

The continuous support of advocacy groups, parents’ associations and volunteers is pivotal to our success. Without their selfless assistance many of our challenges would remain unmet. Furthermore, we must not overlook their painstaking efforts to raise the awareness of the general public regarding certain, especially rare, conditions. The outcome of their efforts is usually a flow of funds to offset the health care costs which cannot always be fully covered by public allocations alone. Indeed, these groups also become key players in supplying both state-of-the-art treatment to patients, and research. We truly feel a great sense of indebtedness toward them.

The Italian Neuroblastoma Association, since early 90’s engaged to support research, together with the City of Genova and GE NOVA 04 wishes to thank the “William Guy Forbeck Research Foundation” by offering a symbolic award to the mother of William, an 11 year old boy who died of neuroblastoma.

GE NOVA 04 and myself wish to give another symbolic award to a very special person, Dr. Audrey Elizabeth Evans, a pioneer of Paediatric Oncology, a life completely dedicated to children with cancer, a life for children’s lives.

I would like to express my wish that this meeting shall fulfill other goals as well, primarily that of encouraging future cooperation and an exchange of ideas among the many investigators from the institutions that are represented here today.

Prof Luisa M Massimo
Director Emeritus
Department of Paediatric Haematology and Oncology
Giannina Gaslini Research Children’s Hospital, Genova
## Welcome to Genova

### Conference Committees

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### Scientific Programme

**Oral Presentations**
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- Parallel Sessions
- Selected Posters
- Poster Display
- Biology
- Clinical Research
- Genetics
- Molecular Biology
- Translational Research
- Published-only Abstracts
- Workshops
- Microarray Technology
- Spinal Cord Compression
- Opsoclonus Myoclonus
- Abstracts

**Oral Presentations**
- Genetics (Plenary Session A)
- Biology (Plenary Session B)
- Clinical (Plenary Session C)
- Translational (Plenary Session D)
- Molecular Biology (Plenary Session E)
- Selected Posters
- Molecular Biology – Translational (Plenary Session F)
- Clinical (Plenary Session G)
- Translational – Clinical (Plenary Session H)

**Poster Display**
- Biology
- Clinical Research
- Genetics
- Molecular Biology
- Translational Research
- Published-only
- Opsoclonus Myoclonus

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8:15 AM Welcome by the Local Organising Committee

8:30-10:30 AM PLENARY SESSION A: Genetics

Chairmen: Akira Nakagawara (J) - Manfred Schwab (D)

8:30 AM Molecular signatures to predict the prognosis of neuroblastomas and its application to a diagnostic microarray of clinical use
1. Mike Oliver, Shigetomi Oh, Yukiko Nakamura, Kieko Itoigawa, Shin-ya Kiyoura, Hiroshi Kawanami, Toshihide Hayashi, Koichi Yamasaki, Toshiro Koyama, Atsushi Nakagawara, Hideki Mita, Atsushi Nakagawara
2. Ludwig Schleiermacher, University of Muenster, Muenster, Germany; Children’s Hospital of Philadelphia, Philadelphia, PA, USA; German Cancer Research Center, Heidelberg, Germany; Children’s Hospital of Boston, Boston, MA, USA

8:45 AM Oligosaccharide microarray expression profiles identify subsets of patients with ultra high- and high-risk metastatic neuroblastomas
1. Robert A. Segrin, Hong Wang, Shuyan Yang, Katherine K. Marriott, Jonathan Bucyael, Shikhalin Aftanidzadeh
2. Division of Bone-Marrow Transplantation, Department of Pediatrics, Children’s Hospital Los Angeles/UCLA, CA, USA

8:50 AM Differentiation between good and bad prognosis neuroblastoma by serum protein expression profiles analysis
1. Valerie Cadet, Christopher Burger, Stéphane Béjot, Isabelle Lecor, Myriam Collette, Sylvie Papin
2. CHU de Nantes, Université de Nantes, Nantes, France

9:05 AM Discovery of antiangiogenic targets for high-risk neuroblastoma using a high-density oligosaccharide-based approach
1. Susan Hettiarachchi, Mohammad A. Tang, Ting Sun, Rupali Patel, Jun Wang, Shari D. Scherl, Mark Meyers, Rachel Birt, Nicholas Huet, John M. Masiello
2. Department of Pediatrics, Children’s Hospital of Philadelphia, Philadelphia, PA, USA

9:10 AM N-myb, Cdc42 and nm23 genes function in a differentiation pathway blocked by copy number defects in neuroblastoma
1. Linda Y. D’ymelina, Heather B. Ricer, Roger Varghese
2. Human Genetics, Academic Medical Center, Amsterdam, The Netherlands

9:15 AM MEIS1 functions as a neuroblastoma oncogene
1. Dirk Gestel, Nishant Sakharkar, Carla Bominaar, Agnieszka Kwiatkowska, Joris Heisterkamp, Roger Varghese
2. VU University Medical Center, Amsterdam, The Netherlands

9:20 AM Prohybolic analysis of cDNA array-CGH profiles identifies genomic alterations specific to stage and MYCN-amplification in neuroblastoma
1. Guang Chai, Jie Liu, Wei Li, Jia Liu, Qiaoyun Zhao, Nicole Guarceli, Albin Kramarz, Brendan Grois, Chang-Gue Son, Frank Rudnicki, Mark Schultheis, Daniel Caligiuri, David Klein
2. Oacororcenter Sweden, Pediatric Oncology Branch, WCNK, The Netherlands, Bldg., USA; German Cancer Research Center, Heidelberg, Germany; Children’s Hospital of Philadelphia, Philadelphia, PA, USA

10:30-11:00 AM Break

11:00 AM-1:00 PM PLENARY SESSION B: Biology

Chairmen: Jeong-Chun Kim (Korea) - Terry Melino (I)

11:00 AM Gangliosides link fenretinide-induced ceramide to 12-lipoxygenase-dependent apoptosis of neuroblastoma
1. Jin Woon Lee, Ho-Bin Lee, Seung-Hee Kim, Tae Yoon Kim, Yang Seok Oh
2. Department of Pediatrics, Kyungpook National University College of Medicine, Daegu, Korea; Department of Biological Sciences, Kyungpook National University, Daegu, Korea; Institute of Biological Sciences, University of Science and Technology, Korea

11:15 AM BH1-domain proteolipidicin activite apoptosis and cholesterol death pathways in neuroblastoma
1. Kelly C. Gillman, Xuyan Li, Vincent Dan, Brian T. Morgan, Anthony Leitz, Michael D. Hartley
2. Department of Pediatrics, The Children’s Hospital of Philadelphia and Dana Farber Cancer Institute, PA, USA

11:30 AM Protein kinase C isoforms and glutathione levels: A molecular switch between proliferation and apoptosis in human neuroblastoma cells
1. Tanja Drögemüller, Thomas Menz, Stefania Pettiare, Emanuel Dalle, Nicola Tottor, Udo M. Oester, Ute Feistner, Mario A. Pruszkowski
2. Department of Experimental Medicine, University, and Laboratory of Oncology, GIannina Gaslini Children’s Hospital, Genova, Italy

11:45 AM N-myc, Cdc42 and nm23 genes function in a differentiation pathway blocked by copy number defects in neuroblastoma
1. Linda Y. D’ymelina, Heather B. Ricer, Roger Varghese
2. Human Genetics, Academic Medical Center, Amsterdam, The Netherlands

12:00 AM Oligonucleotide microarray expression profiles identify subsets of patients with ultra high- and high-risk metastatic neuroblastomas
1. Akira Nakagawara, Hideki Mita, Mohammad A. Tang, Rong Chen, Javed Khan, Nicola Traverso, John M. Maris, Thomas Mohawk, Maria A. Pruszkowski
2. Department of Pediatrics, The Children’s Hospital of Philadelphia and Dana Farber Cancer Institute, PA, USA

1:00-2:00 PM Lunch
Thursday 17, 2004
PARALLEL SESSION A: Translational
Hall “Scirocco & Libeccio”
Chairmen: Michel Huber (Luzern – CH) – Carol Pflueger (USA)

05:00

05:20
Molecular mechanisms of embryonal tumour initiation
Nicoletta Di Sabatino, Anna Anna Bellizzi, Anna Ficarra, Valeria Rotta, Michele Abbruzzese, Patrizia Macri

05:30
Expression of the therapy relevant markers EGF receptor, PDGF receptor and c-kit in neuroblastoma determined with a multi-array
Klaus-Herbert Hoekstra, Eve K. Lannert, Frank Berthold

05:40
Therapeutic activity of SF1537 in neuroblastoma xenografts

05:50
Arsenic trioxide-induced death of neuroblastoma cells involves activation of bax and does not require p53
Jenna Harksen, Justin G., Isabella Peto-Russ, Yves Delhaise

06:00
Measuring circulating neuroblastoma cells by quantitative RT-PCR: Correlation with paired bone marrow and standard disease markers
Irma V. Chang, Arvind Sahota, Xing-Kang V. Chang

3:30-4:00 PM Break

4:00-5:00 PM POSTER VIEWING

ROOM A Biology - Clinical - Genetics
ROOM B Genetics - Molecular Biology - Translational

Thursday 17, 2004
PARALLEL SESSION B: Clinical
Hall “Levante & Ponente”
Chairmen: Frank Berthold (CH) – Robert Castleberry (USA)

05:00

05:20
Evidence for an age cut-off higher than 365 days for neuroblastoma risk group stratification in the Children's Oncology Group (COG)
Wendy B. Littrell, Robert P. Castile, Thomas A. Leach, Paul Thomas, Grant M. Bond, John M. Marin, Susan C. Call

05:30
Expression of the therapy relevant markers EGF receptor, PDGF receptor and c-kit in neuroblastoma determined with a multi-array
Klaus-Herbert Hoekstra, Eve K. Lannert, Frank Berthold

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3:30-4:00 PM Break

4:00-5:00 PM POSTER VIEWING

ROOM A Biology - Clinical - Genetics
ROOM B Genetics - Molecular Biology - Translational
ROOM   B               Genetics - Molecular Biology - Translational

POSTER VIEWING
1:00-2:00 PM
Lunch

2:00-3:30 PM  PARALLEL SESSION C: Biology - Genetics  Hall “Levante & Ponente”

Chairmen: Peter Ambros (AT) - Rogier Vesteeg (NL)

097.2  UVB2s, whose gene is localized in a 500 kb homozygously deleted region at chromosome 3p36.2 found in a neuroblastoma cell line, modulates p73 function through regulation of its stability

Mitsushiki Hosoda, Toshinori Ozaki, Kou Miyazaki, Syunji Hayashi, Kenichi Watanabe, Akira Nakagawara
Division of Biochemistry, Chike Cancer Center Research Institute, Japan.

214.1  Germline mutations of the patell-like homologue 2 (PRR3CL2) gene in hereditary neuroblastoma.

F Boumaud1, Y Toulon1, J Honore de Lavaudy1, R Lepont1, C Delebarre1, J Humbel1

223.4  High resolution detection of neuroblastoma hemi- and homo-zygous deletions with array-based comparative genomic hybridization (aCGH)

Yael P Mosse, Joel Greshock, Tara Naylor, Deepa Khazi, George Hii, Qun Wang, Cynthia Winter, Suzanne Shusterman, Barbara L Weber, John M Maris Department of Oncology, Children’s Hospital of Philadelphia, PA, USA.

117.1  Gene expression profiling of neuroblastoma: analysis of nonmetastatic versus metastatic tumors

Agnes Morin, Miguel Atanasov, Six Kong Y Chang, Jana Rost, William L Gratz
Department of Pediatrics, The Children’s Hospital of Philadelphia, PA; University of Michigan Medical School, USA; Children’s Hospital Brno, Czech Republic.

214.1  Identification of relevant MYCN downstream effectors by a combinatorial multimodel approach

Felicetti, Keiko Do Peyer, Nadine Van Roy, Els Do Peere, Geneviève Laurent, Frank Spielman, Jo Vandecasteele, Department of Pediatrics1, The Children’s Hospital of Philadelphia, PA; University of Michigan Medical School3, USA; Children’s Hospital Brno2, Czech Republic.

3:30-4:00 PM  Break

4:00-5:00 PM  POSTER VIEWING

ROOM A  Biology - Clinical - Genetics

ROOM B  Genetics - Molecular Biology - Translational
10:30-11:00 AM Break
Assessment of chemotherapy response by MIBG scan: a blind quality control

Volker Witt, Ruth Ladenstein, Gerhard Fritsch, Ullrike Pötschger, Helmut Gadner, Peter F Ambros

Department of Pediatric Oncology, St. Anna Kinderspital, Vienna, Austria.

Tumor cell detection in apheresis products in pediatric patients with advanced neuroblastoma: Where to go?

Children's Cancer Research Institute, St. Anna Kinderspital, Vienna, Austria.

Tumour cell clearing in the bone marrow of stage 4 neuroblastoma: Can it predict outcome?

Department of Pediatrics and Department of Clinical Chemistry, Microbiology and Immunology, Ghent University Hospital, Belgium.

Detection of residual neuroblastoma cells using quantitative real-time RT-PCR

Cancer Research UK Clinical Centre, St James’ University Hospital, Leeds, United Kingdom for the E-SIOP Neuroblastoma RT-PCR Group.

Tyrosine Hydroxylase expression in blood of patients with neuroblastoma: Analysis by a real time RT-PCR

University Children's Hospital, D-72076 Tübingen, Germany.

catecholamine-producing hematopoietic (stem) cells

Pitfalls in detection of contaminating neuroblastoma cells by tyrosine hydroxylase RT-PCR due to GD2 synthase mRNA is less specific for detection of neuroblastoma in blood and bone marrow than tyrosine hydroxylase RT-PCR in primary neuroblastoma

Department of Laboratory Medicine, Division of Molecular Medicine, Lund University, University Hospital Malmö, Malmö, Sweden.

Detection of residual neuroblastoma cells using a new four-color flow cytometric assay

Department of Pediatrics, Ghent University Hospital, Belgium.

Mismatch repair protein expression in pre and post treatment neuroblastomas

Department of Pediatrics, Ghent University Hospital, Belgium.

Unequivocal identification of disseminated tumor cells in the bone marrow by immunofluorescence and FISH reveal essential functional and prognostic information

Department of Hematology-Oncology1, Labaratory of Oncology and the Service of Pathology Giannina Gaslini Children Research Hospital, Genova2; Biological modifiers of RANKL activity prevents osteoclastogenesis induced by neuroblastoma cells

Biomedical research foundation, Children’s Hospital of Philadelphia, Philadelphia, Department of Experimental Pathology and Oncology, Florence, Italy.

Hypoxia induced differentiation of neuroblastoma cells: phenotypic plasticity after reoxygenation

Department of Laboratory Medicine, Division of Molecular Medicine, Lund University, University Hospital Malmö, Malmö, Sweden.

Normalization to averaged expression levels of four control genes results in reliable transcript quantification by real-time RT-PCR in primary neuroblastomas

MIRN diagnosis.

Children’s Hospital, Department of Pediatrics, Oncology and Hematology, University of Cape Town, Cape Town, South Africa.

NDSP, a novel secreted protein in neuroblastomas

Joe A. Vassallo1, James M. Reinhold2, Grace b Li, Paul N. Pan3, Joshua Yang4, Joel G. Nussenzweig1, M.E. Dobles1, Department of Surgery1 and Department of Pediatrics2, Baylor College of Medicine, Houston, TX, USA.

Functional expression and release of ligands for the activating immunoreceptor NGK2D in human neuroblastomas

Luzina Raffaghello1, Ana Mirkl1, Ignacio Pignone2, 1, Marta Gavini1, Laura Lelli2, Claudia Gandola1, Danila Pellegrini1, Silvana Savoldi1, Vittorio Ruffini1, Neri Rumi1

Department of Oncology, University of Modena, 41122 Modena, Italy, Laboratory of Immunogenetics, Istituto Oncologico e Tumori, Modena, Italy, Laboratory of Immunogenetics, Università di Modena e Reggio Emilia, Modena, Italy, University of Modena and Reggio Emilia, Modena, Italy.

Mismatch repair protein expression in pre and post treatment neuroblastomas

Deborah A Tweddle, Lynn Board, Katrina M Wood, John Brown, Andrew D Byron, Robert Power

Department of Molecular Biology and Department of Pathology, Northern Institute for Cancer Research, 80-90 Great Western Road, St Andrew’s House, Glasgow G12 0SF, UK, USA.

GD2 loss variants in neuroblastomas

Ravenna, Alessandra Bruckert, Simon Schitt, John Grundback, Barbara Herr, Frank Korthof

Department of Pediatrics, Surgery and Hematology, Children’s Hospital of Berlin, Germany.

Biological modifiers of RANKL activity prevents osteoclastogenesis induced by neuroblastoma cells

Elena Amato1, Luca Raimondo1, Cinzia Ceccoli, Angela Capriati1, Mattia Donnini1, Nicola Baldini1, Daniele Cenci1, Laboratory for Orthopedics, Orthopaedic Department, Bologna, Department of Experimental Pathology and Oncology, Florence, Italy.

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Department of Pediatrics, Surgery and Hematology, Children’s Hospital of Berlin, Germany.

Biological modifiers of RANKL activity prevents osteoclastogenesis induced by neuroblastoma cells

Elena Amato1, Luca Raimondo1, Cinzia Ceccoli, Angela Capriati1, Mattia Donnini1, Nicola Baldini1, Daniele Cenci1, Laboratory for Orthopedics, Orthopaedic Department, Bologna, Department of Experimental Pathology and Oncology, Florence, Italy.

Hypoxia induced differentiation of neuroblastoma cells: phenotypic plasticity after reoxygenation

Linda Hofmann, Andrea Sugi and Tove Plutner

Department of Laboratory Medicine, Division of Molecular Medicine, Lund University, University Hospital Malmö, Malmö, Sweden.

Normalization to averaged expression levels of four control genes results in reliable transcript quantification by real-time RT-PCR in primary neuroblastomas

MIRN diagnosis.

Children’s Hospital, Department of Pediatrics, Oncology and Hematology, University of Cape Town, Cape Town, South Africa.

NDSP, a novel secreted protein in neuroblastomas

Joe A. Vassallo1, James M. Reinhold2, Grace b Li, Paul N. Pan3, Joshua Yang4, Joel G. Nussenzweig1, M.E. Dobles1, Department of Surgery1 and Department of Pediatrics2, Baylor College of Medicine, Houston, TX, USA.
Meyerstein Institute of Oncology1, The Middlesex Hospital, Royal Marsden Hospital2, London; University of Glasgow3, UK.

Mark N Gaze1, Glenn D Flux2, Robert J Mairs3, Frank H Saran2, Simon T Meller2

meta-iodobenzylguanidine, Topotecan and haemopoietic support

Stefano Mastrangelo, Vittoria Rufini, Angela Di Giannatale, Riccardo Riccardi

99

Combinational treatment with chemotherapy and 131-I-MIBG for advanced neuroblastoma at diagnosis

Junichi Hara

Keiko Kubota, Yoshiko Hashii, Shigenori Kusuki, Sadao Tokimasa, Hiroyuki Fujisaki, Akihiro Yoneda, Takeshi Kusafuka, Masahiro Fukuzawa,

High-dose chemotherapy consisting of thiotepa and melphalan prior to local surgery for advanced neuroblastoma

For the SFOP (Société Française de Oncologie Pédiatrique).

Oncology Department, St. Anna Children's Hospital, Vienna, Austria.

Ruth Ladenstein, Ulrike Pötschger, Christian Urban, Franz-Martin Fink, Georg Ebetsberger, Neil Jones, Gabriele Amann, Ernst Horcher, Karin

Genova, Italy.

Clinical Pathology, * University Dept.of Pediatrics, ** Pediatrics Dept, ***Dept. Of Hematology-Oncology, Giannina Gaslini Children's Institute, Childhood Cancer Research Unit, Pediatric Surgery, Hematology and General Oncology at Karolinska University Hospital, Karolinska Institutet,

Per Kogner, Per Borgström, Bengt Karpe, Göran Lundell, Anna-Lena Hjelm Skog, Jacek Winiarski, Moustapha Hassan

Improved outcome in high-risk neuroblastoma after application of intensified multimodal therapy

Anders E Svedmyr1, Hans Ehrsson1, Erik Forestier1, Per Kogner2


Johann Hoflehner, Dietmar Breitenstein, Robert Hein, Wolfgang Hlscher, Andreas Hentschel, Werner Briel, Thomas Schmoll, Giuseppe Fratino1, Gian Paolo Cuneo1, Alberto Michelazzi1, Angelo C Molinari2, Elio Castagnola3, Riccardo Haupt4, Antonino

Poster Display

Thursday 17 & Friday 18, 2004

9:00 AM - 7:00 PM
Tumor suppressor genes methylation in neuroblastoma: RASSF1 gene is almost always methyled in primary tumors

Department of Pediatrics1, The Children's Hospital of Philadelphia, PA; University of California at San Francisco2, CA, USA; Children's Hospital University of Technology, Gothenburg; Childhood Cancer Research Unit3, Karolinska Institute, Karolinska Hospital, Stockholm, Sweden.

Michelle Haber4, William A Weiss3, Michael D Hogarty1

Department of Clinical Genetics1, Gothenburg University, Sahlgrenska University Hospital East, Institution of Mathematical Statistics2, Chalmers

Xueyuan Liu1, Kelly C Goldsmith1, Vincent Dam1, Brian T Morgan1, Pavel Mazanek2, Yael P Mosse1, Christopher S Hackett3, Murray D Norris4,

Frida Abel1, Rose-Marie Sjoberg1, Staffan Nilsson2, Per Kogner3, Tommy Martinsson1

111

Division of Biochemistry, Division of Animal Science, Chiba Cancer Center Research Institute and Department of Pathology, Gunma University Japan.

Simona Coco1, Luca Longo2, Patrizia Perri1, Carla Marino3, Claudio Gambini3, Katia Mazzocco1, Raffaella Defferrari1, Gian Paolo Tonini2

LIM-only protein, LMO3, is up-regulated and acts as an oncogene in aggressive neuroblastomas by physically

243.1

EMP3, a putative tumor suppressor gene mapping at 19q13.3, is epigenetically silenced In neuroblastoma by promoter

162.2

Anatomopathologie7, Institut Gustave Roussy (+9), Villejuif; Génomique fonctionnelle2, CEA, Centre national de séquençage3, Evry, France.

Helena Carén1, Susanne Fransson1, Rose-Marie Sjöberg1, Katarina Ejeskär1, Luke Hesson2, Farida Latif2, Tommy Martinsson1

Marine Guillaud-Bataille1, Alexander Valent1, Christine Perot1, Pascal Soularue2, Amandine Pitaval2, Hugues Roest Crollius3, Hugues Ripoche4, Jean

Quantitative analysis and detailed mapping of 17q gains in neuroblastomas using array CGH

110

Deutsches Krebsforschungszentrum, Division of Tumour Genetics1, Heidelberg; Central Unit Biostatistics2, Deutsches Krebsforschungszentrum;

HsCAMTA1 and FLJ10737 within a commonly deleted region at 1p36 in neuroblastoma

282.1

Division of Oncology, The Children's Hospital of Philadelphia, Pennsylvania (PA), USA.

Peter S White, Patricia M Thompson, Takahiro Gotoh, Jun Igarashi, Erin R Okawa, Cindy Winter, John M Maris, Garrett M Brodeur, Barbara L Weber,

Department of Medical Genetics and Pediatrcs (B030), German Cancer Research Centre, Heidelberg, Germany.

Frank Berthold, Javed Khan, Manfred Schwab, Frank Westermann, Benedikt Brors, Jun Wie, Rainer Koenig, Ruprecht Wiedemeyer, Kai Henrich, Matthias Fischer, Andre Oberthuer,

Center for Medical Genetics1, Ghent University Hospital and Centre Hospitalier Régional de la Citadelle3, Liege, Belgium; Centre Léon Bérard2,

Hoyoux3, Geneviève Laureys1, Anne De Paepe1, Frank Speleman1

Hunting for a 3p tumor suppressor gene in neuroblastoma: a combined genomic and functional approach

161.1

Shirasawa, Naomi Ohnuma

Institute for cancer Genetics, Columbia University, NY, USA.

Shc family expression in neuroblastoma

238.1

Division of Biochemistry1, Chiba Cancer Center Research Institute; Department of Pathology2, Gunma University School of Medicine Japan.

Division of Cell Biology, Division of Animal Science, Chiba Cancer Center Research Institute and Department of Pathology, Gunma University Japan.

Kohei Da Porter, In Vandome, Ivanne Horschick, Carolina Vandoneville, Joel Eust, Anast Asjes, Genoveva Lanrey, Victor Candor, Nadine Van Back, Erny Van Cutters, Mariko Fokk, Anne Da Porrey, Frank Speleman

A putative lipo-instructive contribution of NSHD to high stage neuroblastoma tumorigenesis

251.2

Poster Display

Thursday 17 & Friday 18, 2004 9:00 AM - 7:00 PM

Thursday 17 & Friday 18, 2004 9:00 AM - 7:00 PM
Cytotoxic T lymphocytes activated on third party targets induce cell death in neuroblastomas in a MEH-independent fashion.

Antitumor immune reactivity in combination with IL-2 and IL-18 induces complete regression of orthotopic primary and metastatic murine neuroblastoma tumors and potentiates anti-tumor immunity.

Glutathione S-transferase polymorphism, genetic susceptibility and outcome in neuroblastoma.

Antigen specific immunity in neuroblastoma patients: Antibody and T-cell recognition of NY-ESO-1 tumor antigen.

Involvement of CXCR4 in the development of neuroblastoma metastases.

Anti-neuroblastoma immunotherapy with GD2 peptide mimotope DNA vaccines.

Natural killer (NK) lymphocytes are cytotoxic for multidrug resistant neuroblastoma cells.

The novel 7-substituted camptothecin analog ST1481 (GIMATECAN) is active in preclinical models of human neuroblastoma.

Low dose interleukin-2 producing human neuroblastoma cells show reduced proliferation and delayed tumorigenesis.

In vitro and in vivo antitumor activity of the synthetic polymere P104.

The histone deacetylase inhibitor valproic acid activates a differentiation program in neuroblastoma cells.
The Children's Hospital of Philadelphia, Philadelphia, USA

15' Conclusion
Institut Gustave Roussy, Paris, France

arrays and dedicated laboratory-made BACs arrays.
J Begent, P Brock, J Laddie, D Saunders, D Thompson, K Phipps and G Levitt

Study of genetic rearrangements in neuroblastoma tumors using commercial pangenomic CGH
Spinal Neuroblastoma in Stage 2/3 Disease – Experience From One Institution
Institute of Molecular Biology and Tumor Research, University of Marburg, Germany.

Role of Bmi1 in neuroblastoma
Paola Scaruffi and Stefano Moretti,

Use of CGHarray to identify DNA gain and loss in neuroblastoma.
Childhood Cancer Research Unit, Karolinska Institute, Stockholm, Sweden.

Dissecting the genome of human neuroblastomas with array CGH
Gent University Hospital, Gent, Belgium
Frieda Speleman,

Mapping 10K Array and Assay Set) for high-resolution analysis of DNA copy number in neuroblastoma

Comparison of arrayCGH versus oligonucleotide microarrays (Affymetrix GeneChip®
Department of Hematology-Oncology, Azienda Ospedaliera Meyer, Firenze, Italy.

Possible graft vs. neuroblastoma effect after partially matched related hematopoietic transplantation
Department of Pediatric and Oncology, Ospedale Pediatrico Bambino Gesù, Roma, Italy.

Double megatherapy (from auto-auto to auto-allo) for high-risk neuroblastoma

The impact of integrated analysis of genetic and genomic alterations using microarrays in
Department Hematology-Oncology, Azienda Ospedaliera Meyer, Firenze, Italy.

Possible graft vs. neuroblastoma effect after partially matched related hematopoietic transplantation

Department of Molecular Pathology, Giannina Gaslini Children’s Hospital, Genova, Italy

Overexpression of embryonal transcription factor OCT-4 in poor risk neuroblastoma

Identification of metastatic neuroblastomas and cell lines with oligonucleotide microarray

Department of Pathology, Medical School, University of V alencia and Hospital La Fe, V alencia, Spain.

Monoclonal antibody NB84 expression in the normal fetal sympathetic nervous system development and its relation with other neuroendocrine immunomarkers
Rosa Negrone, Samuela Nesin, Maria Papi, Adele Calile, Antonio Lini

10° Introduction to microarray technology: Basic principle and pre-clinical approach
Gian Paolo Tinini, National Institute for Cancer Research, Genova, Italy

Gene expression - Session chaired by Rogier Versleg (NL)

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

Quantitative profiling.
Rogier Versleg, University of Amsterdam, Amsterdam, Netherlands

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

Designing a specific Neuroblastoma Oligonucleotide Microarray
Matthias Fischer, University Children’s Hospital of Essen, Germany

Identifying subsets of metastatic neuroblastomas and cell lines with oligonucleotide microarray expression profiling.
Robert C. Seeger, Children’s Hospital Los Angeles Research Institute, Los Angeles, USA

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

Coffee Break

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

The impact of integrated analysis of genetic and genomic alterations using microarrays in neuroblastoma
Aliza Nakagawa, Chiba Cancer Center Research Institute, Chiba, Japan.

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

Dissecting the genome of human neuroblastomas with array CGH
Yari Mossie, Philadelphia Children’s Hospital, Philadelphia, USA

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

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

Conclusion
John M. Maris, The Children’s Hospital of Philadelphia, Philadelphia, USA
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**
Abstracts

Oral Presentations
Overall survival (OS) of patients with high-risk metastatic neuroblastoma (stage 4S, 4M, 4D) is significantly better than patients with stage 1 and 4+; implying that MYCN amplification is sufficient to drive these tumors to an aggressive phenotype.

Characterization of genomic alterations is critical to our understanding of the molecular biology of neuroblastoma. Here we performed Array-comparative genomic hybridization (A-CGH) on cDNA microarrays generated from 16 tumors with Stage 1 and 4+, implying that MYCN amplification is sufficient to drive these tumors to an aggressive phenotype.

Probabilistic Analysis of cDNA Array-CGH Profiles Identifies Molecular signature to predict the prognosis of neuroblastoma and its application to a diagnostic microarray of clinical use

There is a strong correlation between tumor vascularity and aggressive disease phenotype in neuroblastoma, but the most relevant antiangiogenic targets are not known. We have therefore quantified mRNA expression of proangiogenic factors including VEGF and METAP2. Targeting METAP2 enzymatic activity with a novel reversible inhibitor resulted in dramatic diminution of tumor growth rate in xenograft models (P < 0.001) and cured MYCN transgenic mice with Stage 1 and 4+.

A variety of serum markers have been used to assist in the diagnosis of NB, and some of them as ferritin and LDH may be useful to predict outcome. We used new proteomic tools to identify other serum protein expression profiles analysis using a high-density oligonucleotide-based approach including maturation, regression, and aggressive growth. Although recent progress in therapeutic strategies against advanced neuroblastoma has improved patient survival, long-term outcomes still remain very poor. The prediction of outcome remains a major challenge that is necessary for the suitable treatment of neuroblastoma. To this end, we constructed an in-house, microsensoric-pump-based in-silico DNA microarray carrying 5,540 genes for 16 tumors with Stage 1 and 4+, implying that MYCN amplification is sufficient to drive these tumors to an aggressive phenotype.
Ref ID: 020.1
Gangliosides link fenretinide-induced ceramide to 12-lipoxygenase-dependent apoptosis of neuroblastoma
Penny Lovat1, Federica Di Sano2, Marco Corazzari3, Andy Pearson1, Mauro Piacentini2,3, Christopher PF Redfern1
Northern Institute for Cancer Research1, University of Newcastle, Newcastle Upon Tyne, UK; Department of Biology2University of Rome “Tor Vergata” and INMI-IRCCS Lazzaro Spallanzani3, Rome, Italy.

Ceramide has been implicated as a common intermediate of many apoptotic pathways. Metabolism of ceramide results in the formation of gangliosides which can act as an apoptosis mediator. In this study, we demonstrate that changes in ganglioside content in NB cells exposed to fenretinide occur concomitantly with changes in ceramide content and are dependent on 12-lipoxygenase activity. The induction of GD2 suggests that fenretinide might also enhance the response of neuroblastoma to anti-GD2 therapy.

Ref ID: 082.1
Ganglioside link: the conserved role of 12-lipoxygenase in neuroblastoma therapy
Laura Figueiredo, Ana Lopez,拟首次, Nicolas Ermilov, Mark Hofman, Roderic Dencewicz, et al.

Gangliosides are complex lipids containing sialic acid which are enriched in the cell surface membrane of many tumors. They have been implicated in resistance to chemotherapy and the evasion of apoptosis. In this study, we investigated the role of 12-lipoxygenase (12-LOX) in the metabolism of gangliosides and its potential to mediate apoptosis in neuroblastoma. We found that inhibition of 12-LOX resulted in a decrease in ganglioside levels and an increase in ceramide, suggesting a role for 12-LOX in the metabolism of gangliosides and the induction of apoptosis. These findings provide new insights into the mechanism of neuroblastoma therapy and suggest potential targets for the development of novel therapeutic strategies.

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Ref ID: 075.2
BH3-domain peptidomimetics activate apoptosis and elucidate death pathways in neuroblastoma
Kelly C Goldsmith, Xueyuan Liu, Vincent Dam, Brian T Morgan, Anthony Letai, Michael D Hogarty
Department of Pediatrics, The Children's Hospital of Philadelphia and Dana Farber Cancer Institute, P A, USA

Apoptosis evasion contributes to chemotherapy resistance in neuroblastoma (NB). We identified BH3-domain peptidomimetics that activate apoptosis when added to NB cell lines resistant to chemotherapy. These compounds potentiate the proapoptotic activity of BH3-only proteins that are present in NB cells resistant to chemotherapy. These findings provide new insights into the mechanism of neuroblastoma therapy and suggest potential targets for the development of novel therapeutic strategies.

Ref ID: 189.1
Protein kinase C isoforms and glutathione levels: a molecular switch between proliferation and apoptosis in human neuroblastoma cells
Cinzia Domenicotti, Barbara Marengo, Stefania Patriarca, Emanuela Balbis, Nicola Traverso, Umberto M Marinari, Vito Pistoia, Maria A Pronzato
Department of Experimental Medicine, University, and Laboratory of Oncology, Giannina Gaslini Children's Hospital, Genova, Italy.

BACKGROUND AND AIMS: Intracellular glutathione (GSH) is critical in balancing the effects of alkylating agents and reactive oxygen species (ROS); GSH levels are crucial for neuroblastoma (NB) proliferation and survival. GSH levels are regulated by the balance between thiol synthesis and ROS production. We have previously shown that NB cells require GSH to evade apoptosis. In this study, we investigated the role of protein kinase C (PKC) isoforms and GSH levels in the regulation of cell proliferation and apoptosis in NB cells.

METHODS: NB cell lines were treated with different concentrations of the GSH synthesis inhibitor buthionine sulfoximine (BSO) and the GSH depletion agent diethylmaleate (DEM). The effects on cell proliferation and apoptosis were measured by MTT assays and Annexin V staining, respectively.

RESULTS: BSO and DEM treatment induced a dose-dependent decrease in GSH levels and a corresponding increase in apoptosis in NB cell lines. PKC isoform expression was also altered by BSO and DEM treatment, with PKC-α, -δ, and -ε levels increasing and PKC-β1, -β2, -γ, and -ε levels decreasing. The decrease in GSH levels and the increase in apoptosis were reversed by the addition of the GSH synthesis inhibitor L-cystine.

CONCLUSIONS: These findings suggest that GSH levels and PKC isoform expression are important regulators of cell proliferation and apoptosis in NB cells. The specific role of each PKC isoform in the regulation of cell proliferation and apoptosis may provide new insights into the mechanism of neuroblastoma therapy and suggest potential targets for the development of novel therapeutic strategies.
Ref ID: 156.1
Expression of the therapy relevant makers EGF receptor, PDGF receptor and c-Kit in neuroblastoma determined with a multitissue array
Karen Ernestus, Barbara Hero, Ivo Leuschner, Frank Berthold
Children's Hospital, Dept. of Pediatric Oncology and Haematology and Dept. of Pathology, University of Cologne, Germany.
AIMS: Alternative therapy is needed for advanced neuroblastoma patients with relapse or progress after chemotherapy. Recently, tyrosine kinase inhibitors have been shown to be potent alternatives for treatment. We investigated neuroblastoma for expression of the tyrosine kinase epidural growth factor receptor (IGF-IR), platelet-derived growth factor receptor (PDGFR) and c-Kit.
METHODOLOGY: 160 patients treated in the German neuroblastoma trials NR05/95 and NB 07 (n=88) were investigated. From the paraffin-embedded neuroblastoma a multi-tissue array was designed and immunohistochemistry was performed for EGFR, PDGFR and c-Kit. The slides were evaluated semiquantitatively.
RESULTS: All but one case were investigated prior to treatment. 154 neuroblastomas were evaluable for EGFR expression. However, none expressed EGFR. PDGFR was positive in 116 from 148 cases (78%) demonstrating a homogenous staining in most tumor cells. In 47 from 48 cases (92%) c-Kit was expressed in the tumor cells. From the 47 c-Kit-positive cases 45 neuroblastomas also expressed PDGFR, while 2 cases were negative for PDGFR. There was no statistically significant correlation between PDGFR or c-Kit expression and event free survival or overall survival or stage. In addition, no correlation existed between the Shimada classification (favorable versus unfavourable) and expression of PDGFR or c-Kit.
CONCLUSIONS: EGFR seems to be not expressed in neuroblastic tumors and therefore no useful therapeutic target. C-Kit and PDGFR are expressed in neuroblastomas and exceptionally PDGFR was expressed in a high percentage of neuroblastomas suggesting the potential usefulness of tyrosine kinase inhibitors in the treatment of advanced neuroblastoma.

Ref ID: 103.2
Therapeutic activity of STI571 in neuroblastoma xenografts
Meco D.1, Servidei T.1, Riccardi A.1, Vitali R.2, Di Francesco A.M.1, Gent M.1, Raschella G.1, Riccardi R.1, Dominici C.1,3,4
Catholic University1; ENEA Research Center Casaccia2; La Sapienza University3 & Bambino Gesù Children’s Hospital4, Rome, Italy.
STI571 (imatinib mesylate, Gleevec) is a selective inhibitor of several structurally related receptor tyrosine kinases including c-Kit and PDGF-R. We investigated in vitro and in vivo STI571 antitumor activity in neuroblastoma. Four human neuroblastoma cell lines (SK-N-BE2c, SK-N-DZ, HTLA230, RNGA) were studied for candidate targets... and suggest that c-Kit may be the critical molecular determinant of this activity. Clinical evaluation is now appropriate.

Ref ID: 102.1
Arsenic trioxide-induced death of neuroblastoma cells involves activation of bax and does not require p53
Jenny Karlsson, Ingrid Øra, Isabella Pörn-Ares, Sven Påhlman
Laboratory medicine, Molecular medicine, Malmö, Sweden.
BACKGROUND AND AIMS: Based on clinical studies showing that arsenic trioxide (Au2O3), via an apoptotic mechanism, and with minimal toxicity, induces complete remission in patients with refractory acute promyelocytic leukemia, and that multidrug resistant and p53-inhibited neuroblastoma cells are sensitive to Au2O3 both in vitro and in vivo, we searched for molecular mechanisms involved in the Au2O3-induced neuroblastoma cell death. METHODOLOGY: We have studied the effect of Au2O3 on the expression and cellular localization of proteins involved in drug-induced death in two neuroblastoma cell lines with intact p53, and with intact p53, and two with mutated p53. RESULTS: Au2O3 provoked Bak expression in all tested neuroblastoma cell lines, including SK-N-BE2(c) cells with mutated p53 and LA-N-1 cells, which have a deleted p53. In all cell lines exposed to Au2O3, p23 Bax was proteinically detected, a protein which readily fits into the more pro-apoptotic Bak. The anti-apoptotic drug cleavage was associated with a decreased Au2O3-induced cell death. CONCLUSIONS: We show that multidrug-resistant neuroblastoma cells differentiated to Au2O3-induced cell death, while inhibition of Bax cleavage was associated with a decreased Au2O3-induced cell death. We suggest that activation of a cytokine pathway different from that induced by conventional apoptogenic agents. We furthermore propose that the proapoptotic activation of Bax is an important event in Au2O3-induced cell death.

Ref ID: 113.1
Measuring circulating neuroblastoma cells by quantitative RT-PCR: correlation with paired bone marrow and standard disease markers
Irene Y Cheung, Arvind Sahota, Nai-Kong V Cheung
Pediatrics, Memorial Sloan-Kettering Cancer Center, New York, NY, USA.
BACKGROUND AND AIMS: Histologic examination of bone marrow (BM) is an accepted clinical standard to detect metastatic neuroblastoma (NB). Circulating tumor cells in peripheral blood (PB) derive from patients with other BM, and its measurement may provide additional information in cancer management.
METHODOLOGY: 120 patients with stage 4B were evaluated for NB cells in PB by quantitative RT-PCR (qRT-PCR) of GD2 and synaptin with a sensitivity of one NB cell in one million normal cells. The findings were compared with qRT-PCR of their simultaneously sampled marrow aspirates and standard modalities of disease detection according to NCCS criteria.
RESULTS: Detection of GD2 synaptin transcript was found in 62 patients; 11 in both BM and PB, whereas the remaining 51 patients were BM-only (n=38) or PB-only (n=13) by qRT-PCR. Paired samples were BM-PB+ had the highest transcript levels. When extent-of-disease was scored by the number of positive evaluation modalities ranging from 0 to 5, 93% of BM+PB+ patients had evidence of disease in 3 or more modalities. All BM-PB+ patients had less evidence of disease, with a positive disease score <=2. Patients with positive marker in BM, regardless of the PB RT-PCR status, correlated with more evidence of disease by disease score. Kaplan-Meier analysis indicated a trend of poorer survival for patients who were BM+PB+ but who were BM-PB-.
CONCLUSIONS: PB monitoring for NB may complement but not replace BM studies. Since blood sampling is less invasive than BM sampling, its clinical utility for routine surveillance deserves further investigation.
Aging continues to be a powerful predictor of EFS and OS in neuroblastoma.

Hypothesis: Patients with NB who are older at diagnosis have a higher risk of death than younger patients.

Methods: 1251 patients enrolled on Children’s Cancer Group protocols. Analyses are ongoing. Localised and non-localised neuroblastoma in infants: excellent outcome with low-dose primary chemotherapy.

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Methods: 1251 patients enrolled on Children’s Cancer Group protocols. Analyses are ongoing. Localised and non-localised neuroblastoma in infants: excellent outcome with low-dose primary chemotherapy.
A novel 1p36.2 located gene, APITD1, with tumor suppressive properties and a putative p53 binding domain, shows low expression in neuroblastoma tumors

Neuroblastomas generally lack TP53 mutations and no other tumor suppressor gene consistently inactivated has yet been identified in this childhood cancer. Ushaped transcript of a new gene, denoted APITD1, in the neuroblastoma suppressor candidate region chromosome 1p36.22 reveals that APITD1 contains a predicted TFX1-binding domain, representing the TATA box binding protein factor-associated protein, TAFII130, which is required for p53-mediated tumor suppression. APITD1 was expressed at low levels and two different transcripts were shown to be ubiquitously expressed, one with an elevated expression in fetal tissues. Primary neuroblastomas of tumor suppressor gene-deficient mice showed weaker or no APITD1 expression. DNA sequencing of the coding regions and the promoter region in 44 neuroblastomas from children with and without TP53 defects did not reveal any mutations in APITD1. Consistent with our results, the APITD1 gene is well conserved. APITD1 was functionally tested by adding APITD1 miRNA to neuroblastoma cells (SK-N-AS and SK-N-BE), which reduced the cell growth by 90% compared to control cells. We suggest that APITD1 may have a role in a cell death pathway. We suggest that low expression of this gene, in defective cells, impairs the ability for apoptosis through the p53 pathway. Based on its cytogenetic location, 1p36.2, and its biological features in primary neuroblastoma and in mouse cells, APITD1 could therefore be considered as a candidate tumor suppressor gene. Further functional studies of the APITD1 gene and protein, and possible interaction with other genes, are ongoing.

Prediction of Clinical Outcome and Biological Subclassification of Primary Neuroblastomas by Expression Profiling

Neuroblastoma (NB) usually occurs as sporadic tumor although 1-2% of cases have a familial recurrence. Linkage studies carried out by us and other groups confirmed that the NB candidate regions chromosome 1p36 and 1p36 region, frequently deleted in NB. Although evidence of linkage was obtained in NB families with sporadic NB and chromosome 1p36 tumors in our and other studies, we did not observe evidence for linkage to this interval in European families, suggesting genetic heterogeneity of NB. A new familial NB case has recently increased the informativeness of an Italian neuroblastoma. To gain further insights into the role of this alteration in oncogenesis, we have constructed a specific chromosome 1p36.2 located gene, denoted APITD1, with tumor suppressive properties and a putative p53 binding domain, shows low expression in neuroblastoma tumors. We suggest that APITD1 may have a role in a cell death pathway. We suggest that low expression of this gene, in defective cells, impairs the ability for apoptosis through the p53 pathway. Based on its cytogenetic location, 1p36.2, and its biological features in primary neuroblastoma and in mouse cells, APITD1 could therefore be considered as a candidate tumor suppressor gene. Further functional studies of the APITD1 gene and protein, and possible interaction with other genes, are ongoing.

A novel region for predisposition to neuroblastoma maps to 1p36.2

The development of risk-adapted and more effective therapy strategies in neuroblastoma requires further improvements in accurate risk assessment. Although numerous prognostic factors have been identified, precise risk scoring in the individual patient remains challenging. To define a reliable predictor for event-free survival after first-line therapy and to identify genes signature characteristic for clinical and biological subgroups, we performed the first ever large-scale expression profiling study on 868 NB tumors using the Affymetrix HG-U133A 2 arrays. Expression data from subgroups were analyzed using the Multi-locus Alleotyping Defines Clinical Sub-types of MYCN-Status according to FISH: Amplification, Gain, and Non-Amplification.

The relevance of IGF axis in neuroblastoma has been clearly established. IGF binding proteins (IGFBPs) are a family of six proteins that inactivate IGFs, thereby tethering it to their receptor. We have previously demonstrated the link between IGFBP-5 expression and outcome in neuroblastoma and now we are determining other modes of IGF-BP regulation and the effects of increase or depletion of IGFBP-5 protein. We characterized different cis-acting elements, located near the promoter of IGFBP-5, and demonstrated that these regulatory elements were conserved across species. We determined that IGFBP-5 expression is regulated by stimulation. In addition, IGFBP-5 transcription by activating PKA/Akt pathway and retinoid acid is phosphorylated BMP-activated SMADs, increasing their binding to the promoter. Protein binding at the 5 UTR of IGFBP-5 mRNA was also suggested as a post-transcriptional regulation of this gene. Furthermore, we gathered evidences that intracellular IGFBP-5 protein is degraded by the 26S proteasome. We carried out experiments in which the endogenous levels of IGFBP-5 in neuroblasts cells were decreased by slable transfaction of expression vectors (dIMR90) driven by U6 promoter that produce microRNA targeted to IGFBP-5. We detected several effects of IGFBP-5 reduction in IGFBP-5-expressing cells: proliferation appeared markedly decreased (assessed by growth curves and viability assays), anchorage-independent growth was diminished in soft agar based colony assays, apoptosis was sharply increased (Hoechst-staining. PI staining, Caspase 3/7 activation assay); retinoic-acid mediated neuronal differentiation was decreased (assessed by neurite length), and a putative role in the tumorigenesis of NB was inferred, suggesting a critical role in neuroblastoma tumorigenesis.
Neuroblastoma (NB) is a childhood neoplasm with heterogeneous behaviour that can often be cured. Several studies have now described specific molecular defects and/or alterations that could be used to detect minimal residual disease (MRD) in neuroblastoma cells, including loss of caspase-8, FAS, or TRAIL-receptor expression and/or overexpression of prosurvival factors. The present study aimed to demonstrate that systemic administration of IL-12 mediates complete regression of advanced orthotopic intrathoracic NB tumors in immunocompetent mice. IL-12 treatment in NB tumor-bearing mice resulted in neovascularization, and ultrastructural changes consistent with tumor and vascular endothelial cell apoptosis. These changes are accompanied by marked intratumoral infiltration of mononuclear cells. This data suggests an expression of the genes encoding caspase-8, TRAIL, FAS/FASL and TNFR1/2 and/or other pro-apoptotic cytokines in NB cells. These studies demonstrate potent antitumor activity by IL-12 against murine neuroblastoma tumors, and provide the first evidence that IL-12 may downregulate and potentially critical survival pathway and mechanism of tumor self-defense in vivo.

Several lines of evidence suggest that NK cell therapy may represent a promising approach to the treatment of neuroblastoma. Several studies have now described active and potential immunotherapeutic agents that could contribute to a better treatment in children with neuroblastoma. To detect circulating neuroblastoma cells we performed flow cytometric analysis of tyrosine hydroxylase (TH), TH-PC12 and TH-PC12/ND4 expression in blood of patients with neuroblastoma. The expression of TH-PC12 and TH-PC12/ND4 was determined in all patients with neuroblastoma and in healthy controls. The expression of TH-PC12 and TH-PC12/ND4 was significantly higher in neuroblastoma patients than in healthy controls. These data suggest that strategies to activate NK cells and target them to neuroblastoma cells may contribute to therapy of this disease.
Ref ID: 129.1
The tumour-associated antigen PRAME is universally expressed in high stage neuroblastoma and associated with poor outcome
André Oberthuer, Barbara Hero, Rüdiger Spitz, Frank Berthold, Matthias Fischer
Department of Pediatric Oncology and Hematology, University of Cologne, Germany.

PURPOSE: The tumour-associated antigen PRAME (preferentially Expressed Antigen in Melanoma) is of interest as a potential target in neuroblastoma. However, no information is available about its expression in neuroblastoma. We therefore investigated PRAME expression in neuroblastoma tumours and assessed its impact on patients’ outcome.

METHODOLOGY: Analysis of PRAME expression in a total of 101 patients diagnosed with neuroblastoma was assessed by both RT-PCR screening, Northern Blotting and Quantitative Real-Time RT-PCR (QPCR). Subsequently, association of tumour stage, patients’ age at diagnosis and MYCN amplification was determined. Moreover, impact of PRAME expression on both Event-Free Survival (EFS) and Overall Survival (OS) was evaluated.

RESULTS: RT-PCR screening determined PRAME expression in 93% of primary neuroblastoma and 100% of patients with advanced disease (stage 3 and 4). Both Northern and QPCR analysis revealed extraordinarily high oscillation of PRAME expression levels and showed significantly associated level of PRAME expression with both advanced tumour stage (p<0.01) and patients’ age at diagnosis (p<0.001). Finally, grouping of patients according to their QPCR values revealed significant impact on patients’ outcome: 3-year EFS was 0.92 ±0.01 (p=0.01) for patients with PRIME values <0.71 (n=80) versus values with patients with values ≥0.71 (n=20-0.00 and 0.45 (n=10) for patients with values >20.00 (p=0.01)-3 year overall survival for these groups was 1.00, 0.86 (n=0.07) and 0.38 (p=0.001). PRIME expression in neuroblastoma is extraordinary consistent and was universally seen in patients with advanced stage disease in our study. Furthermore, significant impact of PRAME expression on patients’ outcome was also observed in patients with completely resected tumours, suggesting extraordinarily attractive target for immunotherapeutic strategies in neuroblastoma.

Ref ID: 192.1
In vivo resistance to CPT-11 in neuroblastoma: Does pleiotrophin play a role? Lorelloy Calvet, Birgit Georger, Alexander Valentin, Gilles Vassal
Department of Pharmacology and New Treatments in Cancer, Institut Gustave Roussy, Villejuif, France.

To study resistance mechanisms to topoisomerase I inhibitors in a therapeutic setting, we established a neuroblastoma xenograft model (IGR-NB8R) with in vivo resistance to CPT-11. Tumor resistance was achieved after 25 passages in nude mice treated with several cycles of 27 months CPT-11.

METHODOLOGY: Continuous delivery of HNFb was established using a gene therapy approach in which adenovirus (Ad-Cre) carrying human HNFb under control of the CMV promoter was used for transduction of IGR-NB8R cells. HNFb expression was confirmed by indirect immunoperoxidase staining. HNFb expression levels were analyzed by Western blot. Resistance results were obtained by in vivo experiments with xenografts in nude mice treated with CPT-11 or vehicle alone.

RESULTS: No overt toxicities were observed. The development of both localized and disseminated disease was prevented in all mice expressing HNFb (n=8 for each group). Established retropitoneal tumors, treated with HNFb when a average tumor size of 280 mm3, grew to only 27% of the size of control treated tumors (p<0.002) after 12 additional days. All mice treated with AAV HNFb once disseminated disease was documented in the liver were still alive 35 days later whereas those treated with a control vector died within 13 days of vector administration. Ten children were treated with AAV HNFb in the naive setting. In 7 out of 10 children, no effects were observed on tumor growth.

CONCLUSIONS: Chronic, genetic AAV-mediated delivery of interferon-β successfully prevented localized and disseminated neuroblastoma engraftment and significantly retarded growth of established retropitoneal and disseminated disease. Considered of this approach should be given for the treatment of patients with neuroblastoma, perhaps in combination with cytokine therapy, and as the setting of minimal residual disease.

Ref ID: 227.3
Interferon-α-mediated anti-angiogenic therapy for neuroblastoma Andrew M Davidoff, Christian J Streck, Catherine YC Ng, Yubin Zhang, Junfang Zhou, Amit C Nathwani
Department of Surgery, St. Jude Children’s Research Hospital, Memphis, Tennessee, USA.

AIMS: Type I interferons (IFN-α) have pleiotropic antitumour activities but have shown limited clinical efficacy and significant toxicity. We hypothesized that the antitumour and anti-angiogenic activity of IFN-α could be enhanced while limiting its systemic toxicity, by chronic, low-dose (metronomic) IFN-α treatment in neuroblastoma patients.

METHODOLOGY: Continuous delivery of HNFb was established using a gene therapy approach in which adenovirus (Ad-Cre) carrying human HNFb under control of the CMV promoter was used for transduction of IGR-NB8R cells. HNFb expression was confirmed by indirect immunoperoxidase staining. HNFb expression levels were analyzed by Western blot. Resistance results were obtained by in vivo experiments with xenografts in nude mice treated with CPT-11 or vehicle alone.

RESULTS: No overt toxicities were observed. The development of both localized and disseminated disease was prevented in all mice expressing HNFb (n=8 for each group). Established retropitoneal tumors, treated with HNFb when a average tumor size of 280 mm3, grew to only 27% of the size of control treated tumors (p<0.002) after 12 additional days. All mice treated with AAV HNFb once disseminated disease was documented in the liver were still alive 35 days later whereas those treated with a control vector died within 13 days of vector administration. Ten children were treated with AAV HNFb in the naive setting. In 7 out of 10 children, no effects were observed on tumor growth.

CONCLUSIONS: Chronic, genetic AAV-mediated delivery of interferon-β successfully prevented localized and disseminated neuroblastoma engraftment and significantly retarded growth of established retropitoneal and disseminated disease. Considered of this approach should be given for the treatment of patients with neuroblastoma, perhaps in combination with cytokine therapy, and as the setting of minimal residual disease.

Ref ID: 299.1
Cyclooxygenase-2 (COX-2) is abundantly expressed in neuroblastoma and its inhibition induces apoptosis and prevents tumour growth in vivo: implications for a novel non-toxic neuroblastoma therapy
John I Johnsen1, Magnus Lindskog1, Frida Ponthan1, Ingvild Pettersen2, Lotta Elfman1, Abiel Orrego3, Baldur Sveinbjornsson2, Per Kogner1
Department of Childhood Cancer Research Unit and Dept. of Pathology, Karolinska Institute, Stockholm; Department Exp. Pathology, University of Tromso, Norway.

INTRODUCTION: The aim of this study was to determine the efficacy of [131I]MIBG in combination with topotecan in vitro and in vivo.

RESULTS: Two cell lines, expressing the noradrenaline transporter (SERT) and [131I]MIBG as the experimental combination treatment of tumours expressing the noradrenaline transporter. [131I]meta-iodobenzylguanidine and topotecan: Experimental combination treatment of tumours expressing the noradrenaline transporter Anthony G McCluskey, Emilio Cosimo, Mark N Gaze, Marie Boyd, Robert J Mairs Radiation Oncology, CRUK Beatson Labs, University of Glasgow, Scotland, UK.

INTRODUCTION: The aim of this study was to determine the efficacy of [131I]MIBG in combination with topotecan in vitro and in vivo.

METHODOLOGY: Analysis of PRAME expression in a total of 101 patients diagnosed with neuroblastoma was assessed by both RT-PCR screening, Northern Blotting and Quantitative Real-Time RT-PCR (QPCR). Subsequently, association of tumour stage, patients’ age at diagnosis and MYCN amplification was determined. Moreover, impact of PRAME expression on both Event-Free Survival (EFS) and Overall Survival (OS) was evaluated.

RESULTS: RT-PCR screening determined PRAME expression in 93% of primary neuroblastoma and 100% of patients with advanced disease (stage 3 and 4). Both Northern and QPCR analysis revealed extraordinarily high oscillation of PRAME expression levels and showed significantly associated level of PRAME expression with both advanced tumour stage (p<0.01) and patients’ age at diagnosis (p<0.001). Finally, grouping of patients according to their QPCR values revealed significant impact on patients’ outcome: 3-year EFS was 0.92 ±0.01 (p=0.01) for patients with PRIME values <0.71 (n=80) versus values with patients with values ≥0.71 (n=20-0.00 and 0.45 (n=10) for patients with values >20.00 (p=0.01)-3 year overall survival for these groups was 1.00, 0.86 (n=0.07) and 0.38 (p=0.001). PRIME expression in neuroblastoma is extraordinary consistent and was universally seen in patients with advanced stage disease in our study. Furthermore, significant impact of PRAME expression on patients’ outcome was also observed in patients with completely resected tumours, suggesting extraordinarily attractive target for immunotherapeutic strategies in neuroblastoma.

Ref ID: 310.1
[131I]meta-iodobenzylguanidine and topotecan: Experimental combination treatment of tumours expressing the noradrenaline transporter Anthony G McCluskey, Emilio Cosimo, Mark N Gaze, Marie Boyd, Robert J Mairs Radiation Oncology, CRUK Beatson Labs, University of Glasgow, Scotland, UK.

INTRODUCTION: The aim of this study was to determine the efficacy of [131I]MIBG in combination with topotecan in vitro and in vivo.

RESULTS: Two cell lines, expressing the noradrenaline transporter (SERT) and [131I]MIBG as the experimental combination treatment of tumours expressing the noradrenaline transporter.
Improved targeted therapeutic approaches to neuroblastoma require a deeper understanding of the molecular pathogenesis of the disease. In order to identify novel therapeutic targets, we investigated the role of tumor necrosis factor receptor-associated factor 6 (TRAF6) in neuroblastoma. TRAF6 is an important mediator of the innate immune response and has been implicated in multiple steps of the immune response, including the induction of proinflammatory cytokines and the regulation of innate lymphoid cell differentiation. We found that TRAF6 expression was significantly associated with improved clinical outcomes in neuroblastoma patients, suggesting that targeting TRAF6 may be a promising therapeutic strategy for this disease.

The Delta-Notch pathway plays an important role in embryonal development and differentiation. This pathway is involved in cell fate decisions during differentiation of cell lineages. The Delta1 gene is highly expressed in normal adrenal medullosa and in a subset of neuroblastoma cell lines. The Delta-Notch pathway may also play a role in the development of neuroblastoma. We found that the expression of Delta1 was correlated with increased aggressiveness of neuroblastoma cells. Our study suggests that targeting the Delta-Notch pathway may be a potential therapeutic strategy for neuroblastoma.

The Delta-Notch pathway integrates the Wnt pathway, the noradrenalin synthesis route and the neurotrophin pathway in neuroblastoma. This pathway is involved in the development and differentiation of neural cells and has been implicated in the pathogenesis of neuroblastoma. Our study suggests that targeting the Delta-Notch pathway may be a potential therapeutic strategy for neuroblastoma.

In summary, our study suggests that targeting the Delta-Notch pathway may be a promising therapeutic strategy for neuroblastoma. Further studies are needed to fully elucidate the role of this pathway in neuroblastoma and to develop novel therapeutic strategies for this disease.
Oral Presentations

Ref ID: 214.1
Germline mutations of the paired-box homeobox 2B (PHOX2B) gene in hereditary neuroblastoma.

Yael P. Mosse, Joel Greshock, Tara Naylor, Deepa Khazi, George Hii, Qun Wang, Cynthia Winter, Suzanne Shusterman, Barbara J. Weber, John M. Maris

Department of Oncology, Children’s Hospital of Philadelphia, PA, USA

The pattern of genomic alterations present in neuroblastomas is strongly associated with tumor behavior and patient outcome. We have used aCGH (4300 BACs, 1 Mb resolution) to perform an unbiased survey of genomic aberrations, and for high resolution mapping of relevant chromosomal loci. We first used a panel of 43 neuroblastoma cell lines (136p, 2p24, 11q23-22, and 17q23-24) and alu repeat cluster data to validate detection of known aberrations. We showed outstanding sensitivity and specificity with all amplicons detected and high concordance with PCR-based hemizygous deletion detection methods. Using a variety of class discovery (eg hierarchical clustering) and prediction (eg k-fold cross-validation) analyses, we have identified frequent recurrent genomic alterations including 1p, 7, 13, and 16. Deletions of 1p were present in 30% of the lines, and we have identified a 5 Mb 1phemizygous deletion. We have also used our platform to map to the critical regions of chromosome 11 required for tumor suppression in functional complementation studies, allowing us to define two critical 11q regions of 2.5 and 4.8 Mb, respectively. These data demonstrate that aCGH can accurately measure copy number in the neuroblastoma genome, refine common regions of discordance among existing approaches, detect hemizygous deletions, and be used to identify novel regions of genomic imbalance.

Ref ID: 225.4
Array comparative genomic hybridization (aCGH) defines genomic subgroups with a set of distinct aberrations which are strongly associated with the prognosis of patients with neuroblastoma.

Nobumoto Tomioka1, Miki Ohira1, Shigeyuki Oba2, Anjan Misra3, Janice Nigro3, Ivan Smirnov3, Jane Fridlyand3, Satoru Todo4, Dan Pinkel3, Donna Albertson3, Yasuhiko Kaneko5, Takeshi Goto6, Shin Ishii2, Burt G. Feuerstein3, Akira Nakagawara1

Chiba Cancer Center Research Institute1 and Nara Institute of Science and Technology2, Japan; Brain Tumor Research Center3, Cancer Center, University of California, San Francisco, CA, USA; Hokkaido University, School of Medicine; Seisakusho, Seikagaku Pharmaceutical Co., Ltd.

To unveil DNA copy number aberrations (CNA) which characterize distinct subsets of neuroblastomas, we applied array-CGH (2,464 human BAC clones) to 244 primary neuroblastomas (120 sporadic, and 114 mass screening) and 25 embryonal rhabdomyosarcoma. ACGH analysis showed the presence of distinct genomic subgroups. Group 1 (G1; n=5), 6-year survival rate was 84%; and DNA ploidy: 35% of the cases. Group 2 (G2, n=6; 9% survival, and 100% diploidy) had a pattern similar to G1, except that they had MYCN amplification. The prognosis of the patients in G2 was externally poor G3 (n=9, 54% survival, and 50% diploidy) only had 1p loss and 17q gain and moderate grades of whole chromosome gains and losses. MYCN amplification occurred in 57% of cases. G4 (n=55, 70% survival, and 47% diploidy) had both 11q loss and 17q gain and other chromosomal abnormalities. MYCN amplification occurred in 15% (G4, 98% survival, and 31% diploidy) had whole chromosome gains and losses, and most were diagnosed by mass screening. Five-year survival rates of 120 sporadic cases were G1 74% (n=23), G2 17% (n=7), G3 38% (n=32), G4 46% (n=31) and G5 38% (n=28). Overall, G1 and G2 had little instability, except G2 had MYCN amplification. Both G3 and G4 had a pattern of genomic instability, and the abnormalities in G5 were more consistent with a pattern of mitotic dysfunction. The presence of G2 itself suggests a new classification of metastatic tumors. Most interestingly, the analysis of the paired primary and recurrent tumor samples suggested progression from G1 and from G2 to G3. These suggest that both 1p loss and MYCN amplification can occur independently in neuroblastoma, and that MYCN amplification might progress into 1p deletion.

Ref ID: 297.1
Identification of relevant MYCN downstream effectors by a combinatorial multimodel approach.

Philip Pytte, Katleen De Preter, Nadine Van Roy, De Di Doro, Anne De Paepe, Genevieve Laureys, Frank Speleman, Jo Vandepaele

Department of Center for Medical Genetics, Ghent University Hospital, Gent, Belgium

The Amplification of the MYCN transcription factor is the hallmark of a subgroup of advanced stage neuroblastomata tumors and was one of the first genetic parameters used for therapy stratification. However, up to now, the molecular circuit governed by MYCN and its target genes is not fully understood. One of the problems that confound deeper insights is the time and cell specific context that influences the MYCN transcriptional program. To address this issue, we designed a multimodel strategy to identify consistent and relevant MYCN downstream effectors. CDA downstream clustering between an amplified and MYCN single copy neuroblastoma cell line generated a list of 300 differentially expressed transcripts (including some novel genes), that were spotted onto a custom CDA neuronal array. Subsequent expression profiling of an extended series of both amplified and MYCN single copy cell lines, and a stably MYCN transfected cell line versus their parent non-amplified cells provided a list of putative MYCN transcriptional target genes. To discriminate between early (direct) and late (secondary) effectors, we profiled different time points in a cell line with tetracycline controllable MYCN expression. Genes that were found consistently differentially expressed in all tested model systems, were further scrutinized by siRNA promoter analysis, and ongoing reporter gene assays. In general, MYCN appears to be a more potent transcriptional silencer, and our data provide possible insights into the way MYCN exerts its oncogenic effects. Further validation in primary tumor biopsies indicated that some MYCN target genes are significantly correlated with reduced patient survival, hence providing potential targets for molecularly oriented therapies for patients with this type of aggressive tumor.
Multivariate evaluation for heterogeneous neuroblastomas: the discrimination of progressing risk tumors detected clinically and through infantile mass-screening program
Takuo Tanaka, Tomoko Iehara, Hiroshi Kuroda, Tohru Sugimoto, Minori Hamakawa, Sonohi Tomioka, Yoshiko Tachida, Mikiro Kato, Tadashi Sawada
Department of Pediatrics, Kyoto Prefectural University of Medicine, Kyoto, Japan.

BACKGROUND: Serum DNA of cancer patients includes a significant amount of tumor-released DNA. Several groups reported that tumor-related aberrations (e.g., loss of heterozygosity) could be detected in the DNA. From serum DNA, the serum M/N ratio appears to be a promising indicator of therapeutic efficacy and relapse for MYCN-amplified cases.

Ref ID: 278.1

Prediction of MYCN amplification in neuroblastoma using serum DNA and real-time quantitative PCR
Takahiro Gotoh, Tomoko Iehara, Hiroshi Kuroda, Tadashi Sawada
Department of Pediatrics, Kyoto Prefectural University of Medicine, Kyoto, Japan.

BACKGROUND: Serum DNA of cancer patients includes a significant amount of tumor-released DNA. Several groups reported that tumor-related aberrations (e.g., loss of heterozygosity) could be detected in the DNA. From serum DNA, the serum M/N ratio appears to be a promising indicator of therapeutic efficacy and relapse for MYCN-amplified cases.

Ref ID: 115.1

Association Of High-Level MRPI Expression With Poor Clinical Outcome In A Large Prospective Study Of Primary Neuroblastomas
Michelle Haber1, Susan Smith1, Sharon Bordow1, Susan L Cohn2, Wendy B London3, Glenn M Mullin1
1Department Pediatrics, University of California, San Francisco, CA; 2University of Miami; 3University of Pennsylvania, USA.

We have previously shown in a retrospective analysis that high expression of the multidrug transporter gene MRPI is strongly predictive of poor outcome in neuroblastoma (NEJM, 334:231-8, 1996). We now present preliminary results from a large prospective study with MRPI expression data. Patients were enrolled into two groups; 226 NBs with MYCN-amplification (MYCN-AM) and 298 NBs without this aberration (MYCN-N). The serum M/N ratio was measured in all cases. The serum M/N ratio was significantly higher in MYCN-AM than in MYCN-N patients (p=0.0025). Although dichotomizing MRPI expression around the median failed to predict outcome, higher outcomemrpi expression became a significant predictor of outcome. Thus, high levels of MRPI (above 0.4) were highly predictive of both event-free survival (p=0.0011) and overall survival (p=0.0003). This cut-off clearly approximated the level of MRPI expression in SK-N-BE cells, which we previously recommended as a reference standard for MRPI. Following adjustment for the effect of MYCN amplification and of other prognostic indicators by multivariate analysis, MRPI expression retained a significant prognostic value for both event-free survival (hazard ratio=0.3; p=0.0011) and survival (hazard ratio=2.6; p=0.0095), whereas MYCN amplification lost prognostic significance. MDRI expression demonstrated no prognostic significance. The results of this prospective study confirm our earlier findings and support a clinically relevant role for MRPI gene expression in drug refractory neuroblastoma.

Ref ID: 123.1

From genotype to phenotype in neuroblastoma: The derivation of clinically different entities
Frank Berteloot1, Israel Khan2, Frank Wotring1, Simon Thörner, Haupounter A, Haupounter A, Haupounter A
Department of Pediatric Oncology and Hematology, Children’s Hospital, University of Cologne, Cologne, Germany; NIH, Bethesda; German Cancer Research Center, Heidelberg.

BACKGROUND: From high throughput genetic studies several characteristic signatures have been proposed. We asked whether or not the suggested genetic categorization is associated with particular clinical phenotypes.

METHODS: The clinical characteristics of 176 patients from three clinically different groups were retrospectively analyzed. Group 1 was defined by no MYCN amplification, no 1p, no 11q, and stage I (n=91). Group 2 comprised tumors without MYCN amplification, with 1p amplification, with 11q amplification and stage 4 (n=87). Group 3 consisted of stage 4 patients with MYCN amplification and without 11q amplifications (n=96).

RESULTS: Marked differences were found between the groups in several aspects. Age: Group 2 patients were older (40 months) compared to group 3 (23 months) and group 1 (10 months, p<0.001). Site: In group 3 the adolescents were more frequently involved (95%, 2; 60%, 1; 44%; p<0.001). No differences were detected in the metastatic patterns between groups 2 and 3. Histology: The tumors of group 3 were more frequently neuroblastoid differented tumors (3: 25%, 2: 41% p=0.02). Unfavorable Shimada histology was similar in groups 2 and 3 (1: 7%; 2: 6%; 3: 69%). Tumor markers: VMA was frequently elevated in group 2 (96%), but only half in group 1 (55%) and 3 (59%) (p<0.001). Outcome: The 3 year overall survival rate was 100%, 73% and 49% (p=0.004) for the groups 1, 2 and 3, respectively. The recurrence of group 2 presented later that those of group 3.

CONCLUSION: The genetically defined neuroblastoma categories demonstrated distinct clinical differences even within the stage 4 subgroups.

Ref ID: 378.1

131-I-MIBG double infusion with autologous stem cell transplant (ASCT) for neuroblastoma: A New Approaches to Neuroblastoma Therapy (NANT) study
Kathleen K Matlak1, John Hribar2, Randall Hawkins1, Gregory Yank1, Marie Charette1, Susan Glasgow1, Robert C Seeger1, Shane Stanimirovic3, John M Maris3
1Department Pediatrics, University of California, San Francisco, CA; 2University of Miami; 3University of Pennsylvania, USA.

131-Metaiodobenzylguanidine (131-I-MIBG) elicits 30% response in refractory neuroblastoma, but the activity infused is limited by radiation safety and hematologic toxicity. The goal of this study is to determine the dose of 131-I-MIBG that can be administered in two consecutive infusions at a 2-week interval, supported by ASCT 2 weeks after the second dose. The dose of 131-I-MIBG was escalated in a 3+3 Phase I trial design, with levels calculated by delivered red marrow radiation index (RMF) from the infusion target. The levels ranged from 400, 600, and 800 cGy. Using dosimetry, the second infusion was adjusted to achieve the target RMF. Seven patients were enrolled; 6 were evaluable for toxicity. Median age was 8 years, all were heavily pretreated, including 6/7 with prior high dose therapy and ASCT. Mean activity administered was 23 μCi/kg for patients at Level 1, with mean measured RMF of 425 ±65. Level 2 activity total 36 μCi/kg, with a mean measured RMF of 571 ±65. Level 3 begins in 3004. No dose-limiting toxicity occurred to date. Hematologic toxicity has been acceptable, with median time to ANC<800 after ASCT of 13 days. All patients required platelet transfusion, with median time to platelet independence of 17 days after ASCT. They have had no infections, hemorrhage, liver with neutropenia, or other non-hematologic toxicity above grade 1. Of 6 evaluable patients, there has been 1 MR and 3 SD. Four patients are alive with stable disease at 20-215 days. The efficacy and safety of this approach will allow dose intensification of 131-I-MIBG, with the possibility of improved response in refractory disease.
The inhibitor of differentiation (Id) 2 is a target of the Retinoblastoma protein (Rb) during embryogenesis. Expression of Id2 is directly activated by Myc oncogenes and plays a key role in the development of human neuroblastomas. Id2 overexpression results in the down-regulation of the anti-apoptotic protein Bcl-xL and up-regulation of the pro-apoptotic protein Bad. Therefore, Id2 is a critical mediator of Rb pathway-dependent cell death in neuroblastoma cells. Inhibition of Id2 expression results in increased cell survival and proliferation, indicating that Id2 plays a key role in the control of cell death in neuroblastoma cells. Therefore, inhibition of Id2 could be a potential therapeutic strategy for the treatment of neuroblastoma.

**Ref ID: 309.1**

**Identification of MYCN transcriptional activity inhibitors yields compounds which preferentially inhibit the growth of neuroblastoma cells**

An effective therapeutic strategy for neuroblastoma is needed. However, the most common approach is chemotherapy, which often results in drug resistance. Therefore, the identification of novel therapeutic strategies is crucial. In this study, we identified compounds that preferentially inhibit the growth of neuroblastoma cells.

**Ref ID: 653.1**

**Methylation Patterns in Ganglioneuroma and neuroblastoma**

The hypermethylation of CpG islands within Gene promoter regions is an epigenetic phenomenon that is often associated with the transcriptional silencing of downstream genes and contributes to carcinogenesis. In this study, we investigated the methylation patterns of genes associated with neuroblastoma, including MYCN, and we found that hypermethylation of these genes was associated with the development and progression of neuroblastoma.

**Ref ID: 374.1**

**Expression of TrkA in SY5Y Neuroblastoma Cells Sensitizes Cells for Chemotherapy-Induced Apoptosis by Up-Regulation of Caspase-8**

In neuroblastoma, the expression of TrkA has been shown to be associated with a favorable prognosis. Therefore, the identification of compounds that sensitize TrkA-expressing neuroblastoma cells to apoptosis is crucial. In this study, we identified compounds that up-regulate caspase-8 expression in TrkA-expressing neuroblastoma cells, indicating that these compounds could be potential therapeutic agents for the treatment of neuroblastoma.

**Ref ID: 338.1**

**p53/MYC/NF-kappaB Signaling Pathway Mediates Anticancer Drugs Induced Cell Death in SY5Y Neuroblastoma Cells**

The p53/MYC/NF-kappaB signaling pathway is a critical target for the treatment of neuroblastoma. In this study, we found that anticancer drugs induce cell death in SY5Y neuroblastoma cells by activating the p53/MYC/NF-kappaB signaling pathway, indicating that targeting this pathway could be a potential therapeutic strategy for the treatment of neuroblastoma.
Enhancement of targeted radiotherapy in neuroblastoma: a novel gene therapy approach

Emilio Consoni, Maria Boyd, Anthony G McElwain, T Robben, Michael R Zalutsky, Robert J Mann

Radiation Oncology, University Of Glasgow, UK.

The radiosurgical 131I-MIBG used as a single agent, has obtained long term eliminations and palliation in neuroblastoma patients. A cureative effect of 131I-MIBG can be achieved by increased concentration of the radiosurgical agent in malignant cells. We previously reported that transfection of the NAT gene into human neuroblastoma cells induced the expression of the functional NAT enzyme, thereby improved the active uptake of 131I-MIBG and resulted in dose-dependent toxicity to the host cells. A natural gene transfer system would be preferable, which should be able to replicate the synthesis of the NAT in neuroblastoma cells. This strategy involves transfected NAT/NAT transfaction followed by an initial dose of radiiodinated MIBG to induce tumour specific expression of the tumour suppressor gene. In this study we transfected a neuroblastoma cell line (SK-N-BE) with a plasmid construct which contained the NFAT gene. By means of a selectable promoter of WAF1 (p21), A G4 extranuclear-brain radio dose increased the GP3 protein level up to 1.4 times the untransfected cells protein level. In the same condition, preliminary studies showed that 4 µg doses of 123I-MIBG and 5 µg doses of 131I-MIBG were able to increase GP3 protein level to 1.5 times the untransfected cells protein level. These encouraging results suggest that the radiation-inducible promoter of WAF1 could be a valid candidate to drive the over-expression of the NAT gene in neuroblastoma cells in a radiosurgical-dependant manner.

Genes associated with multi-drug resistance in neuroblastoma cell lines identified by gene expression profiling

Niko Kovalchik, Bo Yang, Xiao Chen, Timothy J Fitch, Patrick C Reynolds

Department of Hematology/Oncology, Children’s Hospital Los Angeles/USC, CA, USA.

Loss-of-function of p53 (p53-LOF) is one mechanism of acquired multi-drug resistance in neuroblastoma. We have previously published that neuroblastoma cell lines (10 drug-sensitive, 7 MDI) to identify other mechanisms of MDR. Higher expression of p53 (p53-HF) correlated with MDR (minimal p53; median: 24) vs. 7 p53 (p53-HF) (median: 24 vs. 7 p53-LOF) cell lines using the human U335 Attentive gene-chips. Included a paired cell lines from patient treated with MDR drugs (N=7) and drug naive (N=19). To compare drug resistance genes, we compared expression data in 2 drug-sensitive and 3 MDR cell lines using the human U335 Attentive gene-chips. Included a paired cell lines from patient treated with MDR drugs (N=7) and drug naive (N=19). To compare drug resistance genes, we compared expression data in 2 drug-sensitive and 3 MDR cell lines using the human U335 Attentive gene-chips. This study was supported by Grant PHS CA1192095-01.

IMPROVED OUTCOME IN HIGH-RISK NEUROBLASTOMA AFTER APPLICATION OF INTENSIFIED MULTIMODAL THERAPY

Per Kager, Per Bengtsson, Bengt Kogel, Gunn Lundell, Anna-Lena Hultén Spiel, Jack Wennberg, Monica Rodolfo

Memorial Sloan-Kettering Cancer Center1 and Department of Pediatric Hematology Oncology2, Istituto Nazionale Tumori, Milano, Italy.

We report the results of the SIOPEN-NB97 protocol in children >1 year of age with high-risk neuroblastoma (HR-NB) and indicate that NY-ESO-1 is a potential target antigen for immune-based intervention strategies in NB patients.

BACKGROUND AND AIMS: Neuroblastoma cells have been shown to express molecularly defined tumor-associated antigens and to be induced to express HLA by cytokines, thus becoming targets for immune elimination. However, the biological factors involved in its development and progression are still unclear. Promoter hypermethylation of the status of CpG island hypermethylation in human primary tumors may have clinicopathological value.

To improve the prognosis of children > 1 year with a stage 4 NB, we developed an intensified multi-modality therapeutic strategy (ICR131) with high-dose iodine-131-labelled meta-iodobenzylguanidine (131I-MIBG) as a novel targeted radiotherapy, topotecan and haemopoietic support. We have conducted an international randomized phase III study (NCRI-IBG97) after application of intensified multimodal therapy (IMRT) with the primary aim to improve survival and cure for children with high-risk neuroblastoma. We conclude that today the majority of children with high-risk neuroblastoma can be cured.

Improvement in high-risk neuroblastoma: MATERIAL. An unselected population-based consecutive series of high-risk neuroblastoma at a single institution. Forty-five not previously treated children were included in the trial from 1994-2003 with high-risk neuroblastoma (stage 4-S or stage 4-SY or stage 4-C with amplified MYCN, or stage 3-or stage 4-C with MYCN amplification).

TREATMENT: Therapy was step-wise intensified over time during three distinct periods. Participation in collaborative trials was given priority. 1984-1991: IFNS-91. The first entered OPEX (neuroblastoma surgery) and in some cases high-dose Melphalan and autologous bone-marrow rescue (ABMT). 1991-1995: children used randomised (ENS05) to OPEX-COE or COEC followed by surgery. Mel-ABMT and, low dose retinoic acid (RA). 1996-2003: children of the first was randomised in ENS05, the rest received COEC. Surgery was aiming at complete removal. Local irradiation was applied as intraoperative radiotherapy (IORT) and/or external irradiation to the pre-operative primary tumour volume. High-dose chemotherapy (Melphalan alone, or with etoposide-carboplatin or liposomal buparlisib) was followed by stem-cell rescue. Local irradiation and six months high-dose RA in two-week pulses. Selected patients received MIBG-therapy from 1999.

CONCLUSION: Event-free survival probability was 0%, 17% and 56% at 36 months for the periods 1984-1994, 1995-1996 and 1996-2003 resp. (p<0.001). The estimate for 5 years was 18% (95% CI 6%) for children from 1984-1994 are alive whereas 1724 from 1994-2003 are alive in COEC (p<0.01). Our study was performed from 1998-2003. Results of NB 97 SFOP Protocol in children > 1 year with a stage 4 neuroblastoma

Domenico Valente1, Johann Andtchou, Van Per, Christoph Benenson, Hartke Rubie, Carole Coze, Chantal Rodary, Olivier Hartmann

For the SFOP (Société Française de Oncoologie Pédiatrique)

In conclusion, the introduction of 131I-MIBG therapy by dose escalation and combination with a radiotherapy with a hematopoietic autograft appears safe and practical. This approach should be tested for efficacy in a Phase II study in patients with refractory stage 4 neuroblastoma.
Hydroxylase promoter causes tumors in transgenic mice that recapitulate human neuroblastoma (EMBO J. 16:2985-95, 1997). To ... of the S-type marker S100A6 supported this hypothesis. These unique murine cell lines thus provide a valuable model for studying neuroblastoma tumorigenesis.

INTRODUCTION: Cyclin D1 regulates G1 cell cycle progression by controlling the phosphorylation of the retinoblastoma protein (pRb). mRNA expression analysis by SAGE and Northern blot analysis confirmed high expression of Cyclin D1 in 75% of the tumors. We screened by using Southern blot analysis for amplifications and rearrangements of the Cyclin D1 gene in these tumors. Amplifications and one rearrangement were found in 202 neuroblastomas.

AIM: To study the biological relevance of Cyclin D1 and the functional significance of its gene amplification.

RESULTS: We developed small interfering RNAs of which one showed a good transient silencing of Cyclin D1 after 48 hours. We could achieve an 80 to 90% reduction of Cyclin D1 in the adherent lines, together with 50% reduction in SK-NB-E, SK-N-FL, SK-N-MS, or SK-N-BE 2 cells. The SK-N-MS cells harboring an activating point mutation of NRAS in exon 13 (G12V) were rendered proliferating by Cyclin D1 siRNA and remained non-proliferating in the presence of Cyclin D1 siRNA. These findings reveal a critical role for Cyclin D1 in the development of neuroblastomas, but also suggest that Cyclin D1 amplification may represent a useful target for the development of novel therapeutic approaches.

CONCLUSION: Cyclin D1 is a valid target for therapy of neuroblastomas, either as a single entity or as a part of a more comprehensive treatment approach.
that this may be especially relevant for patients with low- and intermediate-risk disease. Identification of the gene(s) targeted by 11q deletions should provide insight into the acquisition of a more malignant neuroblastoma clinical phenotype.

The clinical heterogeneity of neuroblastoma provides a therapeutic challenge. CD1d-restricted V-alpha24/J-alpha18-invariant Natural Killer T cells (iNKT) are potentially important in tumor immunity. However, little is known about their localization to tumors. We analyzed... cells in a CCL2/MCP-1-dependent manner, preferentially infiltrating MYCN-non-amplified tumors that express CCL2/MCP-1.

AIM: To analyze the role of SRF in the surgical approach and in the outcome with the N6 regimen for NB (JCO 12:2607, 1994). We test if using 5 instead of 7 chemotherapy cycles while adding... to achieve CR/VGPR in the majority of children with high-risk NB. Early use of 3F8 may improve molecular remission.

BACKGROUND AND AIMS: We previously reported a high response rate... patients with neuroblastoma: a Children's Oncology Group Study. Further work is required for tumour dosimetry. Quantitative analysis will form a strong basis for multi-centre trials.

Chromosome 11q deletions are an independent marker for decreased survival probability in patients with neuroblastoma: a Children's Oncology Group Study

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Promising results of a pilot trial of a GD2 directed anti-idiotypic antibody as a vaccine for high risk neuroblastoma

Alice L Yu, Ayse Batova, Douglas Strother, Paola Angelini, Robert Castleberry
Pediatric Hematology/Oncology, University of California San Diego, California, USA.

We conducted the first pediatric trial of mAb1A7, an anti-id antibody directed against anti-GD2, 14G2a, as a vaccine for high risk neuroblastoma. Thirty one patients with high risk neuroblastoma who achieved a first or subsequent complete response, or a very good partial response were entered into this trial Patients received SQ injection of mAb1A7 (Titan Pharmaceutical Inc.) q.2w (Aquilla Pharmaceutical Inc.) q.2w x 4, q x 11. The treatment was well tolerated with only transient local reactions, transient fever and chills in 4 patients, and serous sickness in 1. Neurotic rash, often seen with immunotherapy, was not observed. Overall survival (OS), and Event free survival (EFS) were analysed by the Kaplan-Meier test while correlations were assessed with Cox regression.

RESULTS: A total of 115 cases were evaluated following INPC criteria. 4 cases (3.2%) were not evaluable and 5 cases (4%) remain unresolved. The 115 evaluated cases were classified in favorable (91 cases-79.1%) and unfavorable (24 cases-20.9%) categories. Regarding OS after 60 months of follow-up, 97.8% [94.7-100] of favorable cases were alive compared with 72.6% [53.2-91] of unfavorable cases (p=0.001).

EFS analysis showed that a relapse rate of 13.2% [6.3-20.2] and 29.2% [11-47.4] was observed in favorable and unfavorable cases (p=0.05) respectively. Survival analysis demonstrated a significant association between INPC and LDHI (p=0.04).

On the contrary, no significant correlation was found between INPC categories and 1p deletion (p>0.2).

CONCLUSION: Histopathological classification therefore has greater prognostic impact than other factors in predicting relapse in localised neuroblastoma.

Ref ID: 426.1

Histoaggregative value of INPC classification in localised resectable neuroblastoma

Emmanouel S D'Amore, Anna Gribble, Klaas Boeke, Carey Cullinane, Claudio Gambini, Jean-Marc Joseph, Nicole Gross
Pathology and Clinical Sub-Committees LNESG, UK.

BACKGROUND: In order to assess the prognostic value of clinical, biological and morphological data, a multinational protocol including a phase II Trial of surgery as the only treatment for INPC stages 2A and 2B Neuroblastoma was initiated in 1995 (LINESG). Localised Neuroblastoma Study Group 94.01. We present the results of the morphological features following INPC classification as prognostic indicators of relapse in those patients included in this protocol.

MATERIAL AND METHODS: Paraffin sections of 124 neuroblastic tumors from the patients included in the trial were reviewed. The pathology review was a step-wise evaluation of morphologic features without knowledge of clinical information. Following the guidelines of the INPC classification, the cases were classified into favorable and unfavorable categories.

Overall survival (OS), and Event free survival (EFS) were analysed by the Kaplan-Meier test while correlations were assessed with Cox regression.

RESULTS: A total of 115 cases were evaluated following INPC criteria. 4 cases (3.2%) were not evaluable and 5 cases (4%) remain unresolved. The 115 evaluated cases were classified in favorable (91 cases-79.1%) and unfavorable (24 cases-20.9%) categories. Regarding OS after 60 months of follow-up, 97.8% [94.7-100] of favorable cases were alive compared with 72.6% [53.2-91] of unfavorable cases (p=0.001).

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On the contrary, no significant correlation was found between INPC categories and 1p deletion (p>0.2).

CONCLUSION: Histopathological classification therefore has greater prognostic impact than other factors in predicting relapse in localised neuroblastoma.
Within neuroblastoma cell lines, multiple cellular phenotypes analogous to those of the embryonic neural crest exist: neuroblasts (N cells), nonneuronal progenitors (S cells), and malignant stem cells (I cells). Since recent evidence suggests that cancer stem cells may play an important role in tumor incidence and progression, we aimed to: (1) Test that SH-SY5Y cells obtained from different sources are similar for a variety of tasks and at the same time can be used for the development of new models for neuroblastoma biology and cell death. We aimed to: (1) Test that SH-SY5Y cells obtained from different sources are similar for a variety of tasks and at the same time can be used for the development of new models for neuroblastoma biology and cell death.

The trice-cloned N-type SH-SY5Y cell line has been extensively used to study the use of neuroblastoma cell lines for research relying on specific cell types and cell population heterogeneity. The trice-cloned N-type SH-SY5Y cell line has been extensively used to study the use of neuroblastoma cell lines for research relying on specific cell types and cell population heterogeneity.

The lack of good animal models for neuroblastoma has impaired testing of new clinical approaches. Although neuroblastoma cell lines and in vivo xenograft models are useful, they are limited by the fact that the vast majority of patients with neuroblastoma die despite various forms of treatment. The lack of good animal models for neuroblastoma has impaired testing of new clinical approaches. Although neuroblastoma cell lines and in vivo xenograft models are useful, they are limited by the fact that the vast majority of patients with neuroblastoma die despite various forms of treatment.

The TH-MYCN Transgenic Mouse As A Preclinical Testing Model For Neuroblastoma

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The histopathology of neuroblastoma (NB) ranges from well-differentiated to undifferentiated malignancies, with stage IVs tumors to undifferentiated malignancies and stage IVs tumors may even spontaneously regress. Lack of TRKA, the receptor for nerve growth factor (NGF), is a marker for undifferentiation and is associated with poor prognosis. Thus, modulation of this signal pathway could influence differentiation.

Multivariate analysis was based on Cox’s proportional hazards model. Survival probabilities and survival functions were compared by the log-rank test. Aberrant promoter methylation and silencing of RASSF1A may contribute to the pathogenesis of high-risk NB.

The presence of MYCN amplification is a common feature of disseminated neuroblastoma (NB) with bone marrow as the main metastatic site. To understand the metastatic process in disseminated NB, we used a novel animal model in which MYCN amplification was introduced in vitro. The aim of our ongoing research is to establish the diagnosis and prognostic potentials of this gene expression for the NB disease.

BACKGROUND: Epigenetic silencing of tumor suppressor genes plays an important role in the pathogenesis of cancer. In NB, we and others have demonstrated that a number of genes are epigenetically silenced in NB cells. The histopathology of neuroblastoma (NB) ranges from well-differentiated to undifferentiated malignancies, with stage IVs tumors to undifferentiated malignancies and stage IVs tumors may even spontaneously regress. Lack of TRKA, the receptor for nerve growth factor (NGF), is a marker for undifferentiation and is associated with poor prognosis. Thus, modulation of this signal pathway could influence differentiation.

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Aims

Aims: To determine the role of RASSF1A expression in NB.

Methods

We used a novel animal model in which MYCN amplification was introduced in vitro. The aim of our ongoing research is to establish the diagnosis and prognostic potentials of this gene expression for the NB disease.

Results

Interestingly, overexpression of Bcl-2 or FADD/ADD did not interfere with Rb-mediated cell cycle arrest or survival death. NB cells were blocked at the G1/S phase but, since no specific kinase has been identified, the role of ERK and AKT in NB survival is still unknown. We found that Bcl-2 enhanced cell survival by activating the AKT pathway and that the expression of Bcl-2 in NB cells is associated with the activation of the AKT pathway.

Discussion

We conclude that Rb-mediated cell cycle arrest or survival death in NB cells is mediated by the ERK and AKT pathways. However, the role of ERK and AKT in NB survival is still unknown. We found that Bcl-2 enhanced cell survival by activating the AKT pathway and that the expression of Bcl-2 in NB cells is associated with the activation of the AKT pathway. Further studies are needed to clarify the role of ERK and AKT in NB survival.
Normalization to averaged expression levels of four control genes results in reliable transcript quantification by real-time RT-PCR in primary neuroblastoma.

Matthias Skowron
Childrens Hospital, Department of Pediatric Oncology and Hematology, University of Cologne, Germany.

Real-time RT-PCR represents a sensitive and efficient technique to determine expression levels of target genes with those of a single control gene with putatively stable expression levels. However, transcript levels of frequently used control genes such as beta-ACTIN or GAPDH have been demonstrated to exhibit strong variations depending on tissue type or experimental conditions. To establish a suitable normalization factor for real-time RT-PCR experiments in neuroblastoma, the expression stability of putative reference genes HPRT1, LAMB1, PBGD, PGK1, PPDA and SDHA were evaluated in 64 tumor samples obtained from primary neuroblastoma of varying biological and clinical behavior. Variation of control gene mRNA levels observed among the samples considerably decreased when expression values were normalized to the geometric mean of multiple reference genes instead of a single control gene. The geometric mean of control genes SDHA, HPRT1, PEA1 and PBGD was demonstrated to represent a reliable normalization factor and in addition was shown to be not associated with stage of disease or MYCN-amplification status of the tumor. In summary, these data indicate that normalization to the geometric mean of more than one control gene can increase reliability of gene expression data determined by real-time RT-PCR significantly and that the geometrical mean of control genes SDHA, HPRT1, PEA1 and PBGD represents a suitable internal control for studies analyzing gene expression in primary neuroblastoma.

MS was supported by a grant of the Kind-Philipp-Stiftung

Supported by Compania San Paolo

### Other Presentations

**Mismatch repair protein expression in pre and post treatment neuroblastoma**

Deborah A. Tweddle, Lynn Braidwood, Katrina M Wood, Adrian R. Brand, Adrian D. Pearson, Robert Brown
Department of Molecular Biology and Department of Pathology, Northern Institute for Cancer Research, CR UK Beatson Labs, Necastle upon Tyne, Tyneside, UK.

**GD2 loss variants in neuroblastoma**

Roswitha Schumacher-Kuckelkorn, Sandra Schmidt, Anke Grabandt, Barbara Hess, Frank Bortlik
Department of Paediatric Oncology and Haematology, Childrens’Hospital University of Cologne, Cologne, Germany.

**Bone is one of the target organs of metastasis in advanced neuroblastoma. Metastatic osteolytic lesions depend on osteoclast proliferation and differentiation, which are mediated by cytokines.**

**Biological modifiers of RANKL activity prevents osteoclastogenesis induced by neuroblastoma cells**

Ilaria Amato1, Luca Battistelli1, Corinne Calia1, Sergio Capaccioli2, Martino Donnini2, Nicola Baldini1, Daniela Granchi1
Laboratory for Pathophysiology1, Istituti Ortopedici Rizzoli, Bologna; Department of Experimental Pathology and Oncology2, Florence, Italy.

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Department of Molecular Biology and Department of Pathology, Northern Institute for Cancer Research, CR UK Beatson Labs, Necastle upon Tyne, Tyneside, UK.

**Biology**

**Hypoxia induced dedifferentiation of neuroblastoma cells: phenotype persistence after reoxygenation**

Linda Helbing, Annika Hegg and Sven Pahlman
Department of Laboratory Medicine, Division of Molecular Medicine, Lund University, University Hospital Malmö, Malmö, Sweden.

**Functional expression and release of ligands for the activating immunoreceptor NK3G2B in human neuroblastoma**

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**Missmatch repair protein expression in pre and post treatment neuroblastoma**

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**In solid fast growing tumours neovascularization is usually insufficient and leads to areas with poorly oxygenated tumour cells. The stabilization of the hypoxia inducing factors HIF-1alpha and HIF-2alpha, has been considered one functional definition of hypoxia and in neuroblastoma cells this occurs between 1 and 5 % O2. We have earlier shown that the expression of marker genes of the sympathetic nervous system were down-regulated whereas marker genes of neural crest cells were up-regulated in hypoxic (1% O2) neuroblastoma cells in comparison to cells cultured at 21 % O2. These findings suggest that the hypoxic tumour cells adopted a less mature phenotype, which in the clinical setting could result in more aggressive tumour cells with increased metastatic potential. Here we have compared changes in gene expression in cells grown at 1 % O2 (hypoxia) and a more physiological oxygen level of 5% O2. We have also examined the stability of the hypoxia induced dedifferentiated phenotype when cells were reoxygenated at 21 or 5% O2, respectively. The dedifferentiated phenotype persisted for at least 24 h of reoxygenation at both 21 % and 5 % O2. Genes like NPY and chromogranin A and B were still down-regulated, and hypoxia-induced genes like tyrosine hydroxylase and k2 remained up-regulated. This, in the case of metastasizing hypoxic tumour cells that have entered the bloodstream, the aggressive phenotype might persist long enough for the cells to be able to home to a secondary site and metastasize, in part due to their immature characteristics.**

**Supported by AIBC and Ministry of Health**
Supported by SIOPEN-R-NET project (EC grant QLRI-CT-2002-01768)

INTRODUCTION: The staging of neuroblastoma patients according to the DSS relies on the detection of tumour cells in the bone marrow, by means of both cytomorphological examination and histological analysis of trephine biopsies. A new and sensitive method was therefore developed and standardized in order to detect single tumour cells in bone marrow aspirates.

METHODS: A comparison between the new technique and the traditional methods of bone marrow examination was made in a series of 303 patients, in order to prove the feasibility of the new method and to evaluate its ability to increase the probability of the occurrence of isolated micrometastasis.

RESULTS: Out of a series of 303 neuroblastoma patients diagnosed in Italy in the period 1997-2002, 150 were staged as localized according to SS (including absence of tumour cells in bone marrow aspirates and bone marrow biopsies). Of these, nine cases were excluded from the study because complete data from the three methods were not available. Of the remaining 141 cases, 26 patients (18%), immunocytochemical assay with anti-GD2 monoclonal antibody showed immunocytologically, in numbers varying from 1 to 1000 out of 1 x 10^6 cells analyzed. Of these 26 patients, 7 (26.9%) relapsed, versus 6 (15.3%) in the group of 125 cases.

CONCLUSION: Our results confirm that combination of immunocytological examination with anti-GD2 monoclonal antibody may be a sensitive and useful tool in identifying patients at high risk of relapse. While immunocytochemical assay of bone marrow aspirates is not a substitute for conventional methods for staging, it can provide additional useful information for patients with localized disease.

Development and standardization of an immunocytochemical assay to detect residual neuroblastoma cells

Karin Szwarc, Peter F Andersen, Charbel Brousse, Jose M Fernandez Navarro, Nicole Gross, Dyanne Rampling, Spratling Schaudens-Kueckel, Angela Sementa, Ruth Ladenstein, Klaus Beiske

BACKGROUND: Since bone marrow (BM) involvement influences staging and evaluation of therapeutic response in neuroblastoma patients, reliable detection of residual neuroblastoma cells is critical. Cytomorphological examination of BM samples is the current approach to detect residual neuroblastoma cells. Therefore, the SIOPEN Immunocytochemistry/Genetics group decided to standardize performance of two currently available new and sensitive immunocytochemical methods based on the D2-40 and D17-12 antibodies. This technique will be used to study the significance of minimal residual disease (MRD) in neuroblastoma patients treated according to the SIOPEN High Risk study. Standardized method: Technical performance: Staining with D2-40 antibody: Cells are incubated for 30 min at room temperature in a mixture of: D2-40 monoclonal antibody (clone 14G2a), detection system (DAKO A001 complex, FuchsionTM Substrate Chromogen System), hematoxylin (hematoxylin counterstain). Evaluation: Immunostained cells are classified according to SIOPEN immunocytochemical scoring system (cell size, shape, nucleolus). Immunocytochemical staining product. Number of positive cells and evaluated mononuclear cells are reported. The sensitivity of this assay is practically unlimited and depends on the number of investigated cells (ideally 1x10^6). In non-conclusive cases, GD2 positive cells are checked by FISH for genetic abnormalities. Controls: To test the validity of our method, quality control rounds were organized among members of the Immunocytochemistry/Genetics group. After application of the standardized staining and evaluation procedure, two main achievements were obtained: (1) in discordant cases, the range between lowest and highest reported result was reduced from 3.5 to 2 times and (2) conclusive results were only found in samples with ≥10 positive cells per 1x10^6.

CONCLUSIONS: Our method is an appropriate tool to assure reliable detection of residual tumour cells in BM from neuroblastoma patients included in the SIOPEN High Risk Protocol.

NEW IMMUNOCYTOCHEMICAL ASSAY TO DETECT RESIDUAL NEUROBLASTOMA CELLS IN THE BONE MARROW

Support Ref: D42.1 #25

Support Ref: D42.1 #26

Support Ref: D42.1 #27

Support Ref: D42.1 #28

Support Ref: D42.1 #29

Support Ref: D320.1 #31

Support Ref: D321.1 #30

Support Ref: D153.1 #29

Support Ref: D125.1 #32

Support Ref: D328.1 #31

Support Ref: D329.1 #32
International quality assurance programmes are important to ensure accuracy. There have been six quality control rounds. Analysis of cDNA across all seven laboratories demonstrated good sensitivity and specificity of the RT-PCR conditions and source of RT enzyme increased the sensitivity of tyrosine hydroxylase mRNA detection to 10 pg or 1 cell. Sample volume collected will be assessed by weight. PAX gene blood RNA tubes are practical for collection, storage and processing. It is insufficient to isolate all the RNA from 0.5 ml of diagnostic bone marrow. A standard method is under development.

b. Determine the common causes of variability in RT-PCR analyses

BACKGROUND: In European protocol of high risk neuroblastoma, the molecular detection of residual minimal residual disease (MRD) cells is associated with relapse and unfavorable outcome. Consequently, detection of MRD is important for risk evaluation and response to chemotherapy. Molecular detection of MRD cells by real time RT-PCR due to catecholamine-producing hematopoietic (stem) cells is considered to be suitable for detection of contaminating neuroblastoma cells in hematopoietic stem cell preparations. Because clinical sample processing is performed not only by clinicians but also by laboratory scientists, effective quality assurance programmes that are not only for TH, but also for DOPA-decarboxylase (DBH), dopaminogen-d, hydroxindole-O-methyltransferase (HOMT) and dopamine transporter. Additionally, primers for tyrosine hydroxylase were included because in some neuroblastoma cell lines, DOPA production is catalyzed preferentially by this enzyme instead of TH. Using this panel of primers, a moderate sensitivity of the heterogeneous neuroblastoma cells is possible with single RT-PCR, that enables a clear discrimination between RT-PCR positive and RT-PCR negative specimens.

Molecular detection of residual minimal residual disease using a sensitive methodology could contribute to a better treatment in children with neuroblastoma. To detect contaminating neuroblastoma cells, we have developed a qualitative assay for the analysis of Tyrosine Hydroxylase (TH).

METHODS: We analyzed 70 samples of peripheral blood (PB) and 13 intracranial products (IPPC) from 25 patients with neuroblastoma in advanced stages (8 stage 3, 11 stage 4). TH mRNA was analyzed by a RT-PCR method and its endogenous reference gene 18S, was determined. Normalized TH value was obtained by dividing TH/18S. Twenty one samples of PB from donor samples were used as negative controls, and the results were analyzed with the TH/18S expression in each sample in relation to the expression in normal samples.

RESULTS: A median correlation index of 0.85 (range 0.71-0.94) was obtained. The median value of TH expression was detected in all but one patient PB at diagnosis. During treatment 6 patients cleared tumor cells, while at the same time TH expression was down regulated to 0.08 of the initial value. Nine of these patients relapsed while none of the negative patients did. Actuarial 2 year event-free survival was 100% for TH-negative patients and 40% for TH-positive patients (p=0.01). Patients with TH-positive PBC had also a worse prognosis than patients negative for TH. Actuarial 5 year event-free survival was 60% for PBC negative patients and 52% for positive patients (p=0.05).

CONCLUSIONS: TH-positive patients after treatment seem to have a worse prognosis than patients with undetectable TH. Further investigation into the detection of circulating tumor cells during follow up of patients with neuroblastoma is warranted.

Reference


Poster Display

Ref ID: 106:1

Quality assurance of reverse transcription polymerase chain reaction (RT-PCR) to detect neuroblastoma cells

Suz A Bach, Maria V Corrias, Boris Beygelz, Silvio Oliva, Marti Steiner, Kamila Sutro, Alois Vich, Annelie Brancatelli; Ruth Laussen

Cancer Research UK Clinical Centre, St John's University Hospital, Leeds, United Kingdom for the E-SIOP Neuroblastoma Group

RT-PCR for tyrosine hydroxylase mRNA to detect neuroblastoma cells will be employed to evaluate low level disease of bone marrow and peripheral blood from children treated for neuroblastoma. A confident and reliable information is acquired from this multi-centre, prospective clinical outcome study. A simple and cost-effective method is used.

a. Create standard operating procedures (SOPs) for optimal clinical sample collection, storage and processing.

b. RT and PCR sensitivity and specificity.

c. There have been six quality control rounds. Analysis of CDNA across all seven laboratories demonstrated good sensitivity and specificity, 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. Standardization of the amount of RNA, RT conditions, and source of RT enzyme increased the sensitivity of tyrosine hydroxylase mRNA detection to 10 pg or 1 cell.

International quality assurance programmes are important to ensure accuracy of molecular assays.

Ref ID: 402:2

Detection of residual neuroblastoma cells using quantitative real-time RT-PCR

Karin Swets, Barbara De Meester, Catharina Dhoge, Yves Boset, Jan Filipi, Genevieve Lhut

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International quality assurance programmes are important to ensure accuracy of molecular assays.
Gene expression profiling of neuroblastoma: analysis of nonmetastatic versus metastatic tumors

Jummi Moon, Miguel Alaminos,1 Neo-Kong V Cheung3, Jose Rios4, William L Gerald5

1. AIM: First, to test the hypothesis that the expression of TH and MAGE is associated with an improved outcome in localized neuroblastoma and with improved overall survival in localized and disseminated neuroblastoma.

2. METHODS: We used the Taqman assay to evaluate MYCN DNA sequences, in particular when malignant cells are not available for molecular analysis. Sensitive and specific assays for neuroblastoma cells in marrow and blood may improve the prognosis of outcome and evaluation of cells used for autologous hematopoietic stem cell transplantation (AHSCT). To explore this, we performed a pilot study to evaluate the sensitivity of PCR and immunocytology to detect 10-6 and 10-5 respectively. Marrows from 20 patients harvested for AHSCT were analyzed by PCR and immunocytology. Purging was evaluated using immunomagnetic purging, tumor cells were decreased, and none were detectable in 35% by PCR (all immunocytology negative).

3. Results: Sensitivity of PCR and FISH was consistent in 8 tumors with deletion 1p, 11q with MYCN amplification and 8 samples without deletions. Tumors with deletion 1p and 11q were detectable by FISH but not by PCR. A single case indicated a deletion 1p by PCR only. The consistency between both techniques was 92% for 1p, 11q amplification and 100% for MYCN. The discordant cases are most likely due to a heterogeneous cell population in the investigated tissue.

4. Conclusion: The use of a quantitative PCR assay enables the simultaneous detection of the three most relevant chromosomal aberrations accurately as confirmed by FISH. As the assay allows the investigation of embedded marrow and blood specimens to reference tissue, it can be regarded as an additional LOH- or Southern Blot analyses. Determination of the tumor cell content is crucial to avoid false-negative results.

Telomere length–a prognostic marker in neuroblastoma


1. AIM: To assess the prognostic value of TH and MAGE expression in primary neuroblastoma.

2. METHODS: We used the Taqman assay to evaluate MYCN DNA sequences, in particular when malignant cells are not available for molecular analysis.

3. RESULTS: NB tumors were heterogeneous for cell proliferation. A heterogeneity in expression profile was detected among NB tumors and cell lines, and between tumors from two groups with different metastatic status. Ten years Progression Free Survival (PFS) of the patients with Low Tumour Length was significantly longer (85%) than that of patients harboring High Tumour Length (40%) (p<0.001). High Tumour expression was identified in 55% (15/27) of all tumors and correlated with metastatic disease.

4. CONCLUSION: We conclude that the use of circulating MYCN DNA as a tumor-specific marker may improve the prediction of outcome and evaluation of cells used for autologous hematopoietic stem cell transplantation (AHSCT).
BACKGROUND: c-kit (CD117) is a transmembrane tyrosine kinase receptor that is upregulated as a target for STI71 (Glivec) therapy. Some c-kit-overexpressing solid tumours have responded favourably to STI71, potentially because of the presence of c-kit expression.

METHODS: To investigate the epidemiology of c-kit overexpression in neuroblastoma, we performed a retrospective study of 61 patients. All tumours were analysed by immunohistochemistry in a tissue microarray format. We compared the expression of c-kit to the expression of the MYCN-gene, analysed using fluorescence in situ-hybridisation.

RESULTS: Nine of the 61 neuroblastomas expressed c-kit in varying amounts and intensities. Twelve of the neuroblastomas were MYCN amplified. Only 2 of the MYCN amplified tumours expressed c-kit, and only 1 of 24 tumours with favourable histology expressed c-kit. None of the 8 benign tumours expressed c-kit (p=0.05).

CONCLUSION: The expression of c-kit in neuroblastomas is not correlated to MYCN amplification, but is still related to unfavourable histology, indicating that it is a prognostic factor independent of MYCN.

Ref ID: 279.1

An implication of TRK-A expression for surgical strategy in neuroblastoma.

Todoroki Munro1, Akiko Nakajima2, Stigim Tamakawa1, Chikaya Tagami1
Department of Surgery1, Kobe Children’s Hospital, Hyogo; Chiba Cancer Center2, Japan.

Neuroblastoma involving the major vessels is challenging for surgeons. We retrospectively evaluated a c-kit expression which reflects neuronal maturation as a role of surgical strategy. Patients and Methods. Tumours were evaluated in all the 61 patients who had successful outcome. Expression of c-kit was defined as positive when provided c-kit expression at the primary section of the tumour tissue and at least 50% of the sections provided c-kit expression. Results: The expression of c-kit was found significantly lower in all patients than in control group, but it was not related to the presence of skeletal metastasis. A significant correlation was found between OPG and c-kit expression (p<0.001). Free sRANKL was found significantly higher in stage III (32/42, p=0.01) and IV (37/10, p=0.02) than stage I/II (2/19, p=0.02). Conclusion: Expression of c-kit as a marker in determining metosteolytic osteosarcoma. The ratio could serve as a marker for monitoring advanced neuroblastoma.

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Supported by AIBC and Italian Ministry of Health.
The patient was a girl 3 months of age. Anemia (36 g/l) and abdominal enlargement were the main clinical manifestations that caused hospitalization. Sonographic examination revealed a tumor (6x4 cm) in the left suprarenal area. On the contrary, the computed tomography scan of the most of liver parenchyma was observed. The CT examination confirmed changes. In spite of that the biochemical parameters remained stable, however platelet level decreased noticeably. Concerning this natural history and platelet's circuit mass transferion the tumor and liver tissues biopsy was performed. On histological examination of tumor tissue a diagnosis of neuroblastoma was made. Microscopically normal portal tracts were absent in liver tissue. A layer of rough fibrotic connective tissue and loss of fibrous septum with lymphocyte infiltration was found instead. A congenital liver development defect was detected. First chemotherapy block was performed due to POG protocol (cytoxan and vincristine). The patient was discharged. On admitting after 21 days since the first block was held the reduction of tumor (4x2 cm) and liver size and the substitution of numerous cystic structures with parenchyma were revealed. Haematological parameters were normal too. The second chemotherapy block was held similarly. A controversial question is to perform radical operation after the sixth chemotherapy block by protocol or to make early tumorectomy and intraoperative heparin biopsy to confirm diagnosis of liver pathology.

Neuroblastoma presents as metastatic disease in about 60% of newly diagnosed children. The usual sites of metastases are the lymph nodes, bone marrow, liver, bones; rarely lungs or CNS. The prognosis is related to the extent of the disease of the primary tumor, involvement of the brain and spinal cord, and the age of the patient. The presence of certain cellular and molecular characteristics in patients with unfavorable biology neuroblastoma may be observed without treatment despite the advanced INSS stage. Some infants with favorable biology neuroblastoma may be observed without treatment despite the advanced INSS stage. Type-2 clinical cases were distributed in older ages than type-3 cases. The usual sites of metastases are the lymph nodes, bone marrow, liver, bones; rarely lungs or CNS.

Brain tumours are the most common solid neoplasms in the paediatric population. Among them, cerebellar neuroblastoma is extremely rare. A case of cerebellar neuroblastoma was recently reported in our institution. A 3-month-old girl was referred to our hospital with a history of strabismus for one week. Physical and neurological examinations revealed a large mass involving the fourth ventricle, and bilateral hypoglossus. The presumptive routine complete blood count, clotting profiles, electroencephalograms, neuron-specific enolase tumour and the amylase were all within the normal limits. Skull x-ray showed cranial enlargement and spreading of the sutures. The CT scan revealed a marked enlargement of the lateral and fourth ventricles and a 6x4 mm mass in the posterior fossa. The MRI confirmed a circumferential cerebellar midline tumour. She underwent gross total resection and the diagnosis of the neuroblastoma was made. On the basis of the presence of numerous syringopleural vesicles in the greater majority of cell processes and occasional complete synapses within the tumour tissue. N-acetyl amplification was negative. She did not receive any postoperative radiotherapy, and was discharged. A magnetic resonance imaging of the tumour was discovered on the routine radiological examination. Our patient was treated with surgery alone. The outcome was consistent of cranioplasty, cyclophosphamide and VP-16. At the end of nine month, she is disease free, however the optimal treatment for patients with this rare tumours remain uncertain.

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BACKGROUND AND AIM. Widespread neuroblastoma include stage 4 and 4s, while it differs profoundly since the former requires chemotherapy for all, whereas the latter undergoes spontaneous regression in most cases. However, the distinction between stage 4 and 4s is not always clear.

BACKGROUND: The clinical stage of neuroblastomas was defined in 25 Italian institutions between 1991 and 1999. All were evaluated at diagnosis with mIBG scintigraphy and bone marrow aspiration. Stage I-II is curable with chemotherapy according to the current protocols. In absence of life-threatening stage IV symptoms Stage I-III was cured in 90% of cases.

RESULTS: Of 100 eligible pts, 68 had stage IV (4w 60mm and 27 were 2m2). Stage II: 34 (2 IVw 60mm and 3 were 2m2). Stage I: 44 pts showed chemotherapy because symptoms and 8 of them died. Other two died before treatment could be started. Of 43 not treated at onset, 10 developed a Stage II-III disease and 34 of them died. 34 pts with MYCN amplification died. 5 yrs survival at 18-124m (median 65). 5-OS survival 77%, IFS 65%. Stage I: 34 of 32 developed PD or relapse of whom one survives. 57 pts who died had MYCN amplification. An additional patient died of toxicity (brain hemorrhage). 23 patients survived from 42-118m (median 83) and one was LFU with OS 72% and EFS 69%.

CONCLUSION: We confirm that stage 4s prevails over stage 4; stage 2B is confined to the first year of age. Stage 4s is the most exclusive of the second semester; (b) as previously reported. OS and EFS are similar in both stages, (c) in stage IVs MYCN amplification is associated with worse prognosis. We still have biochemical determinants to clearly differentiate stage 4 and 4s in infants.

Neuroblastoma in the adult. A Report on 24 Cases

BACKGROUND AND AIMS: NBL in adolescents is rare, and in adults is exceedingly rare. The term NBL in adults, due to the different nature of the disease, may cause incorrect therapeutic decisions. The aim of the report is to describe histological characteristics of cases with NBL in adults.

METHODOLOGY: In the study period 1980-2002, 33 consecutive cases of NBL in adults were selected. Median age was 17 years (range 8-97). Survival was calculated from diagnosis to last follow-up.

RESULTS: In 24 cases NBL cells were monomorphic and had neuroblastic features with variable amount of myeloid cells. In 13 cases NBL cells were predominantly composed of neuroblasts and in 11 cases they were predominantly composed of myeloid cells. In 2 cases, both components were present. In all cases, however, NBL cells had a neural origin and were derived from the sympathetic nervous system. In all cases, the primary tumor was located in the abdomen, and in 16 cases it was associated with regional lymph nodes. In 2 cases, the primary tumor was located in the bones. In 9 cases the disease was disseminated at diagnosis, and in 15 cases it was localized at diagnosis. In 4 cases, the disease recurred at a later stage. The disease was staged according to the TNM classification. The median follow-up was 7 years (range 1-12). Overall survival at 5 years was 40% (95% CI 20-60). In 3 cases, the disease was in remission at the end of follow-up. In 21 cases, the disease was progressive. In 1 case, the disease was stable at the end of follow-up. In 3 cases, the disease was in remission at the time of the report.

CONCLUSION: NBL in adults is a rare disease with a poor prognosis. The current treatment is based on surgery, radiotherapy, and chemotherapy, and the prognosis is influenced by the stage of the disease at diagnosis and the response to treatment. A multidisciplinary approach, including surgery, chemotherapy, and radiation therapy, is recommended. Further studies are needed to improve the outcome of these patients.

Clinical and biological factors associated with local staging in neuroblastoma

BACKGROUND: Local staging in neuroblastoma is a critical aspect of patient management. The purpose of this study was to evaluate the clinical and biological factors associated with local staging in neuroblastoma.

METHODS: The study included 98 children with localized neuroblastoma who were treated at a single institution. The clinical and biological factors assessed included age, gender, tumor size, stage, MYCN amplification, and serum alpha-fetoprotein (AFP) levels.

RESULTS: The median age of the patients was 2 years (range 6 months-15 years). The majority of patients were males (60%). The most common stage was stage 1 (52%). MYCN amplification was detected in 27% of patients, and high serum AFP levels were found in 45% of patients. The local staging was found to be significantly associated with MYCN amplification (p = 0.03) and high serum AFP levels (p = 0.01).

CONCLUSIONS: Local staging in neuroblastoma is associated with MYCN amplification and high serum AFP levels. These factors should be considered in the clinical decision-making process for managing localized neuroblastoma.

Neuroblastoma and other malignancies in the same patient

BACKGROUND: Neuroblastoma and other malignancies in the same patient are rare. The clinical presentation, diagnosis, and management of these cases can be challenging. The aim of this study was to report a case of neuroblastoma and other malignancies in the same patient.

METHODS: A case report of a patient with neuroblastoma and other malignancies in the same patient is presented.

RESULTS: A 5-year-old boy presented with symptoms of neuroblastoma and other malignancies in the same patient. The patient was diagnosed with neuroblastoma and other malignancies in the same patient and was treated accordingly.

CONCLUSIONS: Neuroblastoma and other malignancies in the same patient are rare. The management of these cases requires a multidisciplinary approach and close follow-up.

Key findings in neuroblastoma: A comprehensive review of clinical characteristics, biologic pattern, chemosensitivity, and survival analysis

BACKGROUND: Neuroblastoma is a malignant tumor of neuroblast cells that arises in the sympathetic nervous system. The clinical characteristics, biologic pattern, chemosensitivity, and survival analysis of neuroblastoma are important for the development of effective treatment strategies. The purpose of this study was to provide a comprehensive review of the clinical characteristics, biologic pattern, chemosensitivity, and survival analysis of neuroblastoma.

METHODS: A comprehensive review of the clinical characteristics, biologic pattern, chemosensitivity, and survival analysis of neuroblastoma was conducted by searching PubMed, Cochrane Library, and other relevant databases.

RESULTS: The clinical characteristics of neuroblastoma include age, gender, stage, MYCN amplification, and serum alpha-fetoprotein levels. The biologic pattern of neuroblastoma includes the expression of neurotrophic factors, growth factors, and cytokines. The chemosensitivity of neuroblastoma is influenced by the stage of the disease, MYCN amplification, and the presence of resistance mutations. The survival analysis of neuroblastoma shows a poor prognosis for patients with advanced-stage disease, MYCN amplification, and high serum alpha-fetoprotein levels.

CONCLUSIONS: The clinical characteristics, biologic pattern, chemosensitivity, and survival analysis of neuroblastoma are important for the development of effective treatment strategies. Further research is needed to improve the outcomes of patients with neuroblastoma.
Spinal cord compression (SCC) in neuroblastoma: the Italian experience 1999-2003

Paola Angiolini, Roberto Lellis, Mario Gallois, Mauropierro Nardini, Mauro Anti, Pietro D'Angelo, Angela Tamberini, Kate Titone, Monica Callisi, Maurizio Bianchi, Alfredo Mercuri, Maria Giuliana Bartalini, Paola Pola, Vittorio Corrado, Andrea Scatena, Anna Maria Ricciardi, Giovanni Pescarmona, Stefano Malaguti, Francesca Rassenti, Claudio De Pasquale, Dario Gabardi, Giuseppe Galli, Carla Frati, Laura Bona, Pasquale Cossu, Anna Maria Pescarmona, Massimo Calcagnini, Roberto De Angelis, Alessandro Santurri, Paolo D'Angelo, Angela Tamberini, Kate Titone, Monica Callisi, Maurizio Bianchi, Alfredo Mercuri, Maria Giuliana Bartalini, Paola Pola, Vittorio Corrado, Andrea Scatena, Anna Maria Ricciardi, Giovanni Pescarmona, Stefano Malaguti, Francesca Rassenti, Claudio De Pasquale, Dario Gabardi, Giuseppe Galli, Carla Frati, Laura Bona, Pasquale Cossu, Anna Maria Pescarmona, Massimo Calcagnini, Roberto De Angelis, Alessandro Santurri, Paolo D'Angelo, Angela Tamberini, Kate Titone, Monica Callisi, Maurizio Bianchi, Alfredo Mercuri, Maria Giuliana Bartalini, Paola Pola, Vittorio Corrado, Andrea Scatena, Anna Maria Ricciardi, Giovanni Pescarmona, Stefano Malaguti, Francesca Rassenti, Claudio De Pasquale, Dario Gabardi, Giuseppe Galli, Carla Frati, Laura Bona, Pasquale Cossu, Anna Maria Pescarmona, Massimo Calcagnini, Roberto De Angelis, Alessandro Santurri.

BACKGROUND: Incidence, clinical course, optimal treatment and late effects of SCC in children with neuroblastoma have not yet been clarified.


RESULTS: The 24 children of this series represent 4.5% of the 469 children with metastatic neuroblastoma who underwent surgery. Two other cases worsened after chemotherapy, and later improved following surgical decompression.

CONCLUSION: Spinal cord compression in neuroblastoma- Experience of Polish Pediatric Solid Tumors Study Group (PPSTSG).

Spinal Cord Compression in Neuroblastoma- Experience of Polish Pediatric Solid Tumors Study Group (PPSTSG)

Ref ID: 033.3 #74

Spinal Cord Compression in Neuroblastoma- Experience of Polish Pediatric Solid Tumors Study Group (PPSTSG)

Ref ID: 035.1 #76

Spinal Cord Compression in Neuroblastoma- Experience of Polish Pediatric Solid Tumors Study Group (PPSTSG)

Ref ID: 037.1 #78

Spinal Cord Compression in Neuroblastoma- Experience of Polish Pediatric Solid Tumors Study Group (PPSTSG)

Ref ID: 039.1 #80

Spinal Cord Compression in Neuroblastoma- Experience of Polish Pediatric Solid Tumors Study Group (PPSTSG)
13 cis-retinoid acid in children with high-risk neuroblastoma: Is more better?

Stefan Aebi, Jerry Bzost, Baris Stark, Yacov Greidel, Liron Kerenstein, Zvi Bar-Sever, Monica Pistorius, Andreas Stoeck, Helen Honeck
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AIMS: Risk adapted treatment according to MYCN amplification, stage, and response for children with localized or metastatic neuroblastoma and evaluation of risk factors.

METHODS AND RESULTS: From July 1994 to January 2002 149 patients (73 females, 76 males) were registered. Staging resulted in 52 stage 1, 47 stage 2, 36 stage 3 and 34 stage 4 patients. There were 83 patients in CR after induction chemotheraphy (IC) and 44 in the continuous-infusion (CI) arm. Successful induction chemotheraphy was achieved in 66% of stage 4 patients. MycN amplified tumours appear to have taken advantage from the intensified concept.

13 cis-retinoid acid (RA) induces differentiation in neuroblastoma (NB) cell lines in vitro. Administration of high-dose RA (two-week pulses for 6 months) improved survival for NB patients with advanced stage NB who are in complete remission following standard and myeloablative chemotherapy. We report our experience with prolonged RA administration in 17 high-risk patients with NB. Patients received an average of 11.3 RA courses (range 2-26) beginning at a median of 93 days after APBSCT. In 5 of 17 patients, complete remission was obtained during RA administration. Six year overall survival is 48% and event free survival is 44%. With a median follow up of 59 months, eight patients have relapsed. Five of 15 patients have relapsed within three months of stopping RA. An additional relapse occurred in a child who did not tolerate RA due to drug-induced hypercalcemia. Side effects of treatment were generally mild. No case of severe hypercalcemia as indicated above. Our cohort of very high-risk patients is exhibiting an unexpectedly good progression free interval after receiving prolonged RA consolidation therapy. In-vitro demonstration of tumor cell differentiation during therapy suggests a role for RA as consolidation treatment for patients even in the presence of small amounts of residual NB. Administration of RA for periods of longer than 6 months should be explored as part of a multi-modal approach to the treatment of high-risk NB.

Compliance of two induction regimens for high-risk neuroblastoma

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Spanish Neuroblastoma Group.

INTRODUCTION: Induction chemotherapy is a pivotal part of high-risk neuroblastoma treatment. Different IC regimens have been used, but the ideal one remains to be found. Continuous infusion has been proposed as a method to treat high-risk neuroblastoma patients. We report our experiences.

METHODOLGY: Patients over 1 year of age with high risk neuroblastoma were consecutively enrolled from 2000 to 2004 in a phase II trial of two different continuous-infusion regimens: vincristine, cisplatin, carboplatin and teiposide (VCTP) in short infusion. Response to IC and grade 3-4 toxicity were compared with our previous study (same drugs, standard infusion). Response was evaluated according to INRC criteria.

RESULTS: 281 patients (161 females, 120 males, age 1.3-12 years) were enrolled. A total of 203 patients (72.3%) achieved CR, 27 (9.6%) VGPR, 39 (14%) PR, and 12 (4%) NoR. Response rates were achieved with a markedly reduced treatment intensity for MycN non-amplified patients. Eleven infants with chemotherapy should be administered before tumor gains chemo-resistance. In our institute, induction and consolidation chemotherapy for neuroblastoma was established for treatment of neuroblastoma. We report here our experience with low dose 131I-mIBG for treatment and evaluation of risk factors.

In an attempt to improve long term results in advanced Neuroblastoma, it may be of more better? A single institution study of low dose 131I-MIBG for stage 4 neuroblastoma – therapeutic implications

Sandro Jecout, Kirsi Black, Gal Asmussen, John W Lougha, Curian Terry, Alexander J Melin, Kajsa Norberg
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BACKGROUND AND AIMS: High-dose therapy regimens with busulphan may improve prognosis in neuroblastoma and Ewing's sarcoma. Busulphan is usually administered orally every six hours over four days as a part of a conditioning regimne for autogous bone marrow transplantation. The drug is toxic but cure rate is excellent in children and adults with acute lymphoblastic leukemia. However, busulphan may cause severe toxicity such as veno-occlusive disease (VOD) and intestinal fibrosis (IF). 20-40% of treated patients experienced severe toxicity, mainly young heavily pretreated children. Another problem connected with oral administration of busulphan is the wide interindividual variability of its pharmacokinetics.

MELPHALAN AND BUSULPHAN TOPOGRAPHY STUDY: We used a new formula of busulphan encapsulated in liposomes (LB) suitable for i.v. administration. We thereby avoid the first passage metabolism of the drug and as our recent study has shown, achieve better systemic exposure and more predictable pharmacodynamics. We have undertaken a randomized controlled study for advanced neuroblastoma, and patients with Ewing's sarcoma, for treatment with LB combined with Melphalan as high dose therapy followed by stem cell rescue. No long-term survivors developed VOD or IF. The pharmacokinetic study showed no significant difference between the first and the last dose of liposomal busulphan when the drug was given as a 2 h infusion twice a day. At follow up, median 14 months, from high dose treatment, 2 patients with neuroblastoma and our patient with Ewing sarcoma died of which one by other reasons than malignant disease. We also treated nude rats bearing human neuroblastoma xenograph tumours with LB. There was a significant difference in toxicity as compared to untreated tumours in control rats. No significant toxicity, except for the expected myelosuppression, were observed. Modified LB formulas i.e. are more predictable than after oral administration. Liposomal busulphan is suitable to use and cause minimal toxicity.

Background of neuroblastoma treatment. Different IC regimens have been used, but the ideal one remains to be found. Continuous infusion has been proposed as a method to treat high-risk neuroblastoma patients. We report our experiences.

Ref ID: 307.1 #89

Ref ID: 308.1 #91

Ref ID: 237.1 #86

Ref ID: 143.1 #81

Ref ID: 381.1 #88

Ref ID: 228.1 #82

Ref ID: 805.1 #1047

Poster Display

Poster Display

Clinical

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**Aims:** New high activity protocols of mIBG alone or in combination with chemotherapy are ongoing to improve the survival of patients refractory to conventional treatments. Open questions are: is the biological response improved? Is the optimal waiting time for stem cell re-infusion different than in other treatments? Which is the optimal waiting time for stem cells re-infusion to assure that residual bone marrow dose and radiation dose do not damage them?

**Methods:** Baseline characteristics were measured using a gamma-counter (Blood and urine), a scintillation probe, and a gammacamera for four days. A measured contamination of the gamma-counter count loss was developed. In 7 year old patient, the marrow dose calculation was performed with two extreme hypotheses: bone involvement, biological half-time, quark spine uptake hypothetically extended to the whole body. Results: Total body radioactivity was satisfactorily measured by probe which avoidance of radiation dose and the presence of gammacamera to stimulate re-ganulation.

**Treatment 2002/102004/10**

Adjuvant MIBG therapy was performed in 7 episodes (5 related to infections, 1 related to thrombosis). CONCLUSIONS: CVC complications occur in almost 26% of children. SL-HB catheters had a significantly higher monthly incidence of complications than usual central venous catheters (CVCs) guarantee a reliable vascular access and are essential for the management of children undergoing anticancer treatment. In 9 of the 49 SL-HB (18%), 8 of the 22 SL-PASV (36%) and 1 of the 3 ports (33%) presented a complication; in one PASV the refitted catheter was needed twice for hemodialysis.

**Dosimeters:** women 0.1a-0.1b (0.05-0.25), men 0.1a-0.3b (0.05-0.45) Red marrow 0.4a-0.5b (0.2-0.75), bladder wall 5.8a-6.1b Treatments: 7.2a-7.5b

Conclusions: Red marrow dose is remarkably different from total body dose. WR curve is identical in the two treatments, while primary tumour dose and metastatic spine dose per GBq are much less in the worst hypothesis (b), with no re-infusion time for 4.3 and 2.6 days for 1st and 2nd treatments.

**Aims:** To determine efficacy of different propensity scores in comparing long-term complications of treatment in survivors of high-risk neuroblastoma.

**Methods:** We retrospectively reviewed 103 children with high-risk neuroblastoma (47% of them with metastasis). We compared two schedules of treatment: chemotherapy (CT) followed by autologous BMT (AbMT) or alloSCT-MA/RI. The 5 year survival was 54% (95% CI: 43-67) in the CT/AbMT group and 60% (95% CI: 49-71) in the CT/RI-AllosCT group. The cumulative incidence of the six long-term complications were similar in both groups.

**Results:** Between September 2000 and October 2002, laparoscopic adrenal surgery for neuroblastomas in children was performed in two centers. The method presents some advantages: easier access to the involved tumor, avoiding invasive transperitoneal approach, and safer procedure. The adrenal tumor was completely removed in 20 cases (95%) with a mean operating time of 93.8 min (range 42-170) and mean blood loss of 80.3 ml (range 30-150). In 2 cases, transperitoneal approach was used. The adrenal tumors were completely resected in all cases. There were no postoperative complications, except one port site infection. Blood transfusion was not required in any case. Average hospital stay was 4.5 days (range 2-10). Histologic analysis of the excised adrenal showed that the adrenal was involved by tumor. There were no regional recurrences or metastases over a mean follow-up of 29 months (range 12-55).

**Conclusions:** Laparoscopic adrenal surgery is feasible in children with neuroblastomas. It can be performed with minimal morbidity and mortality and can be considered as a good alternative to the open approach performed in high risk neuroblastomas.

**Aims:** To study the pharmacokinetics, toxicity, and radiation dose to the liver, bladder wall, red marrow, and total body dose in patients treated with high-dose chemotherapy (HDC) and autologous peripheral blood stem cell transplantation (PBSCT).

**Methods:** Twenty-eight patients treated with high-dose chemotherapy and autologous PBSCT were included in this study. Absorbed doses were calculated for liver, bladder wall, red marrow, and total body using GEANT-4 Monte Carlo simulations. Treatment-related acute and late toxicities were also evaluated.

**Results:** The highest dose was received by the liver (8.6 Gy), followed by the bladder wall (5.1 Gy), red marrow (4.3 Gy), and total body (1.0 Gy). The cumulative incidence of grade III-IV toxicities was 64% (95% CI: 51-76) and 46% (95% CI: 31-60) for liver and bladder wall, respectively.

**Conclusions:** High-dose chemotherapy and autologous PBSCT for neuroblastoma is feasible, with significant morbidity and mortality. We studied the pharmacokinetics, toxicity, and radiation dose to the liver, bladder wall, red marrow, and total body dose in patients treated with high-dose chemotherapy and autologous PBSCT.

**Aims:** To determine whether the use of indwelling central venous catheters (CVCs) guarantee a reliable vascular access and are essential for the management of children undergoing anticancer treatment. In 9 of the 49 SL-HB (18%), 8 of the 22 SL-PASV (36%) and 1 of the 3 ports (33%) presented a complication; in one PASV the refitted catheter was needed twice for hemodialysis.
Functional genomics in neuroblastoma

Danieli Spano1, Vokodok Aggi1, Maria D’Apuzzo1, Giuseppe Rau2, Vittoria Selvaggio1, Michele Falcinelli1, Vincenzo Zelato1

C.L.E.N.G.E.2, Università degli Studi di Napoli Federico II, and T.I.G.L.E.2 Napoli, Servizio di Genomica Umana e Genoma del Sviluppo, Istituto Nazionale per la Ricerca sul Cancro, Unità di Studio per la Neuroblastoma e il Sistemi Neuroendocrini, and Università degli Studi di Pavia, Pavia, Italy.

Neuroblastoma represents the most frequent solid extracranial neoplasia in children and, from an etiopathogenetic and molecular analysis of neuroblastoma demonstrated that several chromosome abnormalities are present in these tumors. Unfortunately, the understanding of the genetic events involved in the genesis of neuroblastoma is not well defined and the involved genes are not known yet. In order to identify genes, proto-oncogenes/proteins responsible for malignant transformation and progression in neuroblastoma, we performed a gene expression profiling approach of un differentiated and retinoic acid differentiated neuroblastoma cell lines. This approach is indicating new genes up and down regulated in neuroblastoma cellular models. We used the GeneChip Human U133 Plus 2.0 Array (Affymetrix) for testing mRNA from LAN-5 cell line: 0.3% of the total genes were differentially expressed in LAN-5 cell line. 81 were up-regulated and 68 were down-regulated in comparison with control. These genes were evaluated for differential expression in 99 neuroblastoma patients. The expression patterns observed in high-risk tumors that parallel expression patterns observed in normal-risk tumors.

Gene expression analysis with Prognosis of Neuroblastoma

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To investigate the biological features associated with prognosis of neuroblastoma, we analyzed gene expression of 59 tumor samples obtained from patients treated in Japan. These tumors included 16 tumors of stage 1, 4 of stage 2, 19 of stage 4-S, 6 of stage 4 and 10 of stage 4-M. For expression data acquisition, we employed an Affymetrix HG-U133A microarray which contains 22,015 probe sets. More than 60% of genes were examined in at least one of the 59 tumors. Under unsupervised principal component analysis (PCA) of the expression data, the majority of the normal and tumor genes associated with immune system, were not correlated to outcome of the tumor. This result implies that genes related to immune system are not likely to be correlated to prognosis. Genes associated with neural differentiation contributed largely to this component. In this paper, using theX-ray detector and the patient's expression profile, we could detect the expression of the gene associated with the prognosis of the tumor. The results are significant (p-value<0.01) and associated with prognosis. Interestingly, most genes except MYCN and its co-amplified genes were expressed similarly in the tumors with poor prognosis regardless of MYCN amplification status. In addition, MYCN target genes such as DIC1 and ribosomal protein genes were highly expressed even in the MYCN amplification-negative tumors with poor prognosis. These results indicate that gene expression analysis is extremely useful for outcome prediction of neuroblastoma patients, and suggest that a molecular function analysis using theX-ray detector might be an effective tool to predict the clinical outcome of neuroblastoma with MYCN non-amplification.

Gene expression Differences Among Stages in Stroma-Poor Neuroblastoma

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BACKGROUND: In the last decade, microarray technology has been extensively used to study the genetic and molecular analysis of neuroblastoma demonstrated that several chromosome abnormalities are present in these tumors. Unfortunately, the understanding of the genetic events involved in the genesis of neuroblastoma is not well defined and the involved genes are not known yet. In order to identify genes, proto-oncogenes/proteins responsible for malignant transformation and progression in neuroblastoma, we performed a gene expression profiling approach of undifferentiated and retinoic acid differentiated neuroblastoma cell lines. This approach is indicating new genes up and down regulated in neuroblastoma cellular models. We used the GeneChip Human U133 Plus 2.0 Array (Affymetrix) for testing mRNA from LAN-5 cell line: 0.3% of the total genes were differentially expressed in LAN-5 cell line: 81 were up-regulated and 68 were down-regulated in comparison with control. These genes were evaluated for differential expression in 99 neuroblastoma patients. The expression patterns observed in high-risk tumors that parallel expression patterns observed in normal-risk tumors.

Use of CGH array to identify MYCN amplification and chromosome 1p36 deletion in neuroblastoma

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BACKGROUND: Neuroblastoma, a developmental tumor that originates from the neural crest, exhibits a broad spectrum of clinical behavior and histologic differentiation. Accurate stage and risk-stratification remains a major clinical challenge. In addition, the genes that determine aggressive clinical behavior remain largely unknown. We therefore have been interested in the development of a model that could stratify patients in high-risk groups. This work also shows the usefulness of combined genomic and transcriptomic analyses for cancer gene discovery.

Identification of a gene signature discriminating stage 4S and stage 4 neuroblastoma using Serial Analysis of Gene Expression

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Differentialization of various subtypes of neuroblastoma (NB) using gene expression profiling has recently been demonstrated by several publications. The aim of this study was to discriminate stage 4S neuroblastoma of stage 4 from stage 4, both of which represent metastasising tumors but usually follow opposite clinical courses. To bring out the molecular differences of stage 4-SB vs stage 4-MB, we performed genome amplification and unfavourable stage 4 NB without MYCN-amplification, gene expression analysis of four stage 4 neuroblastoma samples generated using the technique of Serial Analysis of Gene Expression (SAGE). In total, 232 genes were differentially expressed between 20,000 and 30,000 tags. A total of about 53,000 unique tags were catalogued, 18,000 of which were detected in at least two SAGE libraries. These genes correspond to tags that were upregulated in at least one sample and downregulated in the other sample, which were overrepresented and showed significant functional enrichment. We validated these results using a set of 428 genes that were uniquely catalogued at 5% false discovery rate. Out of these 428, we detected 395 differentially expressed genes, which at least 84% of the stage 4S tumors and 80% of the stage 4 NB developed from cells of distinct stages of neural differentiation.
Patients with neuroblastoma (NB) are carefully risk-stratified in order to maximize cure rates for this most common pediatric cancer. Although numerous factors including patient age, stage of the disease and genetic profile are used to classify risk, little is known about distinct pathological genes and molecular mechanisms underlying spontaneous regression of this disease. We tested the expression pattern of aggressive neuroblastomas and their benign variant tumors of other entities. The direct visualization of all transcribed sequences along the chromosomes was performed by comparative genomic hybridization (CGH). In neuroblastoma, MYCN is the most frequent amplified oncogene. A new technology that allows determination of DNA copy number alterations. In neuroblastoma, MYCN is the most frequent amplified oncogene. Two independent tumor cell populations, one of which corresponds to the genomic region of the MYCN amplification (3p21.3), were identified in these tumors. One population corresponded to two different populations of double-minute chromosomes, one of which also contains the MYCN locus.

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The PPM1D gene, encoding a serine/threonine protein phosphatase, locates at a mechanism in the development and maintenance of cancer cells. Until recently, identification of the targeted genes relied on the assumption that activation of proto-oncogenes by DNA amplification is an important approach for identification of overexpressed genes in DNA amplification. Combined subtractive cDNA cloning and array CGH: an efficient approach to elucidation of the translation of specific genes.

We carried out genome-wide expression profiling of 108 NB tumors (stages 1 through 4, MYCN-amplified (AMP) as well as non-amplified (NA) using 42KcDNA micro-arrays. Utilizing permutation t-test we found that N-myc mRNA decreased by a significant increase in the expression of the genes involved in the cell cycle, which opens perspectives for improved identification of hitherto unknown targeted oncogenes in cancer cells.
Expression of Trk receptors is an important prognostic factor in neuroblastoma. Activation of the TrkA receptor by NGF mediates growth inhibition and differentiation of neuroblastoma cells, whereas TrkB/TrkD signaling promotes cell survival and inhibits apoptosis. Neuroblastoma patients with poor prognosis demonstrate higher levels of TrkA/TrkB receptor expression.

A constitutional translocation t(1;17)(p36.2;q11.2) in a neuroblastoma patient

ID2 promotes angiogenesis in neuroblastoma

Regulation of gene expression by N-myc in neuroblastoma

A Constitutional Translocation (t(11;17)(p13.2;q11.2)) in a Neuroblastoma Patient Disrupts NBG1, a Putative Tumor Suppressor Gene

Proteome Center Bochum2, Germany.

Myeloid differentiation factor 88 (MyD88), a key component of the MyD88-dependent signaling pathway, and the expression in NB differentiation raises the possibility that hCas may be a putative NB tumor suppressor gene on 1p.

Increased susceptibility to high-risk neuroblastoma is associated with amplification of the N-myc gene, which codes for a nuclear transcription factor that regulates numerous genes involved in cell growth, differentiation, and apoptosis. Amplified MYCN, which results in overexpression of MycN protein, is a powerful prognostic marker and identifies a group of high-risk patients with poor prognosis. MYCN amplified tumours, although sensitive to initial treatment, show a high rate of relapse and a poor long-term outcome with current cancer therapies. Most of the currently used chemotherapeutic drugs in neuroblastoma are not effective against MYCN amplified tumours and death via p53 signaling pathways. Although p53 is rarely mutated in neuroblastoma tumours compared to other cancer types, p53 signaling pathways seem to be impaired in drug-sensitive neuroblastoma cell lines. Understanding the mechanisms underlying normal vs. tumor differentiation is important for developing novel therapeutic strategies.

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A Constitutional Translocation (t(11;17)(p13.2;q11.2)) in a Neuroblastoma Patient Disrupts NBG1, a Putative Tumor Suppressor Gene

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Myeloid differentiation factor 88 (MyD88), a key component of the MyD88-dependent signaling pathway, and the expression in NB differentiation raises the possibility that hCas may be a putative NB tumor suppressor gene on 1p.

Increased susceptibility to high-risk neuroblastoma is associated with amplification of the N-myc gene, which codes for a nuclear transcription factor that regulates numerous genes involved in cell growth, differentiation, and apoptosis. Amplified MYCN, which results in overexpression of MycN protein, is a powerful prognostic marker and identifies a group of high-risk patients with poor prognosis. MYCN amplified tumours, although sensitive to initial treatment, show a high rate of relapse and a poor long-term outcome with current cancer therapies. Most of the currently used chemotherapeutic drugs in neuroblastoma are not effective against MYCN amplified tumours and death via p53 signaling pathways. Although p53 is rarely mutated in neuroblastoma tumours compared to other cancer types, p53 signaling pathways seem to be impaired in drug-sensitive neuroblastoma cell lines. Understanding the mechanisms underlying normal vs. tumor differentiation is important for developing novel therapeutic strategies.
We used a functional selection strategy with human neural cells to identify the key role of p73 in neuroblastoma cell apoptosis. Our in vitro findings were validated in vivo with MYCN transgenic mice. The selected apoptosis-resistant cell line had a clonal alteration [unbalanced t(17;13) with trisomy 1-q21, and t(18) (1q21)] that led to gene amplification and induced transformation [AACR 2005]. At 1q gain has been described in NBs we wished to investigate MCL1 as a candidate oncogene. Human IRB aCGH was used to assess aberrations gain in 45 NB cell lines. 1q gain occurred in 12/26 MYCN amplified and 69 non-amplified lines (p=0.044) and defined a large SRO including MCL1. NBs arising in the transgenic TH-MYCN mouse were studied with murine aCGH [Hackett, Cancer Research 2003] and demonstrated gain of orthologous chromosome 3 in 16/39 (41%), with subchromosomal gain around MCL1 in additional tumors. Two of 6 TH-MYCN NB cell lines also showed chromosome 3 gain including MCL1. Human NB cell lines expressed more MCL1 mRNA than control cells, and more MCL1 than BCL2. Mcl1 protein was high in 41% MYCN-amplified and 24% nonamplified lines. Expression of BIRC5 in primary NBs was higher than fetal brain 2 months and the trend was higher for Stage 4 high-risk tumors versus others. We studied low-grade MYCN (1q21) tumors with low MYCN copy number, gain was more common in human NBs and correlated with MYCN amplification. Gains are high and include MCL1. In support, gain of orthologous chromosome 3 occurs with the high-risk NBs. We identified a MYCN-independent role for MCL1 studies, including tumor tissue microarrays, to assess MCL1 expression with respect to MYCN status, tumor phenotype and 1q status or gene copy number.

**References:**


**Poster Display**

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High expression of novel HECT-type E3 ubiquitin ligases, NEDLI and NEDL2, which target Dishevelled-1 and p73, respectively, is associated with favorable neuroblastomas with spontaneous regression.

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Somatic genetic events occurring in infant with neuroblastoma during the first year of life

Gian Paolo Tonini1, Katia Murazzu2, Raffaella Defferrari2, Annina Cozza1, Luca Longi1, Yael P Mosse3, Patrizia Perri2, Maria Lastowska1, Yeun-Jun Chung2, Ngan C Cheng3, Michelle Haber3, Murray D Norris3, Patricia Dahia, Garrett M Brodeur, Barbara L Weber, John M Maris4

Inherited predisposition to neuroblastoma shows incomplete penetrance and high lethality, resulting in relatively small pedigrees and contributing to the rarity of this disease. Therefore, understanding the genetic basis of neuroblastoma to 16p12-13 (NB1; LJD = 3.3) is important. We have now identified additional neuroblastoma families, including one with 7 affected individuals. In this study, we report on a new case of neuroblastoma on 16p12-13. To date, we have constructed the neuroblastoma cDNA libraries, from which we identified a novel gene, Nbla0078, which showed a similar expression pattern with Nbla0079.

Jo Vandenompele, Bjorn Hvelplund, Nadine Van Roy, Evi Michels, Els De Steen, Stefan Vermeulen, Anne De Paepe, Geneviève Laureys, Frank Speleman

Additional evidence for linkage of hereditary neuroblastoma to 16p13 and discovery of cooperating mutations

Yufei Zhou, Matt B. Lautenbacher, Deepak Khati, George Hii, Haosheng Zhou, Eric Rappaport, Patricia Dahia, Nathan M. Breslau, Jo B., John Maris4

This study is supported by Italian Neuroblastoma Foundation and AIRC.
In neuroblastoma, the most frequent genetic alterations are unbalanced chromosome 17 translocations leading to gain of distal 17q, which is thought to occur by chromosome 17(q) reciprocal translocations. This effect of genes located on this chromosome. However, little is known about the mechanism leading to these unbalanced translocations, and to date, no such translocation has been characterized at a molecular level. In order to analyze their molecular structure, we have now cloned the breakpoints of the full-length 17q translocations: a der(17)(11;17)(p34;q21) and a der(11;17)(p32;q12) in cell lines CLB-Bar and CLB-Ma, a der(1;17)(q21;p13) in SK-N-AS, and a der(17)(11;17)(p32;q11) in SK-N-BE. Our approach consisted of a delineation of the chromosome 17q breakpoints, which was facilitated by the use of long template, linearized FISH using PCR generated probes was performed. Southern analysis identified rearranged fragments in all cell lines, and pluge libraries were constructed, which were then screened for recombinant clones containing the rearranged fragments. Positive recombinant clones were sequenced, and the exact position of breakpoints on 17q and the reciprocal chromosomes could be identified in all four cases. Distinct molecular features could be identified at the translocation breakpoints, suggesting that different molecular mechanisms may lead to unbalanced chromosome 17 translocations in neuroblastoma. The disrupted genes and the molecular structure at the fusion sites will be further described.

BACKGROUND: Inactivation of tumor suppressor genes may occur by aberrant hypermethylation. In neuroblastoma, the most frequent genetic alterations are unbalanced chromosome 17 translocations leading to gain of distal 17q, which is thought to occur by chromosome 17(q) reciprocal translocations. This effect of genes located on this chromosome. However, little is known about the mechanism leading to these unbalanced translocations, and to date, no such translocation has been characterized at a molecular level. In order to analyze their molecular structure, we have now cloned the breakpoints of the full-length 17q translocations: a der(17)(11;17)(p34;q21) and a der(11;17)(p32;q12) in cell lines CLB-Bar and CLB-Ma, a der(1;17)(q21;p13) in SK-N-AS, and a der(17)(11;17)(p32;q11) in SK-N-BE. Our approach consisted of a delineation of the chromosome 17q breakpoints, which was facilitated by the use of long template, linearized FISH using PCR generated probes was performed. Southern analysis identified rearranged fragments in all cell lines, and pluge libraries were constructed, which were then screened for recombinant clones containing the rearranged fragments. Positive recombinant clones were sequenced, and the exact position of breakpoints on 17q and the reciprocal chromosomes could be identified in all four cases. Distinct molecular features could be identified at the translocation breakpoints, suggesting that different molecular mechanisms may lead to unbalanced chromosome 17 translocations in neuroblastoma. The disrupted genes and the molecular structure at the fusion sites will be further described.

EMPA, A Putative Tumor Suppressor Gene Mapping At 19q13.3, Is Involved in Tumorigenesis of Primary Tumors by Promoter Hypermethylation and May Have Prognostic Value

Matias Alatanci, Veronica Davidek, Nai-Kong V Cheung, William L Gerald, Manel Migiel Alaminos, Miguel Marie-Christine Favrot, Dominique Plantaz, Anick Nuyts, Geneviève Laureys, Valérie Combaret, Nadine Van Roy, Rudy Van Coster, Anne De Paepe, Frank Speleman

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anatomical pathology3, kuLeuven, Leuven, Belgium.

Cancer Epidemiology Laboratory1, Spanish National Cancer Center (CNIO), Madrid, Spain; Memorial Sloan-Kettering Centre2, New York, USA.

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A number of systems for conditional gene expression in mammalian cells have been described, however, their use is often difficult due to early integration into the host genome and background transcription in the absence of the inducing drug. We constructed a tetracycline expression system that combines single-copy neo-regulated transgenes with tet-rx regulated transpositional silencers in one retroviral vector. Tet-transactivator, tet-repressor and selection marker genes are transcribed as tricistronic mRNA allowing efficient selection of those infected cells that express high cellular levels of transcriptionactivator/repressor-mRNA. The reporter genes are inserted in a self-inactivating viral vector that contains a tetCMV promoter and a selection marker. More than 92% of infected neuroblastoma cells, selected for stable insertion of both virus vectors, expressed the reporter gene EGFTR in a tetracycline-regulated manner. Both silenced promoters, tetKrbA and TRSD, reduced background transcription of EGFTR to auto-fluorescence. Interestingly, while cells with a tetKrbA repressor had only poor induction rates when stably integrated into the host genome, a combination of the two tetM2 transactivator and the TRSD repressor allowed for up to 1100 fold induction and an almost linear regulation of transgene expression in neuroblastoma cells.
Clotrimazole, Simon Prochazka, Fabio Costa, Salvador Corea, Francesco Pollesi, Giulia Marini, Alessandra Curti, Alex Pizzuti, Elisabetta Di Lernia, Marco Pozza, Giuseppe Russo, Piero Tinti

Genetica, Biologia, Biochimica, Università degli Studi di Torino, Torino, Italy.

The proteomic techniques were applied in the comparison between active beta-catenin and inactive beta-catenin. The rationale of these studies is to identify beta-catenin targets that might represent interesting molecular markers for the diagnosis of neuroblastoma and other pathological conditions. The results showed that beta-catenin affected the expression of several proteins, including apolipoprotein D, beta-actin, and cofilin. These proteins are involved in the regulation of cell growth and viability, and their expression is affected by beta-catenin. The identification of these proteins could be useful in the development of new diagnostic and therapeutic strategies for neuroblastoma.

Ref ID: 570.1 #173

Studies Using 17-Allylamino-17-demethoxygeldanamycin (17-AAG) to Understand the Heterogeneity of Hsp90 Linked Survival and Signalling Pathways in Neuroblastoma

Ana Naredo, Linley Hawkins

Consilium for Experimental Therapeutics for Childhood Cancers, Alberta Children’s Hospital, Calgary, Canada.

Clinical heterogeneity is a salient feature of neuroblastoma that is reflected in a diversity of growth regulatory pathways in NB and provide evidence for the use of agents such as 17-AAG in its treatment.

Ref ID: 572.1 #174

BMP signaling in human neuroblastoma cells: the role of p27KIP1

Vicky Nakazawa, Tomohiro Osaki, Atsuki Nakagawa,Shinya Sakamoto

Department of Orthopaedic Surgery, Tokyo University of Medicine, Tokyo, Japan.

The role of BMP signaling in human neuroblastoma is not well characterized. In this study, we investigated the role of p27KIP1, a cyclin-dependent kinase inhibitor, in BMP signaling in human neuroblastoma cells. The results showed that BMP signaling upregulated p27KIP1 expression, which suppressed cell proliferation and induced morphologic differentiation. These findings suggest that BMP signaling might be a potential therapeutic target for neuroblastoma.

Ref ID: 574.1 #175

Investigation of endoplasmic reticulum inactivation in response to stress and modulation of cell death in neuroblastoma cells

Miroslava M Kovaříková, Li Li, Jackie Buckovski, Lina M Obeid, Besim Ogretmen

Department of Pediatric Hematology Oncology, UCSF, San Francisco, CA, USA.

Bone morphogenetic proteins (BMPs) are members of the TGF-beta superfamily, which play important roles in the development and differentiation of osteoblasts and chondrocytes. In this study, we investigated the role of BMP signaling in the modulation of cell death in neuroblastoma cells. The results showed that BMP signaling suppressed cell death and induced morphologic differentiation. These findings suggest that BMP signaling might be a potential therapeutic target for neuroblastoma.

Ref ID: 576.1 #176

Involvement of endoplasmic reticulum in the inhibition of telomerase activity in human neuroblastoma cells

Jacqueline M Kraveka, Li Li, Jacek Bielawski, Lina M Obeid, Besim Ogretmen

Division of Biochemistry, Chiba Cancer Center Research Institute, Japan.

The role of the endoplasmic reticulum (ER) in the regulation of telomerase activity in human neuroblastoma cells is not well characterized. In this study, we investigated the role of the ER in the inhibition of telomerase activity in human neuroblastoma cells. The results showed that the inhibition of telomerase activity was associated with the ER stress response, which was induced by the ER stress inducers thapsigargin and tunicamycin. These findings suggest that the ER stress response might be a potential therapeutic target for neuroblastoma.

Ref ID: 886.1 #177

The role of Wnt signaling in neural crest (NC) development

Tomoyuki Sato, Tetsuo Noda, Yutaka Hayashi

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During development of NC, which is the origin of neuroblastoma, Wnt signaling is also known to regulate cell expansion and/or determination of cell fate. To elucidate the function of Wnt signaling in the development of NC, we investigated the role of Wnt signaling in the expansion and/or determination of NC cells. The results showed that Wnt signaling promoted the expansion and/or determination of NC cells, which might be associated with the development of neuroblastoma.

Ref ID: 888.1 #178

BMP signalling in neuroblastoma cells: the role of p27KIP1 in inducing morphologic differentiation

Vicky Nakazawa, Tomohiro Osaki, Atsuki Nakagawa, Shinya Sakamoto

Department of Orthopaedic Surgery, Tokyo University of Medicine, Tokyo, Japan.

Bone morphogenetic proteins (BMPs) are members of the TGF-beta superfamily, and play important roles in the development and differentiation of osteoblasts and chondrocytes. In this study, we investigated the role of BMP signaling in the modulation of morphologic differentiation in human neuroblastoma cells. The results showed that BMP signaling induced morphologic differentiation, which was associated with the upregulation of p27KIP1 expression. These findings suggest that BMP signaling might be a potential therapeutic target for neuroblastoma.

Ref ID: 140.1 #179

Proteomic and gene expression studies in neuroblastosoma and Ewing sarcoma

Cristina Zanoni, Simon Prochazka, Fabio Costa, Salvador Corea, Francesco Pollesi, Giulia Marini, Alessandra Curti, Alex Pizzuti, Giuseppe Russo, Piero Tinti

Genetica, Biologia, Biochimica, Università degli Studi di Torino, Torino, Italy.

The proteomic techniques were applied in the comparison between active beta-catenin and inactive beta-catenin. The rationale of these studies is to identify beta-catenin targets that might represent interesting molecular markers for the diagnosis of neuroblastoma and other pathological conditions. The results showed that beta-catenin affected the expression of several proteins, including apolipoprotein D, beta-actin, and cofilin. These proteins are involved in the regulation of cell growth and viability, and their expression is affected by beta-catenin. The identification of these proteins could be useful in the development of new diagnostic and therapeutic strategies for neuroblastoma.

Ref ID: 396.1 #180

Small molecule inhibitors of PI3-Kinase suppress growth of neuroblastoma cell lines. No obvious malformation of heart suggested beta-catenin may be unnecessary in the development of cardiac NC cells.

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Department of Pediatric Hematology Oncology, UCSF, San Francisco, CA, USA.

Bone morphogenetic proteins (BMPs) are members of the TGF-beta superfamily, and play important roles in the development and differentiation of osteoblasts and chondrocytes. In this study, we investigated the role of BMP signaling in the modulation of morphologic differentiation in human neuroblastoma cells. The results showed that BMP signaling induced morphologic differentiation, which was associated with the upregulation of p27KIP1 expression. These findings suggest that BMP signaling might be a potential therapeutic target for neuroblastoma.
In neuroblastoma (NB), p53 and p73 are rarely mutated. In recent studies we showed that an overexpression of DeltaNp73alpha in the NB line SH-SY5Y with wt-p53, interestingly, induces p53 protein but also cooperates with wild-type p53 in playing a physiological role through the activation of BTG2 gene expression.

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Silencing of MYCN expression in human neuroblastoma cell lines

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BACKGROUND AND AIMS: The most important predictor of poor outcome in neuroblastoma is amplification of the MYCN oncogene. There are indications of a direct role for the MYCN protein in neuroblastoma pathogenesis. Studies using specific antisense oligonucleotides to investigate the role of MYCN expression in neuroblastoma cells have suggested that it is possible to reduce the production of MYCN protein and cell proliferation by approximately 50%. With a strategy using RNA interference (RNAi), which provides a more efficient means of reducing protein levels, we wanted to examine if it was possible to reduce the production of MYCN RNA and cell proliferation in neuroblastoma cells.

METHODOLGY AND RESULTS: Our RNAi strategy is based on SHAGgy (G-to-C) activated gene silencing. We designed vectors expressing shRNAs targeting the coding region of the MYCN oncogene. shRNA-expressing plasmids were transiently transfected into MYCN amplified human neuroblastoma cell lines (Kelly, SK-N-BE). Transfection efficiencies were examined by transfecting a plasmid expressing the green fluorescent protein (GFP). Transfection efficiencies were typically 50-70%. There was a reduction of 40-70% of the MYCN protein in transfected cells compared with control cells. Real-time RT-PCR showed that MYCN mRNA levels were reduced by approximately 40% compared with the negative control transfectants. This experiment confirms that the reduction of MYCN protein observed on Western blots was the result of reduced MYCN mRNA levels. Conclusion: We have shown that shRNA targeting the coding region of the MYCN oncogene is able to efficiently reduce its expression. Consistently, we show that knockdown efficiencies of 50-60% of our results indicate that the MYCN specific shRNA eludes the vast majority of both the mRNA and protein code for by the MYCN gene. Transfected neuroblastoma targeted therapies may be clinically important MYCN amplified neuroblastomas.
MOLECULAR BIOLOGY

Poster Display

Ref ID: 781.3 #198
Charaterization of p53 mutation in neuroblastoma cell lines

The tumor suppressor gene p53 is rarely mutated in neuroblastoma (NB) tumors at diagnosis. Its dysfunction leads to non-functional cell-cell communication and cytotoxicity resulting in resistance to NB tumors. To investigate the p53 functionality, we performed direct sequencing of the 3 primary somatic mutation regions (exons 5, 7, 8, 9, 10) and identified a total of 125 mutations, 124 of which were directly deoxyribonucleotide fragment (dNFSs) by dideoxynucleotide sequencing. The remaining 1 mutation was a direct sequencing of the 8 additional regions of interest (NRas, MYCN, PIK3CA, ARN, BCL2L1, ERBB2, FOXO3A, and RB1). The mutations were categorized as missense, nonsense, and splice site mutations.

In neuroblastoma there is a strong correlation between low stage of tumor cell differentiation and poor outcome. Our group has previously shown that increased hypoxia is a predictor of poor outcome. In this study, we present two new markers for hypoxia assessment: 1) SK-N-BE(2), which is known to be induced by cytokotic therapy leading to resistance in NB tumors. To investigate the p53 functionality, we performed direct sequencing of the 3 primary somatic mutation regions (exons 5, 7, 8, 9, 10) and identified a total of 125 mutations, 124 of which were directly deoxyribonucleotide fragment (dNFSs) by dideoxynucleotide sequencing. The remaining 1 mutation was a direct sequencing of the 8 additional regions of interest (NRas, MYCN, PIK3CA, ARN, BCL2L1, ERBB2, FOXO3A, and RB1). The mutations were categorized as missense, nonsense, and splice site mutations.

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In a subset of neuroblastomas, a neuronal to neuroendocrine lineage shift is important therapeutic option to manage advanced neuroblastoma. However, inhibition of angiogenesis might lead to hypoxia, which may increase resistance to apoptosis and consequently further the risk of invasion, metastasis and death. The tumor suppressor gene p53 is frequently mutated in NB cancers, and a small number of these mutations lead to a decrease in the expression of hypoxia-inducible factors (HIFs), which results in increased hypoxia and decreased angiogenesis. This study investigated the expression of hypoxia-inducible factors (HIFs) in NB cell lines and its correlation with p53 status.

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Bak: A downstream mediator of fermitin-induced apoptosis of SH-SY5Y neuroblastoma cells

Fenretinide increases ceramide induction through acidic sphingomyelinase-dependent hydrolysis of sphingomyelin resulting in downstream apoptosis of neuroblastoma cells

Studies on lymphangiogenesis in human neuroblastoma

Bone Marrow Derived Matrix Metalloproteinase-9 Regulates Normal and Tumor-Related Neovascularization

Vessel characterisation in an orthotopic model of neuroblastoma

Understanding the molecular pathways that regulate apoptosis of neuroblastoma cells may provide important information about drug resistance mechanisms and may be ultimately lead to the development of novel therapeutic strategies. We have been studying the transcriptional profiles of neuroblastoma cells in response to fenretinide and we have found an anti-apoptotic, anti-angiogenesis and anti-proliferative effect of this drug. We have demonstrated that fenretinide induces the expression of the BH3-only genes HRK and BBC3, which are involved in the pro-apoptotic activity of the BCL2 family. In addition, we have found that fenretinide induces the expression of the BH3-only genes HRK and BBC3, which are involved in the pro-apoptotic activity of the BCL2 family. In addition, we have found that fenretinide induces the expression of the BH3-only genes HRK and BBC3, which are involved in the pro-apoptotic activity of the BCL2 family. In addition, we have found that fenretinide induces the expression of the BH3-only genes HRK and BBC3, which are involved in the pro-apoptotic activity of the BCL2 family.

Ceramide is an important lipid-signaling molecule implicated as a common intermediate of many apoptotic pathways. Fenretinide is thought to induce apoptosis and reactive oxygen species (ROS) via the endoplasmic reticulum via ceramide synthesis or by hydrolysis of sphingomyelin into ceramide and phosphorylcholine. We have shown that fenretinide increases the expression of these pathways to fermitin-induced ROS and apoptosis in neuroblastoma cells.

The data suggest that inhibition of MMP-9 could contribute to neuroblastoma therapy by altering tumor vascularization.

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Like 13-cis retinolic acid, the synthetic retinoid fenretinide (4-HPR) is a potent inducer of apoptosis in neuroblastoma cells through mechanisms involving retinoic acid receptors (RARs) and oxidative stress. After screening a cDNA array for apoptosis-related genes, the 4-HPR-related protein Bak was identified as a fermitin-inducible gene in SH-SY5Y neuroblastoma cells and this was confirmed by Western blotting and flow cytometry. Although fermitin acts synergistically in vitro with chemotherapeutic drugs, these drugs did not induce Bak expression. RAR antagonists did not block the induction of Bak by fermitin. Conversely, Bak induction was blocked by the antioxidant, vitamin C. Overexpression of Bak increased apoptosis in both the presence and absence of fermitin, whereas expression of antisense Bak inhibited fermitin-induced apoptosis. Bak expression was also induced in cells over-expressing the stress-induced transcription factor GADD153, but inhibited in cells expressing an antisense-GADD153 construct. These results suggest that Bak is a downstream mediator of an oxidative stress pathway leading to apoptosis of neuroblastoma cells in response to fermitin.

Fenretinide (4-HPR) is a synthetic retinoid whose apoptosis-inducing effects have been demonstrated in vitro and is currently being tested in clinical trials for a number of malignancies including neuroblastoma (NB). In an in vitro study, we have shown that fenretinide induces the expression of the BH3-only genes HRK and BBC3, which are involved in the pro-apoptotic activity of the BCL2 family. In addition, we have found that fenretinide induces the expression of the BH3-only genes HRK and BBC3, which are involved in the pro-apoptotic activity of the BCL2 family. In addition, we have found that fenretinide induces the expression of the BH3-only genes HRK and BBC3, which are involved in the pro-apoptotic activity of the BCL2 family. In addition, we have found that fenretinide induces the expression of the BH3-only genes HRK and BBC3, which are involved in the pro-apoptotic activity of the BCL2 family.

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Inhibition of Angiogenesis by the Epidermal Growth Factor (EGF)-module of the Follistatin Domain of SPARC is Conformation-Dependent

Takahiro Iwai, Hironori Sako, Tsuyoshi Fujita

BACKGROUND: SPARC (Secreted Protein Acidic and Rich in Cysteine) is a multi-functional matrix glycoprotein. Previously, we demonstrated that neuroblastoma (NB) tumor-derived Schwann cells produce SPARC and that punctuated silencing of SPARC in NB cells suppresses angiogenesis and impairs NB cell growth in vivo. The SPARC protein is comprised of three highly conserved domains that are responsible for its functions: EGF, follistatin (FS) and Ig domains. To investigate the biological activity of these domains, we prepared transiently overexpressed forms of domain-specific SPARC and investigated their angiostatic and anti-tumor activities.

METHODS: SPARC domains were synthesized as cysteine-linked peptides FS-E8, FS-K, and EC N- and C-terminally designed to correspond to the EGF-like module, part of the Kazal module of the follistatin domain, and the conserved Asp955-Ile956 in the C-terminal extracellular calcium-binding domain, respectively. A non-fused peptide FS-K in which the cysteines were not linked during the synthesis, and a scrambled peptide FS-E8, were used as controls. The folded peptides and controls were tested for their ability to inhibit endothelial cell migration and angiogenesis in vivo.

RESULTS: Peptide FS-E8 strongly inhibited basic fibroblast growth factor-(FGF)-induced endothelial cell migration (3E50–10^3 μM) and potently blocked angiogenesis in vivo in the rat corneal assay and the Matrigel plug assay. The anti-angiogenic activity was completely abrogated with the non-fused scrambled FS-E8-peptide. Peptides FS-K and EC-N had no inhibitory activity. CONCLUSION: Our results establish that the EGF-like module of the SPARC follistatin domain is a powerful inhibitor of angiogenesis, and that its structure is essential for this biological activity. Because of its physiological relevance to Schwann cells, SPARC peptides like FS-E8 may be promising candidates for the development of anti-angiogenic treatment strategies.

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Fenretinide [N-(4-hydroxyphenyl)retinamide] induces apoptosis of neuroblastoma cells in vitro and interacts synergistically with the chemotherapeutic drugs cisplatin and etoposide. The stress-inducible transcription factor GADD153 is induced in response to fenretinide, and in other studies modulated apoptosis and pro- or anti-apoptotic actions of the BCL2 family. Since BCL2 family proteins are important in apoptosis induced by growth factors, viral infection or chemotherapy, the interaction between fenretinide and chemotherapeutic drugs to investigate. This study shows that fenretinide induces apoptosis of neuroblastoma cells in vitro, and interacts synergistically with the chemotherapeutic drugs cisplatin and etoposide. This result suggests that fenretinide and chemotherapeutic drugs work in synergy and may be a promising therapeutic combination for the treatment of neuroblastoma.

Inhibition of neuroblastoma cell proliferation by combination of STI-571 with 9-cis retinoic acid in vitro

Many neuroblastoma express the tyrosine kinase receptors for PDGF and c-kit, both of which can be targeted by imatinib mesylate. We evaluated dose- and time-dependent inhibition of c-kit and PDGF receptor-α expression and activity in STI-571-resistant neuroblastoma cells. Continuous low-dose treatment with STI-571 blocks N-myc expression in neuroblastoma cells leading to cell-cycle inhibition and apoptosis.

Digoxin inhibits neuroblastoma tumor growth in mice

Digoxin inhibits neuroblastoma tumor growth in mice. These effects were observed at low concentrations of digoxin, and the mechanism of action may involve inhibition of c-myc expression, which is known to be upregulated in neuroblastoma cells. The results suggest that digoxin may be a potential therapeutic agent for the treatment of neuroblastoma.

Anti-gene peptide nucleic acid (PNA) specifically and persistently blocks N-myc expression in neuroblastoma cells leading to cell-cycle inhibition and apoptosis

We evaluated dose- and time-dependent effects of treatment with imatinib mesylate in a panel of 6 neuroblastoma cell lines. Treatment with 5 µM imatinib significantly inhibited the growth of 5/6 neuroblastoma cell lines, with the exception of SH-SY5Y cells. Treatment with low doses of imatinib resulted in a dose-dependent decrease in the growth of all cell lines, with the exception of SH-SY5Y cells. These results suggest that imatinib and related compounds may be potential therapeutic agents for the treatment of neuroblastoma.

Sensitising effect of MYCN to different chemotherapeutic drugs in vitro

We evaluated the sensitising effect of MYCN to different chemotherapeutic drugs in vitro. Our results suggest that MYCN can be sensitised to different chemotherapeutic drugs, including doxorubicin, vincristine, and cyclophosphamide. These findings indicate that MYCN may be a potential therapeutic target for the treatment of neuroblastoma.

Multiple drug resistance is a major complication especially in patients with advanced neuroblastoma tumours with amplified MYCN. Dysfunctional apoptotic pathways appear to be involved in this mechanism. We investigated 6 NB cell lines (SH-EP, SH-SY5Y, Kelly, LAN-5, SK-NFI, NMC) in vitro in order to study the drug response of chemotherapy-resistant neuroblastoma cells. We found that the combination of imatinib and chemotherapy drugs may be a promising therapeutic approach for the treatment of neuroblastoma.

Continuous low-dose treatment with imatinib mesylate as a possible therapeutic strategy for neuroblastoma

We evaluated the sensitising effect of MYCN to different chemotherapeutic drugs in vitro. Our results suggest that MYCN can be sensitised to different chemotherapeutic drugs, including doxorubicin, vincristine, and cyclophosphamide. These findings indicate that MYCN may be a potential therapeutic target for the treatment of neuroblastoma.

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INTRODUCTION: MYCN amplified neuroblastoma cell lines exhibit two morphologically distinct cell types, the neuronal (N-cells) and fibroblastoid like so-called flat cells (F-cells). It has been shown that F-cells are generated by active expulsion of extracellularly amplified MYCN copies by micropinocytosis forming tumor cell revertant. We tested whether it is possible to induce F-cells by the exposure of cells to low concentrations of hydroxyurea and whether these morphological changes are associated with changes in the MYCN copy number, telomere lengths, p16 and SA-β-gal.

MATERIAL AND METHODS: The two cell lines STA-6B-N9 and STA-6B-10 were treated with 75 - 150 nM hydroxyurea for several weeks. Automated RJ-FISH (interphase quantitative fluorescence in situ hybridization) was performed using MYCN and telomere specific probes and appropriate references. The quantification of p16 expression was performed by static intensity measurement on immunofluorescence stained cells.

RESULTS: F-cells showed, in contrast to N-cells, a dramatically reduced MYCN copy number or single copies of MYCN while p16 deletion and 17q gain was found in both sublines. While p16 had solely been expressed in N-cells and no SA-β-gal activity had been observed, the F-cells showed a marked reduction of p16 expression but were positive for SA-β-gal.

CONCLUSION: The induced F-cells are characterized by a dramatic reduction of the MYCN copy number and of the telomere lengths. Together with the upregulation of SA-β-gal and the reduced p16 expression in F-cells we hypothesize that the replicative pathway is operating but that the p16 senescence pathway is not involved. The entering of the tumor cells into cellular senescence can be enhanced by the hydroxyurea.

Reference: [Ref ID: 368.1]

Dramatic reduction of MYCN gene copy number and telomere lengths in F-cells induced by hydroxyurea

Rita Nahal, Ingo M Amthor, Peter F Amthor

Tumornecrosis, CRC Children’s Cancer Research Institute, Vienna, Austria.

Purpose of the present study was to evaluate different strategies for immunotherapy of high risk NB based on vaccination or transfection with tumor mRNA represent promising strategies for development of cancer vaccines in treatment of NB.

Neuroblastoma is the most common solid tumor of childhood that can arise anywhere in the sympathetic nervous system. Children with high-risk neuroblastoma (NB) have a poor clinical outcome. The Ricerca sul Cancro2, Istituto Giannina Gaslini3, 16148 Genova, Italy.

Sensitivity of neuroblastoma to NK-mediated killing

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Celecoxib, a selective cyclo-oxygenase-2 (COX-2) inhibitor, is currently being studied for both chemoprevention and treatment of adult cancers in numerous clinical trials. It has been demonstrated that celecoxib doses of at least 20 mg are required to inhibit COX-2.

We have correlated drug resistance in neuroblastoma (NB) with mRNA expression of glucocorticoid receptor (GR), glucocorticoid-induced enzyme (GCE), and glucocorticoid-induced enzyme 2 (GCE-2) in a panel of NB cell lines. We also determined by digital imaging microscopy microarray: 10 of the cell lines constitute 5 pairs established from 5 patients.

We treated 20 NB cell lines containing 10 sensitive and 10 multi-drug resistant lines. Sensitivity to methylpiperazin, cisplatin, carboplatin, and 5-fluorouracil was determined by quantitative RT-PCR for 11 enzymes and substrates included in ceramic synthesis [LCB-1 and LCB-2 substrates of Senoi Palmitoyl Transferase, Acyl and Neutral Sphingomyelinase, Dihydrolipoamide Desuccinylase] and metabolism [Sphingosine Kinase, Sphingosine Kinase 2, Ceramide Kinase, Acyl Ceramide Kinase, Ceramide Synthase, Glucose Synthase, and Acyl Ceramidase].

We found that a number of NB cell lines are susceptible for lysis by terminally differentiated CD8+ T lymphocytes activated on third party targets induce cell death in neuroblastoma with limited toxicity. We suggest that the drug warrants further clinical testing in patients with neuroblastoma.

Reference: [Ref ID: 331.1]

Analysis of susceptibility of neuroblastoma to NK-mediated killing

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Department of Hematology-Oncology, Children’s Hospital Los Angeles, CA, USA.

BACKGROUND: Celecoxib is known to induce apoptosis in a number of different cell types, including neuroblastoma cells, and this effect can be mediated in an MHC-independent fashion.

RESULTS: We found that a number of NB cell lines are susceptible for lysis by terminally differentiated CD8+ T lymphocytes activated on third party targets.

CONCLUSIONS: The mechanism by which celecoxib inhibits neuroblastoma tumor growth is not yet fully understood. The finding that celecoxib induces tumor cell death in NB cells suggests that celecoxib may be a promising therapeutic agent for the treatment of NB.

Reference: [Ref ID: 145.1]

Analysis of susceptibility of neuroblastoma to NK-mediated killing

Roberta Carminati1,3, Sergio Rutella3, Maurizio D’Incalci4, Riccardo Riccardi1

Division of Paediatric Oncology1 and Division of Haematology3Catholic; Department of Oncology/Pathology, Cancer Centrum Karolinska, Stockholm, Sweden.

Neuroblastoma is the most common solid tumor of childhood that can arise anywhere in the sympathetic nervous system. Although many efforts have been made, Neuroblastoma remains the tumor with the highest risk of death in children. The aim of our study was to value neuroblastoma cells susceptibility to Natural Killer (NK) cells cytotoxicity. This analysis could provide solid basis for the development of an effective therapeutic strategy.

Smac agonists as novel therapeutics to overcome resistance of neuroblastoma cells against radiation therapy

Osnat Zmora, Michael J Cascio, Zesheng Wan, Bo Yang, Nino Keshelava, C Patrick Reynolds

Department of Paediatric Research1, National Hospital of Norway, Oslo, Norway; Section for Tumorcytogenetics, CCRI Children’s Cancer Research Institute, Vienna, Austria.

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CONCLUSIONS: The mechanism by which celecoxib inhibits neuroblastoma tumor growth is not yet fully understood. The finding that celecoxib induces tumor cell death in NB cells suggests that celecoxib may be a promising therapeutic agent for the treatment of NB.

Reference: [Ref ID: 058.1]

Analysis of susceptibility of neuroblastoma to NK-mediated killing

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Children with high-risk neuroblastoma (NB) have a poor clinical outcome. The chemotherapy treatment is usually associated with significant toxicity and immunotherapy of high risk NB based on vaccination with antigen-loaded dendritic cells (DCs) has been explored in clinical trials. DCs are professional antigen-presenting cells with the ability to activate naive T cells. However, clinical responses are disappointing. We have developed DCs loaded with apoptotic tumor cells or transfected with NB-cell mRNA were both able to induce T-cell responses against neuroblastoma antigens with apoptotic NB cells or transfection with tumor mRNA represent promising strategies for development of cancer vaccines in treatment of NB.
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BACKGROUND: Spontaneous differentiation is one of the biological hallmarks of childhood neuroblastoma. The degree of differentiation toward ganglionic cells is recognized as the principal morphological feature to be of główczak, 2011. It is characterized by the presence of ganglion-like structures among proliferating neuroblastoma cells. We demonstrated that exogenously overexpressed c-KIT positive neuroblastoma cells can differentiate into ganglionic phenotypes. We hypothesized that neuroblastoma cells contain a differentiation program which is altered by gain or loss of function mutations. In particular, we tested the role of c-KIT, which is expressed in a subset of particularly aggressive neuroblastomas for which selective therapeutic targeting is needed.

We showed recently that c-KIT is preferentially expressed in MYCN-amplified neuroblastomas. One member of the P2X receptor family, P2X7 receptor (P2X7R), is expressed in hematopoietic cells and it is able to allow the transduction of extracellular signals to the cytoplasm. The purinergic P2X receptors are ligand-gated ion channels activated by ATP. As a rare neurological disease, the paraneoplastic form of OMS in children is an autoimmune disease of unknown etiology characterized by a wide spectrum of neurological symptoms, including opsoclonus, myoclonus, and ataxia. Autoimmune sera from pediatric patients with opsoclonus-myoclonus-syndrome (OMS) contains autoantibodies against a variety of tumor cell autoantigens, including neuronal antigens, gangliosides, and cytoskeletal proteins. The autoreactive immune response toward tumor cell antigens might be enhanced by tumor cell autologous immune system that recognizes self-antigens and also the tumor cell antigens.

As a rare neurological disease, the paraneoplastic form of OMS in children is an autoimmune disease of unknown etiology characterized by a wide spectrum of neurological symptoms, including opsoclonus, myoclonus, and ataxia. Autoimmune sera from pediatric patients with opsoclonus-myoclonus-syndrome (OMS) contains autoantibodies against a variety of tumor cell autoantigens, including neuronal antigens, gangliosides, and cytoskeletal proteins. The autoreactive immune response toward tumor cell antigens might be enhanced by tumor cell autologous immune system that recognizes self-antigens and also the tumor cell antigens.

One of the prominent features of pediatric patients with opsoclonus-myoclonus-syndrome (OMS) is their cellular immune response against tumor cells autoantigens. The cellular distribution of autoantigens and functional activity of the polyamine biosynthetic pathway as a drug target for neuroblastoma therapy

We tested the effects of P10 in nude mice injected with 10^5 NXS2 murine NB cell line. Tumour xenografts with P10 were smaller and slower growing compared to the control group. The data suggest that P10 holds promise as an anti-cancer compound, independent of its drug carrier suitability.

Cellular distribution of autoantigens and functional activity of the polyamine biosynthetic pathway as a drug target for neuroblastoma therapy

We synthesised an amphiphilic polymer based on a polyvinylalcohol backbone and showed that it is able to form nanoparticles. The nanoparticles can be internalized by neuroblastoma cell lines and can be targeted to the mitochondria. The nanoparticles were then tested in a murine model of neuroblastoma metastases. The results showed that the nanoparticles can be used to deliver anticancer drugs to neuroblastoma cells, and that this approach is effective in reducing tumor growth.

Involvement of CXCR4 in the development of neuroblastoma metastases

Polyamine Biosynthetic Pathway as a Drug Target for Neuroblastoma therapy

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Glutathione S-transferase polymorphism, genetic susceptibility and outcome in neuroblastoma

Marina Lanciotti, Paola Di Michele, Simona Coco, Riccardo Haupt, Luca Boni, Carlo Dufour, Alberto Garaventa, Gian Paolo Tonini

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The glutathione S-transferases (GSTs) activities are involved in the metabolism of carcinogens and of some anticancer drugs and may also confer resistance to them. The GSTT1 and GSTM1 genes exhibit a deletion polymorphism, which in case of homozygosity leads to absence of the protein (null genotype), whereas the GSTP1 gene displays a polymorphism (Ile105Val) that confers a different catalytic activity. Subjects with a modified ability to metabolize carcinogens are at increased risk of cancer. Furthermore, in case of cytotoxic treatment, the low enzymatic activity, reducing the detoxification of anticancer drugs, may increase the cytotoxic effect of them. In our study we hypothesized that GSTs genotype may have a role in the susceptibility and outcome of neuroblastoma. We compared GSTs genotypes of 264 children with neuroblastoma with those of 392 normal subjects. Within the neuroblastoma group we further analyzed if any particular GSTs genotype was correlated to different risk factors or to disease outcome. No significant differences of allele frequencies were found between neuroblastoma patients and controls (GSTT1 null 19% vs. 18%, p=0.66, GSTM1 null 52% vs. 55%, p=0.81, GSTT1 null + GSTM1 null 9% vs. 10%, p=0.81; and GSTP1 Ilele 47% vs. 53%, Ile/Val 46% vs. 38%, Val/Val 7% vs. 9%, p=0.14). We did not detect any particular association in the analysis of the relationship of GSTM1, GSTT1, and GSTP1 genotypes, and age at diagnosis, sex, primary site and stage of the disease, levels of VMA, HVA, LDH, Ferritine, NSE, MYCN amplification, 1p/16 deletion and 1p36 imbalance. No correlation with outcome was observed. Our data do not support an important effect of GSTs genotype on neuroblastoma susceptibility.
Abstracts

Published-only
Flt-3 expression in neural crest-derived tissues and tumor cells
Cristina Zanini1, Francesco Pulerà1, Nicoletta Crescenzio2, Marika Crudelini1, Luca Cordero di Montezemolo2, Marco Forni1, Fabio Timeus2
Dipatimento di Genetica, Biologia e Biochimica1, Dipartimento Oncoematologia2, Università di Torino, Italy.

Flt-3 is a class III tyrosine kinase receptor that plays with its ligand FL a central role in proliferation and survival of human hematopoietic progenitors and immature thymocytes. We have previously shown that Flt-3 expression is reduced in human neural crest-derived tumor cells and that FL promotes their survival and proliferation, suggesting an antiapoptotic role. We have also observed by RTPCR Fl-3 expression in a subset of neural crest (NCC) Flt-3 negative (NB), while there is no data about flt-3 expression in normal human neural crest-derived tissues. To confirm these findings in a larger cohort of patients, we analyzed flt-3 expression in 160 children (88 boys and 72 girls) were diagnosed. The median follow-up was 118 months (range, 20 months to 200 years) and at last clinical data available were in the year 2016. We observed a progression free survival of 0.42 at 5 years. There was a significant increase in the 5-year PFS between children who survived periods from 0.28 in 1981-1985 to 0.61 in 1996-2000 (p=0.007). Also when analyzing the results of children with stage 4 the PFS increased significantly after the 2-year time periods (0.14 from 1981-1990 and 0.29 in 1991-2000, P=0.057). Age was a significant prognostic factor both when analyzing the entire population (p=0.001) and also when considering only st. 4 (p=0.02). Stage was a significant prognostic factor, the 5-year progression free survival was: st. 1: 1.00; st. 2: 0.86; st. 3: 0.42; st. 4: 0.22 and st. 4: 0.50. Also the localisation of the primary tumor had prognostic significance (p=0.012).

This observational study could detect any significant impact of autologous stemcell transplantation.

Analysis of neuroblastoma treatment results of children between 1 and 2 years old in the aspect of molecular, histopathological and biochemical diagnostic markers
Anna Raciborska, Tadeusz Izbicki, Wojciech Wozniak, Magda Rychlowska, Teresa Klepacka, Elzbieta Michalak
Children Oncology Surgery Clinic, Mother and Child Institute, Warsaw, Poland.

PURPOSE: At present it is agreed that the diagnosis of NBL after completion of 1st year of life changes the prognostics and has a meaningful impact on the treatment procedures. Literature data concerning children in the age of 1-2 years old is still sparse and the results are difficult to analyse to the NBL treatment results among children in the age of 1-2 years old in the aspect of tumors in relation to the nerve tissue, and other factors. We have also observed differences in the treatment outcome of the children in the above-mentioned age group.

MATERIALS AND METHODS: The study included 74 children with diagnosed neuroblastoma, whose age between 1 and 24 months, treated at Children’s University Hospital in the period of the years 1990-2000. NBL was diagnosed based on histopathological test of the primary tumour or metastases. The stage of advancement was determined in accordance to Evans’ classification and recently based on INSS. 3,12,20 and 31 children were respectively classified into stages I-II, IVS, IVS2, IVF. Pathological material underwent histogenetic, biochemical test and urine analysis was conducted in accordance with the genetical-approved standards.

RESULTS: The frequency of failures as well as the number of load factors would increase with age growth. Children in stage I, IVS made up for a total of 24%, the 18% children belonged to stage III and IV. 21 children of the studied group died, in that 17 in IV stage of advancement. All of the children in stage I, II, IVS are alive and are under observation.

CONCLUSIONS: In the analysed material, children up to 2 years old would make up for a rather good prognostic group. Age cannot be the only prognostic factor.

Monoclonal antibody NB84 expression in the normal fetal sympathetic nervous system development and its relation with other neuroendocrine immunomarkers
Rosa Noguera, Samuel Navarro, Marta Piqueras, Adela Cañete, Antonio Llombart-Bosch
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Neuroblastoma (NB) is an embryonal, sympathetic nervous system (SNS)- derived tumor that may originate at any site where SNS tissue is located. The NB84 monoclonal antibody, raised to NB84 proteins from neuroblastoma and ganglioneuroblastoma, reacts with all SNS and ganglionic SNS cells. This antibody recognizes a common epitope in normal and ganglionic SNS cells. These marker profiles may provide an explanation for the putative progenitor cell types of NB.

Funded by Grant FIS 030089, Madrid, Spain.

Neuroblastoma in Denmark. A 20 years population based study
Henrik Schroeder1, Jeanette Wacher1, Jørn Atterman2, Steen Rosthøj1, Niels Carlsen1, Catherine Rechnitzer3
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All patient charts from patients below 15 years of age diagnosed with neuroblastoma (NBL), ganglioneuroblastoma (GNB) and ganglioneuroma (GNR) in Denmark from 1981 to 2000 were reviewed. A total of 140 children (88 boys and 72 girls) were diagnosed. The median follow-up was 118 months (range, 20 months to 200 years) and at last clinical data available were in the year 2016. We observed a progression free survival of 0.42 at 5 years. There was a significant increase in the 5-year PFS between children who survived periods from 0.28 in 1981-1985 to 0.61 in 1996-2000 (p=0.007). Also when analyzing the results of children with stage 4 the PFS increased significantly after the 2-year time periods (0.14 from 1981-1990 and 0.29 in 1991-2000, P=0.057). Age was a significant prognostic factor both when analyzing the entire population (p=0.001) and also when considering only st. 4 (p=0.02). Stage was a significant prognostic factor, the 5-year progression free survival was: st. 1: 1.00; st. 2: 0.86; st. 3: 0.42; st. 4: 0.22 and st. 4: 0.50. Also the localisation of the primary tumor had prognostic significance (p=0.012).

This observational study could detect any significant impact of autologous stemcell transplantation.
Neuroblastoma and related hematopoietic transplantation

Luisa M Massimo

Department of Pediatric Hematology-Oncology, Giannina Gaslini Children’s Hospital, Genova, Italy.

BACKGROUND AND AIM: To report on frequency and severity of neutropenia and infections in children treated with the European HR-NBL-1 Protocol (highly intensive COJEC regimen, directed against 2-3 copies of MYCN, and autologous peripheral blood stem cell reinfusion (aPBSCR)).

METHODS: During the first years of treatment, we reported a high incidence of neutropenia (absolute granulocyte count <1000/mm3) in children at our center. From January 2002 – January 2003, 57 children with neuroblastoma received antineoplastic chemotherapy. Among them 20 (35%) were treated according with the European HR-NBL-1 Protocol. RESULTS: In the period January 2002 – January 2003, among the 57 children with neuroblastoma treated at Tata Memorial Hospital, Mumbai, India

Neutropenia and fever in children with neuroblastoma treated with the European HR-NBL-1 Protocol. A mono-institutional experience

Elio Castagnola, Ilaria Caviglia, Silvia Caruso, Carla Manzitti, Massimo Conte, Riccardo Magri

Giannina Gaslini Children’s Hospital, Genova, Italy.

BACKGROUND: Among cases of undifferentiated and poorly differentiated neuroblastoma with bone metastases and/or multiple copies of MYCN, as well as poor responders for current chemotherapeutic drugs, the role of hematopoietic transplantation has not been established.

METHODS: We treated 2 cases of relapsed stage IV NB (age 6 and 7 years) with a stem cell transplant from a haploidentical family donor. Both patients lacked a matched sibling donor and in the first case a matched unrelated donor. Conditioning consisted of thiotepa 400 mg/m2, fludarabine 90 mg/m2, ATG and melphan 140 mg/m2 followed by purified CD34+ peripheral blood stem cells (CliniMACS, Miltenyi Biotec, Bergish Gladbach, Germany). Neither GVHD prophylaxis was administered. Hematopoietic recovery was prompt in both cases with >80% donor chimerism at day 30 post transplantation. Pt 1, a 6 year old boy, was transplanted after a delayed conditioning, using busulfan 12 mg/kg and fludarabine 75 mg/m2 followed by purified CD34+ peripheral blood stem cells (CliniMACS, Miltenyi Biotec, Bergish Gladbach, Germany). Pt 1 was in complete remission (CR) at day 150 post transplantation with decreasing MRD. Pt 1 had no evidence of disease at 2 years follow up. Pt 2 had both local (lymphadenopathy) and bone marrow relapse. He was transplanted with an initial course of thiotepa and melphan (same doses as the conditioning regimen) and autologous stem cell rescue with a partial response. Marrow disease became undetectable following hematopoietic transplantation while local mediodisease remained CR 5 months post transplant. Both patients survived with no evidence of disease. Pt 1 was alive and well with no evidence of disease, complete CR at 2 years from transplant. Both cases lacked an NK alloreactive setup as predicted by HLA mismatch. In conclusion hematopoietic transplantation can provide a graft vs. neuroblastoma effect.

Possible graft vs. neuroblastoma effect after partially matched related hematopoietic transplantation

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Department Hematology-Oncology, Azienda Ospedaliera Meyer, Firenze, Italy.

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Published-only Abstracts

Ref ID: 057.1
Role of Bmi1 in neuroblastoma
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We recently identified the MYCN oncogene as a tissue-specific target gene of E2F-1 in neuroblastomas (Strieder et al., 2003; Kramps et al., 2004). A search for additional oncogenic target genes of E2F-1 using human neuroblastoma cells expressing a 4-hydroxy-tamoxifen (4-OHT) regulated E2F-1-ER fusion protein and cDNA microarray analysis identified the polycomb group gene BMI1. Real-time RT-PCR and Western-blotting confirmed the induction of BMI1 by E2F-1. Cycloheximide did not inhibit induction of BMI1 by E2F-1-ER suggesting that BMI1 is a direct target of E2F-1. Indeed, the human BMI1 promoter contains a putative E2F binding site that is conserved in the mouse. In transient assays, this binding site was required for the activation of a BMI1-driven reporter construct by E2F-1. In addition, chromatin-immunoprecipitation revealed binding of E2F-1 to the BMI1 promoter in vivo. These data establish BMI1 as a direct E2F target gene. BMI1 in turn, via repression of p16INK4a, can stimulate E2F activity suggesting a positive feedback loop between E2F-1 and BMI1. BMI1 was recently shown to be essential for the self-renewal of both hematopoietic and neuronal stem cells and to act as an immortalizing oncogene early in tumorigenesis. Moreover, BMI1 co-operates with c-Myc in the pathogenesis of lymphomas by blocking Myc-induced apoptosis. Thus, a failure to down-regulate BMI1 may be a critical event in the initiation of neuroblastomas. On the one hand, BMI1 can promote immortalization, on the other hand BMI1 may pave the way for MYCN amplification by simultaneously stimulating E2F-activity and blocking Myc-induced apoptosis. Consistent with a role of BMI1 in neuroblastoma, BMI1 expression was detected in all primary neuroblastomas analyzed. We will present results of loss-of-function and gain-of-function studies addressing the role of BMI1 in neuroblastoma cells.

Ref ID: 416.1
Spinal Neuroblastoma in Stage 2/3 Disease – Experience From One Institution
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Spinal cord compression secondary to neuroblastoma can be considered an emergency. There is no treatment consensus for symptomatic presentation. Aim: to analyse management and outcome of children with stage 2/3 neuroblastoma with spinal involvement treated at Great Ormond Street Hospital (GOSH) over 20 years. Data was acquired by retrospective note review.

Results: 387 children had neuroblastoma, 315 had stage 2/3 disease. 22 patients had evidence of an intraspinal component. 16 presented <1 year of age (mean 0.74 years, range 0 to 1.8 years); 14/22 had stage 3 disease, 8/22 had stage 2 disease. 9/22 were thoracic tumours and 7/22 were pelvic. Presentation: time from first symptoms to GOSH - 24 hrs to 3 months (mean 18 days). 11 children had limb neurology, 4 had bladder / bowel symptoms and 7 non-neurological symptoms.

Treatment: 5 had steroids, 21 had chemotherapy; 7 underwent laminectomy (3 at presentation, 4 after chemotherapy); 3 required further surgery to primary disease; 15 children underwent surgery on the intra thoracic/abdominal component of the primary; 2 received radiotherapy. Follow up: the interval is wide (1 to 20 years; mean 9.3 years); all but one of these patients are alive; one has possible localised disease progression. 6 patients cannot walk, all presented with limb neurology. 12 have urinary incontinence / bowel control problems. 6 children have no significant sequelae. Overall survival from disease is 100% however morbidity free survival is 27%.

Conclusion: 19% of children with stage 2/3 neuroblastoma had spinal involvement. Management of stage 2/3 neuroblastoma involving the spinal cord has not been consistent; prompt treatment may improve outcome. Although prognosis from this disease is good, long-term morbidity is high. We need to recognise this early and investigate prospectively which treatment best reduces long term sequelae.
Ref ID: 376.2
Neuroblastoma-associated Opsoclonus, Myoclonus, Ataxia syndrome: A Clinical and Biologic Dilemma
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Opsoclonus-myoclonus ataxia syndrome (OMS), also called “dancing eyes-dancing feet”, is a rare paraneoplastic disorder that occurs in less than 2-4% of patients with neuroblastoma. This syndrome is characterized by neurologic findings including the presence of involutionary, conjugate, chaotic eye movements and myoclonic limb jerking. These symptoms are often accompanied by significant mood and sleep disturbance. Although OMS may also occur in association with other tumors approximately 50% of children with OMS are diagnosed with neuroblastoma. The majority of children present with localized, favorable biology tumors. Most children present between age 1-4, with a median of 2 years. Data from patient tumors and sera suggest an immune-mediated process, with an excess of lymphoid infiltrates in the tumors and a higher frequency of anti-neuronal antibodies in sera at diagnosis. MRI examinations over time show progressive cerebellar atrophy. Although the outcome from the standpoint of the malignancy tends to be favorable, with greater than 90% survival, 70% of children with OMS inevitably develop significant neurologic sequelae and developmental and speech delay. Recent approaches to treatment have focused on intensive immunosuppression rather than specific anti-tumor therapy. The current management, usually successful in arresting the acute symptoms, is tumor resection, followed by steroid or ACTH therapy and often other immunosuppressive therapy, cyclophosphamide or intravenous immunoglobulin.

Newer approaches being tested include rituximab. Future efforts must include further investigation of the pathogenesis of this disorder, and careful assessment of the effectiveness of therapies on prevention of the late neurologic and developmental deficiencies resulting from OMA.

Ref ID: 429.1
Longitudinal neurodevelopmental evaluation of children with opsoclonus-ataxia
Wendy G. Mischel, Veronique L. Brunet, Colleen Azra, Kirsten E. Peterson, Jonny Rodríguez
We previously reported upon children with opsoclonus-ataxia due to neuroblastoma who found prolonged neurodevelopmental deficits, years after onset, with unclear relationships to treatment modality or timing of treatment. A significant negative correlation of functional status with age at testing raised a question of whether OAs are the result of cerebellar hypomyelination, which we are trying to clarify with repeated testing.

METHODS: Thirteen of 17 children previously reported were reevaluated a second time 2-4 years later. In addition, 5 new subjects (2 with NB, 3 without NB) were enrolled, and were evaluated twice at a minimum interval of one year between assessments. Intercurrent medical events, immunosuppressive therapy, medication (ACTH, oral steroids, IVlg, other immuno suppressants) and other important medical changes were noted. Cognitive, adaptive behavior, speech and motor abilities were assessed.

RESULTS: Generally, younger subjects’ cognitive and adaptive behavior scores were stable over time. Some children in our cohort responded to a monophasic course of steroid treatment or ACTH, but none of 14 evaluated children showed improvement in all areas. Only four children are currently functioning in the average range with FSQ of 90 or above. All four were unusual, in that despite severe initial symptoms each appeared to have a monophasic course of gradual improvement on steroids or ACTH. None experienced a relapse upon medication taper or with intercurrent illnesses. These four subjects were the only subjects without any relapses.

The results continue to be raise concern that opsoclonus-ataxia represents a progressive encephalopathy, at least in some children. It appears that some children have a monophasic course despite initial severity of symptoms. However, the patients may be biologically different, with a more benign prognosis. There is some evidence that aggressive intervention may improve overall outcome, but this does not reach statistical significance in this observational study. An ongoing randomized clinical trial comparing different modalities of immunosuppression in OSA syndrome is needed.


Acknowledgments: Initial testing was supported by a grant from Aventis- Behring (formerly Centeon Pharmaceuticals). The second phase was supported in part by the NIH NCRR GCR Grant MO1 RR-43 and was performed at the GCRC at Children’s Hospital Los Angeles.

Ref ID: 141.3
Immunopathogenesis of Opsoclonus-Myoclonus Syndrome
Franz Blees
Department of Neurology, Justus-Liebig-University, Giessen, Germany.

Opsoclonus-myoclonus syndrome (OMS), also known as Dancing eye syndrome or Kinsbourne’s syndrome is characterized by chaotic, omnidirectional synchronous eye movements (opsoclonus), sudden unprovoked postural and locomotor lurching (myoclonus) and ataxia of voluntary movement. In children, additional symptoms like irritability, behavioral disturbances and language difficulties may also occur. As a paraneoplastic syndrome, about 50% of the childhood OMS are associated with neuroblastoma and vice versa, 1-3% of neuroblastoma patients have OMS. Most OMS patients respond to immunosuppressive or immunomodulatory drugs, which led to the suspicion of an autoimmune pathogenesis. Additional factors support this hypothesis. Inflammation: Some OMS patients have been found to have lymphocytic pleocytosis or oligodendroglial bands in the CSF. Relapses and deterioration in OMS often occur in association with infections. Tumour immunity: Neuroblastoma of OMS patients have more lymphocytic infiltration than neuroblastoma without OMS and children with OMS have a better prognosis of the tumour disease, suggesting a cross-reactive immunity between neuroblastoma and nervous system tissue.

The acute side effects of treatment with low dose cyclophosphamide appear moderate, but the extent of long term side effects with those low doses is not well investigated. For a treatment protocol of patients with opsoclonus-myoclonus syndrome, the risk of late side effects caused by cyclophosphamide has to be balanced against the potential positive influence of cyclophosphamide and cyclosporine A to reduce the total amount of corticosteroids with their considerable acute and chronic toxicity.

Ref ID: 370.2
Longitudinal neurodevelopmental evaluation of children with opsoclonus-ataxia
Wendy G. Mischel, Veronique L. Brunet, Colleen Azra, Kirsten E. Peterson, Jonny Rodríguez
We previously reported upon children with opsoclonus-ataxia due to neuroblastoma who found prolonged neurodevelopmental deficits, years after onset, with unclear relationships to treatment modality or timing of treatment. A significant negative correlation of functional status with age at testing raised a question of whether OAs are the result of cerebellar hypomyelination, which we are trying to clarify with repeated testing.

METHODS: Thirteen of 17 children previously reported were reevaluated a second time 2-4 years later. In addition, 5 new subjects (2 with NB, 3 without NB) were enrolled, and were evaluated twice at a minimum interval of one year between assessments. Intercurrent medical events, immunosuppressive therapy, medication (ACTH, oral steroids, IVlg, other immuno suppressants) and other important medical changes were noted. Cognitive, adaptive behavior, speech and motor abilities were assessed.

RESULTS: Generally, younger subjects’ cognitive and adaptive behavior scores were stable over time. Some children in our cohort responded to a monophasic course of steroid treatment or ACTH, but none of 14 evaluated children showed improvement in all areas. Only four children are currently functioning in the average range with FSQ of 90 or above. All four were unusual, in that despite severe initial symptoms each appeared to have a monophasic course of gradual improvement on steroids or ACTH. None experienced a relapse upon medication taper or with intercurrent illnesses. These four subjects were the only subjects without any relapses.

The results continue to be raise concern that opsoclonus-ataxia represents a progressive encephalopathy, at least in some children. It appears that some children have a monophasic course despite initial severity of symptoms. However, the patients may be biologically different, with a more benign prognosis. There is some evidence that aggressive intervention may improve overall outcome, but this does not reach statistical significance in this observational study. An ongoing randomized clinical trial comparing different modalities of immunosuppression in OSA syndrome is needed.


Acknowledgments: Initial testing was supported by a grant from Aventis-Behring (formerly Centeon Pharmaceuticals). The second phase was supported in part by the NIH NCRR GCR Grant MO1 RR-43 and was performed at the GCRC at Children’s Hospital Los Angeles.

Ref ID: 429.2
COG Therapeutic trial in OMS: A neurologist’s view
Dr Michael Pike MA, MD, FRCPCH, Consultant Paediatric Neurologist
John Radcliffe Hospital, Oxford, UK
The COG therapeutic trial is a welcome attempt to evaluate the role of treatment options, particularly intra-venous immunoglobulin, in the management of this distressing disorder. From the perspective of a pediatric neurologist the following issues deserve discussion:• Inclusion criteria specifically in relation to children without associated neuroblastoma.
• Age range – particularly in relation to the inclusion of young adults.
• The acceptability of cyclophosphamide to families whose children do not have neuroblastoma and to treating clinicians.
• The difficulties of OMS evaluation resulting from (a) the intermittent nature of opsoclonus in some children, (b) The natural fluctuation of all symptoms of OMS in relation to a variety of factors including intercurrent infections in many children.

These and related issues will be discussed with colleagues.

Ref ID: 410.1
Lymphoid infiltration in neuroblastoma-associated Opsoclonus Myoclonus Syndrome (OMS)
Lizzia Raffaghello, Claudio Gambini, R. Boldrini, Vito Pistoia
Laboratories of Oncology and Pathology, G. Gaslini Institute, Genoa, and Laboratory of Pathology, Bambin Gesù Institute, Rome, Italy
OMS is an autosomal disorder of the central nervous system that may affect children with neuroblastoma. This association is observed in 1.3% of neuroblastoma cases in the Italian population. distinctive features of OMS-related neuroblastoma are the predominant ganglioneuroblastoma histology, the lack of MYCN amplification and the presence of abundant lymphoid infiltrates. These are often organized in lymphoid follicles with a well defined network of follicular dendritic cells, a mantle and a germinal centre, indicating the occurrence of a process of lymphoid neogenesis. It cells are concentrated in the follicles, whereas T marker and CD45+ markers are found in extrafollicular location. Similar features may be observed in OMS-unrelated ganglioneuroblastoma, in which less infiltrating lymphoid cells were detected. Absence of HLA class I from the surface of neuroblasts in OMS-associated tumours militants against the hypothesis that T cell reactivity to the tumour antigen is driven. Key players in lymphoid neogenesis are the CLL19, CCL21, CXC12, and CXCL13 chemokines, that interact with their cognate receptors, i.e. CCR7/CXCR4 and CXCR5. In preliminary experiments we have investigated expression of these receptors in OMS-related tumours by immunohistochemistry, CCR7, CXCR4 and CXCR5 were found to be expressed on the major tumour cells, with higher intensity on the more differentiated ganglionic component. CXCR5, but not CXCR4 or CCR7, was detected on infiltrating lymphoid cells. Studies are now in progress to investigate expression of the ligands of these receptors in tumour tissue.
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Social Programme

Opening Ceremony & Welcome Reception

Date: Thursday, June 17, 2004
Time: 7:00 - 09:30 PM
Location: Magazzini del Cotone Congress Centre and Italian Navy's Amerigo Vespucci
Fee: Included in the Senior, Young A, Young B and Accompanying person fee.

The Welcome Reception will be held on the "Molo Vecchio" Pier (the Congress venue's Pier in the Old Harbour) and aboard the spectacular Amerigo Vespucci tall ship, the only square-rigged three-decker sailing ship still in existence and one of the largest that is active nowadays. The pride of the Italian Navy, she is a full ship-rigged steel hull (and masts) frigate built during 1930-31 in the former Royal Shipyard at Castellammare di Stabia (Naples) as a sail-training ship. Inspired by the big 19th Century frigates, with high freeboard, stern gallery and white-painted strakes, her bow and stern decorated with intricate gilt carvings, the Vespucci is one of the grandest of the tall ships and a spectacular sight under full sail. The ship is employed for the annual Italian Naval Academy training cruise.

Gala Dinner

Date: Friday, June 18, 2004
Time: 8:30 - 11:00 PM
Location: Aquarium of Genoa
Fee: € 100,00 (included in the Senior, Young A and Accompanying person fee).

The Gala Dinner will take place inside the spectacular marine environments of the Aquarium of Genoa, the largest aquarium in Europe and one of the most frequently visited cultural sites in Italy, thanks to its dimensions of 10,000 square metres and 71 tanks. Most tables will be arranged along two 25-metre long tanks (1,300,000 litres of water), face to face with the world’s most feared and beloved marine creatures, so that the sharks’ elegant gait and the dolphin’s contagious curiosity will set the scene for a most extraordinary evening, unique and unforgettable, in the only such setting in the world, which will leave you speechless.

Sponsors

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TEVA PHARMA ITALIA S.R.L.
Hall “Maestrale”
Auditorium, First Floor
- Workshop on Microarray Technology (June 16)
- Plenary Session (June 17-18-19)
- Parallel Session E: Molecular Biology - Translational (June 19)

Hall “Scirocco & Libeccio”
Module 9 - Third Floor
- Parallel Session A: Translational (June 17)
- Parallel Session C: Biology and Genetics (June 18)

Hall “Levante & Ponente”
Module 9 - Second Floor
- Parallel Session B, D, F: Clinical (June 17-18-19)
- Workshop on Spinal Cord Compression (June 19) Hall “Levante”
- Workshop on Opsoclonus Myoclonus (June 19) Hall “Ponente”

Posters Exhibition
Room A Biology - Clinical - Genetics 1
Room B Genetics 2 - Molecular Biology - Translational
Under the auspices of

Giannina Gaslini Children’s Hospital and International School of Paediatric Sciences, Genova

IST - National Cancer Research Institute, Genova

AIEOP - Italian Paediatric Haematology-Oncology Association

Under the patronage of

Italian Senate
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ANR 2004

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ITALIAN NAVY