Dear all,

We are so happy that you all finally made it here, to ANR 2010, to Scandinavia, and most of all, to our beautiful capital of Sweden, Stockholm!

We are excited to have received more than 400 abstracts which is the basis of our scientific programme. We are therefore confident that this year’s programme will satisfy every single participant, being a researcher, a clinician, a nurse, a student or a parent. Numerous sessions are prepared, spanning all aspects of neuroblastoma from bench to bedside.

We are thrilled to have such excellent invited lecturers including four Nobel laureates visiting us, together with several future ones, we are sure!

We are grateful to our sponsors and all others who have contributed to make the meeting possible.

Please enjoy the multitude of oral and poster presentations. Discuss and interact! Enjoy the social programme, explore the light summer in the beautiful city of Stockholm. After the meeting you should go home exhausted with important experiences, new ideas and friends.

On behalf of the local organising committee,

Per Kogner

CONTENTS

| General Information | 2 |
| Programme | Abstract Book |
| Monday June 21\textsuperscript{st} | Contents Abstract Book | 7 |
| Tuesday June 22\textsuperscript{nd} | Author Index | 14 |
| Poster session | | 29 |
| Wednesday June 23\textsuperscript{rd} | Keyword Index | 53 |
| Poster session | | 29 |
| Thursday June 24\textsuperscript{th} | | 70 |
Committees*

ANRA, Advances in Neuroblastoma Research Association
ANRA President: Susan L. Cohn
ANRA Past President: Frank Berthold
ANRA Incoming President: Michelle Haber

ANRA Steering Committee
Frank Berthold
Garrett M. Brodeur
Susan L. Cohn
Audrey E. Evans
Michelle Haber
Per Kogner
Akira Nakagawara
Patrick C. Reynolds
Manfred Schwab
Frank Speleman
Carol J. Thiele

ANRA Advisory Board (North/South America)
Rochelle Bagatell
Sylvain Baruchel
Garrett M. Brodeur
Susan L. Cohn
Michael Hogarty
Wendy London
John M. Maris
Katherine K. Matthay
Julie Park
Patrick C. Reynolds
Carol J. Thiele
Darell Yamashiro

ANRA Advisory Board (Russia/Europe)
Klaus Beiske
Bruno De Bernardi
Huib Caron
Angelika Eggert
Ruth Ladenstein
Geneviève Laureys
Jean Michon
Andrew Pearson
Gudrun Schleiermacher
Dominique Valteau-Couanet
Rogier Versteeg
Frank Westermann

ANRA Advisory Board (Asia/Australia/Africa)
Chan C.F. Godfrey
Michelle Haber
Hitoshi Ikeda
Kenji Kadomatsu
Purna Kurkure
Glenn Marshall
Akira Nakagawara
Murray D. Norris
Nili Peylan-Ramu
Cai Weisong

ANR2010 Local Organising Committee
President: Per Kogner
Nibb Baryawno
Birgitta de Verdier
Ann-Mari Dumanski
Lotta Elfman
Helena Gleissman
John Inge Johnsen
Catharina Karlsson
Lova Segerström
Malin Wickström

ANR2010 Scientific Committee
Per Kogner
Marie Arsenian Henriksson
John Inge Johnsen
Bertil Kågedal
Tommy Martinsson
Sven Pålman
Ingrid Öra

ANR2010 Nurses Committee
Lisa Burström
Karin Enskår
Pernilla Pergert
Eva Turup

* as before election spring 2010.
Please see www.anrmeeting.org for updates and contact details.
Acknowledgements and Sponsors

The Advances in Neuroblastoma Research Conference would like to thank the following sponsors for their generous support and contributions

Co-organisers and main contributors
Swedish Childhood Cancer Foundation/Barncancerfonden
Karolinska Institutet
ANRA
Swedish Cancer Society/Cancerfonden
Swedish Institute/Svenska Institutet

Silver sponsorship
Mary Béve Foundation

Bronze Sponsorship
Pierre Fabre Oncology
Visual Sonics

Exhibiting Organisations

The Advances in Neuroblastoma Research Conference would like to thank the following exhibiting organisations for their contribution and participation. Exhibiting organisations are listed in alphabetical order.

ACGT Advancing Clinico Genomic Trials on Cancer
ANR2012
Covidien
Hospira
Pierre Fabre Oncology
Ridgeview Instruments AB
Swedish Childhood Cancer Foundation/Barncancerfonden
Visual Sonics

Additional contributors
Amgen Oncology
Medac
Scandinavian Airlines SAS
The Vasa Museum
Waldemarsudde

Social Programme

The Social Programme is open to all registered participants and accompanying persons, and must be pre-booked. Additional tickets may be booked and/or purchased at the Registration desk, should space be available.

Welcome Programme (Welcome to Stockholm and Welcome to Sweden)
The Welcome Programme will take place at the Stockholm City Conference Centre Monday June 21st at 16:30. The programme includes an introduction to Stockholm, Karolinska Institutet and the Nobel Prize, followed by a lecture by the Swedish Nobel Laureate, Professor Bengt Samuelsson. This will be followed by traditional Swedish music and performances by various artists, and you will get to know Sweden culturally and historically. The Welcome Programme is followed by a Welcome Reception at the Stockholm City Hall. The Welcome Programme is open to all participants and registered accompanying guests.

Welcome Reception at Stockholm City Hall
The Welcome Reception, Monday June 21st, at 19:00, is hosted by the City of Stockholm and the County Council at Stockholm City Hall, the venue for the Nobel Prize dinner. A buffet dinner will be served followed by a guided tour of the building.

Please note: The reception is open to participants and registered accompanying guests, only if marked on the registration form. Please bring your ticket.
Opening Ceremony
All participants and registered accompanying guest are invited to attend the Opening Ceremony on June 22nd at 08:00 in the Stockholm City Conference Centre where ANR 2010 officially will be opened.

Archipelago Boat Tour
The Archipelago Boat Tour, Tuesday June 22nd, will depart from, and return to Nybrokajen, in Stockholm. The duration of the Tour is approximately three and a half hours and you will be given the opportunity to see some of the most beautiful scenery of Stockholm. A light dinner, including a glass of wine or non-alcoholic beverage and coffee, will be served on-board. A cash bar will be open, at own expense, during the Tour.

Please note: The Archipelago Boat Tour is open for participants and registered accompanying guests. The boat will depart at 19:00 sharp. Please bring your ticket. A light jacket and/or scarf is recommended.

Gala Dinner
The Gala Dinner will take place at Solliden, a classic restaurant with beautiful decor and a stunning view of Stockholm and south inlet heights. The Gala Dinner will be held on Wednesday June 23rd, at 19:00 and buses will depart from City Conference Centre/Norra Bantorget from 18:45. Buses will return to Norra Bantorget from 22:45.

Solliden is located at Skansen, Sweden's largest outdoor museum, where there is always something happening. Every season has its own charm. Summer and winter, every day, all year round, there are lots to see and experience. Skansen is known for its wild animals and culture and out-door exhibitions of Sweden's cultural heritage. It is also the stage from where entertainment programmes are often shown live on TV.

Registered Accompanying Guests
The registration fee for accompanying guests includes admission to the Welcome Programme at the City Conference Centre and Welcome Reception at the City Hall on Monday June 21st, as well as the Opening Ceremony on Tuesday June 22nd. Registered accompanying guests are also invited to listen to the lectures “The Road to Stockholm and Beyond” 1 and 2 in conjunction with the above mentioned events. In addition it gives accompanying guests the possibility to attend the Archipelago Boat Tour and the Gala Dinner for an additional fee.

Please note that the fee for accompanying guests does not include admission to the scientific sessions, the commercial- or the poster exhibitions nor does it entitle them to any Conference documentation or meals during breaks.

Exhibitor Registration Fee
The registration fee for exhibitors includes daily tea/coffee and lunch as well as the Welcome Reception at Stockholm City Hall.

Please note that the fee for exhibitors and sponsors does not include admission to the scientific sessions.

General Information
Badges
Each participant will receive a name badge upon registration at the Conference venue. For security reasons all participants are requested to wear their badge during all the Conference activities and social events. The cost for replacing a lost badge is SEK 100.

Banks, Credit Cards and Currency Exchange
Banks are open between 09:30/10:00 and 15:00 on weekdays. Some banks in central Stockholm are open from 09:00 to 17:00. Major credit cards are accepted in hotels, restaurants, taxis and shops. It is advisable to carry an identity card or some form of photo identification.

The official currency is Swedish Krona (SEK). USD 1 = SEK 7.75, EUR 1 = SEK 10.00 (May, 2010). For money exchange, the companies Forex and X-change have offices at the airports and in the city. Opening hours and other information about exchange can be found at www.forex.se and www.x-change.se.

Certificate of Attendance
A certificate of attendance will be given to registered participants upon request.
Climate and Dress
The weather in Stockholm at this time of the year is usually warm and sunny with temperatures of approximately 15–20 degrees Celsius; showers may occur. Informal dress is recommended for the Conference sessions and smart casual is recommended for the Welcome Reception and the Gala Dinner. A light jacket and/or scarf is recommended for the Archipelago Boat Tour.

Commercial Exhibition
A commercial exhibition will be arranged in the Conference Hall Foyer in conjunction with the Conference. Please see the list of exhibitors and further details on the cover.

Disclaimer/Liability
The Organising Committee and Congrex Sweden AB accept no liability for any injuries/losses incurred by participants and/or accompanying persons, nor loss of, or damage to, any luggage and/or personal belongings.

Electricity
Electrical current in Sweden is 220 V/50 Hz. Round, European-style two-pin plugs are used. Appliances designed to operate on 110/120 Volts need a voltage converter and a plug adapter.

European Green Capital
Stockholm is the first city to be awarded European Green Capital, 2010, by the European Commission. The award marks a city's wish and capability to solve environmental problems in order to both improve the quality of life of its citizens and reduce the impact it makes to the global environment as a whole. Everything from water protection plans, to public transport and work to omit fossil fuel emissions was reviewed. In all aspects, Stockholm is considered a front-runner.

Internet
High-speed wireless Internet is available at no charge in the Conference venue.

Language
The official language of the Conference is English. No simultaneous translation will be provided.

Meals
Daily tea/coffee and lunches are included in the registration fee.

Official Airline
SAS is proud to be the Official Airline for the Advances in Neuroblastoma Research meeting – ANR 2010 and welcomes you to Stockholm.

Professional Congress Organiser
Congrex Sweden AB is the official organiser of the Advances in Neuroblastoma Research Congress – ANR 2010. Congrex Sweden AB is a leading international management company offering comprehensive services for meetings, events, conferences, association management, travel and accommodation. Let’s meet, visit www.congrex.com

On-site Registration at the City Conference Centre
On-site registration will start Sunday June 20th, at 15:00. The registration desk and Conference secretariat will be open for the duration of the Conference.

Time Zone
The time zone in Stockholm is GMT + 1 hour. Daylight Saving Time is used during the summer.

Tipping
A gratuity is included in the price of hotels and taxis. It is common, however, to leave a tip of around 10%. When visiting restaurants, you can show your appreciation for good service by leaving a little extra.

Tourist Information
The Conference secretariat will be available to give you more information about Stockholm. For additional information please contact the official tourist guide of Stockholm at:

Web site: www.stockholmtown.se
Visiting address: Sverigehuset, Hamngatan 27
E-mail: info@svb.stockholm.se
Phone: +46 8 508 28 508
Scientific Information

Poster Sessions
All posters will be displayed for the duration of the Conference. Special time is set aside for poster viewing only, and presenting authors of the abstracts in focus are asked to stand by their posters at this time.

Time for mounting and dismantling
Mounting: June 22\textsuperscript{nd}, 10:15 – 12:00
Dismantling: June 24\textsuperscript{th}, 13:00

Poster discussion time with presenting author present by their poster board:
June 22\textsuperscript{nd} 16:45 – 17:30 (odd numbered/left aligned posters, see page 29)
June 23\textsuperscript{rd} 16:45 – 17:30 (even numbered/indented posters, see page 29)

Selected Posters
In addition to the oral presentation of selected posters, all selected posters will be displayed throughout the Conference. Selected posters will be displayed at the “Mezzanine level” on the day of the lecture, and in the main poster area the other days.

Selected posters 1–24 will be displayed at the “Mezzanine level” on June 22\textsuperscript{nd} and selected posters 25–48 on June 23\textsuperscript{rd}. Therefore, a switch of posters from the “Mezzanine level” to the main poster area needs to be done after the poster session on June 22\textsuperscript{nd}. We ask all authors for their kind assistance to make this switch run smoothly.

Detailed instructions for mounting/dismantling Biology 1, Clinical 1 and Translational 1 (selected posters 1–24):
Mounting: June 22\textsuperscript{nd}, 10:15 – 12:00 on Mezzanine level
Dismantling: June 22\textsuperscript{nd}, 18.30 (posters can be stored at the venue over night)
Mounting: June 23\textsuperscript{rd}, 07:30 – 10:00 in main poster area
Dismantling: June 24\textsuperscript{th}, 13:00

Detailed instructions for mounting/dismantling Biology 2, Biology 3 and Translational 2 (selected posters 25–48):
Mounting: June 22\textsuperscript{nd}, 09:00 – 10:15 in main poster area
Dismantling: June 22\textsuperscript{nd}, 18.30 (posters can be stored at the venue over night)
Mounting: June 23\textsuperscript{rd}, 07:30 – 10:00 on Mezzanine level
Dismantling: June 24\textsuperscript{th}, 13:00

Selected poster discussion time (presenting author present by their poster board):
Authors to selected posters SEL1–24, presented in oral session on June 22\textsuperscript{nd} should be present by their poster
June 22\textsuperscript{nd} 16:45 – 17:30 (Mezzanine level)
Authors to selected posters SEL25–48, presented in oral session on June 23\textsuperscript{rd} should be present by their poster
June 23\textsuperscript{rd} 16:45 – 17:30 (Mezzanine level)

Speakers Preview room and AV information
The Speakers Preview room is located back stage of the Conference Halls and will open one hour before the first session each day and stay open throughout the day.

Speakers are requested to use this facility before their session to ensure that their slides project clearly and are in correct order. Slides should then be handed over to the technical staff no later than three hours before the start of that particular session.

Awards
Awards for oral presentations
The Conference Organisers offered abstract presenters to apply for an award. Among these applicants, the abstracts with best rating were selected for a second round of rating by unbiased experts and a final set of candidates were selected. After final assessments of the oral presentations during the conference, awardees will be appointed and honored at the Gala Dinner, June 23\textsuperscript{rd} (Biology and Translational award winners) and the Closing and Award Session June 24\textsuperscript{th} (Clinical award winners).

Poster Prizes
Two kinds of poster prizes will be awarded. One set of awardees will be selected by three different committees (Biology, Translational and Clinical). Another set of awardees will be selected by all participants by using the voting form enclosed in your conference bag. All votes must be returned to the registration desk no later than Thursday lunch, 13:00. All poster prizes will be presented during the Closing and Award Session on Thursday June 24\textsuperscript{th}.
# Neuroblastoma Update Course

**Monday June 21**

**09:00 – 16:00 Monday June 21**

**Hall A**

**Neuroblastoma Update Course**

**Organisers:** Susan L. Cohn and Andrew Pearson

**Chairs:** Susan L. Cohn and Rani George

## Neuroblastoma biology

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00</td>
<td>C1 Using genome-wide strategies to discover new gene aberrations</td>
<td>Frank Speleman, Belgium</td>
<td>78</td>
</tr>
<tr>
<td>09:25</td>
<td>C2 Biological and clinical relevance of ALK mutations</td>
<td>Rani George and Yaël Mosse, United States</td>
<td>78</td>
</tr>
</tbody>
</table>

## Risk classification

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:05</td>
<td>C3 INRG: Next steps</td>
<td>Andy Pearson, United States</td>
<td>78</td>
</tr>
</tbody>
</table>

## Imaging

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:50</td>
<td>C4 Using PET and MIBG to evaluate disease and response</td>
<td>Sue Sharp, United States</td>
<td>78</td>
</tr>
</tbody>
</table>

## Minimally invasive surgery

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>11:15</td>
<td>C5 Is there a role for laparoscopic surgery?</td>
<td>Jed Nuctern, United States</td>
<td>78</td>
</tr>
</tbody>
</table>

## Low and intermediate-risk disease

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>11:40</td>
<td>C6 Therapy and segmental aberrations for treatment stratification</td>
<td>Gudrun Schleiermacher, France</td>
<td>79</td>
</tr>
<tr>
<td>12:05</td>
<td>C7 When should we use a “wait and see” approach</td>
<td>Frank Berthold, Germany</td>
<td>79</td>
</tr>
</tbody>
</table>

## High-risk disease

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>13:30</td>
<td>C8 Immunotherapy plus retinoic acid: A new standard of care</td>
<td>Alice Yu, United States</td>
<td>79</td>
</tr>
</tbody>
</table>

## MIBG radiotherapy

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>13:55</td>
<td>C9 Update on MIBG therapy</td>
<td>Kate Matthay, United States</td>
<td>79</td>
</tr>
</tbody>
</table>

## Late Effects

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:20</td>
<td>C10 Late effects in neuroblastoma</td>
<td>Lisa Diller, United States</td>
<td>80</td>
</tr>
</tbody>
</table>

## Relapsed Disease

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>15:05</td>
<td>C11 Factors that predict outcome in relapsed disease</td>
<td>Wendy London, United States</td>
<td>80</td>
</tr>
</tbody>
</table>

## Novel Treatments

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>15:30</td>
<td>C12 Molecularly targeted therapy</td>
<td>Louis Chesler, United Kingdom</td>
<td>80</td>
</tr>
</tbody>
</table>

## Closing

Made possible thanks to a special grant from Mary Béve Foundation.
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Organiser(s)</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:30</td>
<td>WS1</td>
<td>Tumor initiating/stem cells, hypoxia and vascularization - what are the connections?</td>
<td>Sven Påhlman, Sweden</td>
<td>Hall B/C</td>
</tr>
<tr>
<td>09:50</td>
<td>WS2</td>
<td>Development of the autonomic nervous system, a molecular view</td>
<td>Herman Rohrer, Germany</td>
<td>Hall B/C</td>
</tr>
<tr>
<td>10:10</td>
<td>WS3</td>
<td>Tumor initiating cells from bone marrow of high-stage neuroblastoma patients</td>
<td>David Kaplan, Canada</td>
<td>Hall B/C</td>
</tr>
<tr>
<td>10:30</td>
<td>BREAK</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:45</td>
<td>WS4</td>
<td>Tumor initiating cells from MYCN amplified neuroblastomas</td>
<td>Jan Molenaar, Netherlands</td>
<td>Hall B/C</td>
</tr>
<tr>
<td>10:55</td>
<td>WS5</td>
<td>The miRNAome of TICs in relation to tumor cells and fetal neuroblasts</td>
<td>Katleen de Preter, Belgium</td>
<td>Hall B/C</td>
</tr>
<tr>
<td>11:05</td>
<td>WS6</td>
<td>Identification and molecular characterization of human neuroblastoma tumor-initiating cells</td>
<td>Aurelie Coulon, Switzerland</td>
<td>Hall B/C</td>
</tr>
<tr>
<td>11:15</td>
<td>WS7</td>
<td>Vascular mimicry in human neuroblastoma: identification of the progenitors of tumor derived endothelial cells</td>
<td>Vito Pistoia, Italy</td>
<td>Hall B/C</td>
</tr>
<tr>
<td>11:25</td>
<td>WS8</td>
<td>Identification <em>in vitro</em> and <em>in vivo</em> of tumoral glial precursor cells in neuroblastic tumors</td>
<td>Jaume Mora, Spain</td>
<td>Hall B/C</td>
</tr>
<tr>
<td>11:35</td>
<td>WS9</td>
<td>Exploiting the embryonic environment to reprogram cancer stem cells in neuroblastoma</td>
<td>Rachel Carter, United Kingdom</td>
<td>Hall B/C</td>
</tr>
<tr>
<td>11:45</td>
<td>WS10</td>
<td>Low Dose Metronomic anti-angiogenic (LDM) oral Topotecan and Pazopanib as a potential model for maintenance therapy neuroblastoma</td>
<td>Sylvain Baruchel, Canada</td>
<td>Hall B/C</td>
</tr>
</tbody>
</table>
| 11:55 | Discussion | – Phenotype of NB initiating/stem cells  
  – Primary vs cell line TICs/SCs  
  – Stem cell niche and angiogenesis  
  – NB TIC/SC location - similar cells in primary tumors and in the bone marrow?  
  – Number of NB TICs/SCs at a given site of prognostic significance?  
  – Targeting of TICs/SCs |
| 12:25 | Concluding remarks | David Kaplan and Sven Påhlman                                        |                                      |                     |
| 12:30 | LUNCH   |                                                                      |                                       |                     |
### Workshop 2 – Contribution of microRNAs to neuroblastoma pathogenesis
Organisers and chairs: Marie Arsenian Henriksson and Angelika Eggert

<table>
<thead>
<tr>
<th>Time</th>
<th>Session Title</th>
<th>Presenter and Location</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>13:00</td>
<td><strong>WS11</strong> The neuroblastoma miRNA map, prioritization and functional evaluation of candidate miRNAs</td>
<td>Pieter Mestdagh, Belgium</td>
<td>83</td>
</tr>
<tr>
<td>13:30</td>
<td><strong>WS12</strong> miRNAs regulating neuroblastoma cell differentiation</td>
<td>Ray Stallings, Ireland</td>
<td>83</td>
</tr>
<tr>
<td>13:50</td>
<td><strong>WS13</strong> MYCN-regulated microRNAs repress estrogen receptor-α (ESR1) expression and neuronal differentiation in human neuroblastoma</td>
<td>Marie Arsenian Henriksson, Sweden</td>
<td>83</td>
</tr>
<tr>
<td>14:05</td>
<td><strong>BREAK</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:25</td>
<td><strong>WS14</strong> Assessing the role of miRNAs in neuroblastoma biology – from expression profiling to functional analysis</td>
<td>Angelika Eggert, Germany</td>
<td>84</td>
</tr>
<tr>
<td>14:40</td>
<td><strong>WS15</strong> Bioinformatic analysis of miRNA profiles of a series of neuroblastomas by the R2</td>
<td>Rogier Versteeg, Netherlands</td>
<td>84</td>
</tr>
<tr>
<td>15:00</td>
<td><strong>WS16</strong> Genomic and proteomic study of microRNAs in pediatric cancers</td>
<td>Jun Wei, United States</td>
<td>84</td>
</tr>
<tr>
<td>15:20</td>
<td><strong>Concluding remarks</strong></td>
<td>Angelika Eggert and Marie Arsenian Henriksson</td>
<td></td>
</tr>
</tbody>
</table>
15:30 – 16:00  Monday June 21st
Hall B/C
Special session – Public web-based analysis tool R2
Chairs: Marie Arsenian Henriksson and Angelika Eggert

SS1  Introduction to practical use of an analysis tool and database for high throughput and clinical data of neuroblastoma series
Rogier Versteeg, Netherlands

16:00 – 16:30  BREAK
PL1  The role of eicosanoids in health and disease
Bengt Samuelsson, Sweden

Dr. Bengt Samuelsson is Professor of Physiological Chemistry at the Karolinska Institute. Dr. Samuelsson’s research led to the discovery of various prostaglandins and related substances. Of particular interest are the thromboxanes which are involved in such common, severe thrombotic diseases as strokes and coronary infarcts. He also discovered the leukotrienes, substances that play a role in inflammation and asthma and other allergic diseases. For his discovery of prostanoids and leukotrienes he was awarded the Nobel Prize in Physiology or Medicine in 1982.

Dr. Samuelsson obtained his Doctor of Medical Science degree in biochemistry and later, his M.D. degree, from the Karolinska Institute. He spent a year as a research fellow in the Department of Chemistry at Harvard University, Cambridge, Mass., USA. In 1972, Dr. Samuelsson was appointed professor at the Karolinska Institute. In 1973–1983, he was Chairman of the Department of Chemistry; in 1978–1983, Dean of the Medical Faculty and in 1983–1995, President of the Karolinska Institute.

Dr. Samuelsson has been a member of the Nobel Assembly and the Nobel Committee for Physiology or Medicine at the Karolinska Institute and in 1993–2005, he was Chairman of the Nobel Foundation in Stockholm.

In addition to the Nobel Prize, Dr. Samuelsson has received a number of worldwide awards and honorary academic degrees. These include the Louisa Gross Horwitz Award, the Gairdner Foundation Award and the Albert Lasker Basic Medical Research Award. He is a Foreign Associate of the US National Academy of Sciences and a Foreign Member of the Royal Society, London. He is a member of the Royal Swedish Academy of Sciences, the Royal National Academy of Medicine, Spain, the French Academy of Sciences and the Institute of Medicine, USA.

PL2  Fatty acids as positive and negative regulators in neuroblastoma development
Per Kogner, Sweden

Per Kogner, MD Karolinska Institutet 1985, Certified Pediatrician 1990, PhD Karolinska Institutet 1993, Postdoc Karolinska Institutet 1993-1994, Full Professor Karolinska Institutet 2005. Present position: Professor of Pediatric Oncology, Childhood Cancer Research Unit, Department of Women’s and Children’s Health, Karolinska Institutet, and Senior Consultant, Astrid Lindgren Children’s Hospital.
17:45 – 18:30  Monday June 21st
Hall A/B/C
Welcome to Sweden!

The programme includes traditional Swedish music and performance by various artists, and you will get to know Sweden culturally and historically.

Performers:
Ann-Marie Sundberg, birch-bark horn
Elin & Edward Anderzon, Swedish keyed fiddle
Ulrika Gunnarsson, Swedish traditional falsetto song
Skansen Folkdance Team
Children's Choir

The Welcome Programme is followed by a Welcome Reception in Stockholm City Hall.
## Programme

### Tuesday June 22nd

<table>
<thead>
<tr>
<th>Time</th>
<th>Hall A</th>
<th>Hall B</th>
<th>Hall C</th>
<th>Room 403</th>
<th>Poster Halls</th>
<th>Reg</th>
</tr>
</thead>
<tbody>
<tr>
<td>07:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Open</td>
</tr>
<tr>
<td>07:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>08:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>08:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>08:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>08:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>09:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>09:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>09:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>09:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Break</td>
<td></td>
</tr>
<tr>
<td>10:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Plenary Session 2 Biology [18]</td>
<td></td>
</tr>
<tr>
<td>11:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lunch</td>
<td></td>
</tr>
<tr>
<td>12:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Parallel 1 Targeting kinases [19]</td>
<td></td>
</tr>
<tr>
<td>13:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Special Clinical Session – Strategies for Progress [20]</td>
<td></td>
</tr>
<tr>
<td>13:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Parallel 2 Tumour initiating stem cells [21]</td>
<td></td>
</tr>
<tr>
<td>13:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Open</td>
</tr>
<tr>
<td>14:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Break</td>
<td></td>
</tr>
<tr>
<td>14:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Parallel 3 Immunotherapy [22]</td>
<td></td>
</tr>
<tr>
<td>15:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Workshop 3 Genome Sequencing [23]</td>
<td></td>
</tr>
<tr>
<td>15:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Parallel 4 p53 and molecular targets [24]</td>
<td></td>
</tr>
<tr>
<td>15:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ANR Nurses Special Session [25]</td>
<td></td>
</tr>
<tr>
<td>15:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Selected Posters</td>
<td></td>
</tr>
<tr>
<td>16:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Biology 1 [24]</td>
<td></td>
</tr>
<tr>
<td>16:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Clinical [27]</td>
<td></td>
</tr>
<tr>
<td>16:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Translational 1 [24]</td>
<td></td>
</tr>
<tr>
<td>17:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ANRA Advisory Board Meeting*</td>
<td></td>
</tr>
<tr>
<td>18:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Concluding Remarks*</td>
<td></td>
</tr>
<tr>
<td>18:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Free Poster Session</td>
<td></td>
</tr>
<tr>
<td>18:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Free evening, arrangements possible</td>
<td></td>
</tr>
<tr>
<td>19:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Archipelago Boat Tour for pre-registered participants</td>
<td></td>
</tr>
<tr>
<td>19:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Boat from Nybrokajen 19:00 sharp</td>
<td></td>
</tr>
<tr>
<td>20:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bus departure from STOCC not arranged</td>
<td></td>
</tr>
</tbody>
</table>

* By invitation only
Tuesday June 22nd

08:00 – 08:30  Tuesday June 22nd
Hall A/B/C
Opening Ceremony

Professor Per Kogner, ANR 2010 President
Professor Harriet Wallberg-Henriksson, President Karolinska Institutet
Professor Susan L. Cohn, President ANRA
Professor Olle Björk, General Secretary, Swedish Childhood Cancer Foundation
Mrs Filippa Reinfeldt, Health Care Commissioner, Stockholm County Council
Elizabeth Blackburn, Ph.D. is the recipient of the 2009 Nobel Prize in Physiology or Medicine for her discoveries in telomere biology that have uncovered a new understanding of normal cell functioning and given rise to a growing field of inquiry. Throughout her distinguished career, whether as the editor of high-profile scientific journals, such as Molecular Cancer Research and Molecular Biology of the Cell, or as a current member of over 30 distinct institutional advisory boards or review committees, Dr. Elizabeth Blackburn has spent countless hours in service to her constituency. Further, she has held leadership positions in several scientific societies, including her current appointment as President of the American Association for Cancer Research.

Dr. Blackburn has been recognized for her seminal contribution to the field of telomere biology with numerous prizes, awards, and honorary degrees, including the 2006 Albert Lasker Award for Basic Medical Research and elections to the American Association for the Advancement of Science and the Institute of Medicine. In 2007, Time magazine named her one of the ‘100 Most Influential People in the World,’ and in 2008 she was the North American Laureate for the L'Oréal-UNESCO For Women In Science. The scientific community bestowed upon her the ultimate recognition of her legacy by honoring Dr. Elizabeth Blackburn with the 2009 Nobel Prize in Physiology or Medicine.

Dr. Blackburn is currently the Morris Herzstein Endowed Chair in Biology and Physiology in the Department of Biochemistry and Biophysics at the University of California, San Francisco. She is also a Non-Resident Fellow of the Salk Institute.

Dr. John Maris is a tenured Professor in the Department of Pediatrics at the University of Pennsylvania. He is a physician-scientist who has developed a translational research program broaching the basic genetic mechanisms of childhood cancer initiation to pivotal clinical trials for these same diseases. He currently serves as Chief of the Division of Oncology at the Children's Hospital of Philadelphia (CHOP) and Director of the Center for Childhood Cancer research, also housed at CHOP. He is also Chair of the Children's Oncology Group Neuroblastoma Committee and Director of the Pediatric Oncology Program in the Abramson Cancer Center at Penn. Dr. Maris’ major research contributions involve providing key insights into the genetic basis of neuroblastoma initiation and progression.
09:30 – 10:15  Tuesday June 22nd
Hall A/B/C
Plenary session 1 – Biology
Chairs: Frank Speleman and Susan L. Cohn

09:30  PL5  Copy number variations (CNVs) in neuroblastoma
Sharon J. Diskin; Kristopher Bosse; Patrick Mayes; Michael LaQuaglia; Edward F. Attiyeh; Yael
P. Mosse; Marci Laudenslager; Maura Diamond; Geoffrey Norris; Cuiping Hou; Kai Wang; Haito
Zhang; Cecilia Kim; Wendy London; Marcella Devoto; Hongzhe Li; Hakon Hakonarson; John M.
Maris
United States

09:45  PL6  A potential role for the pluripotency factor LIN28B and Let-7 signalling in
neuroblastoma
Molenaar, Jan1; Ebus, Marli1; Koster, Jan1; Sluis van, Peter1; Schulte, Johannes2; Egger, Angelika3;
Mestdagh, Pieter4; Sompele van de, Jo5; Speleman, Frank5; Caron, Huib5; Versteeg, Rogier6;
1University of Amsterdam, Human Genetics, Amsterdam, Netherlands; 2University Childrens
Hospital Essen, Pediatric oncology, Essen, Germany; 3Gent University Hospital, CMGG, Gent,
Belgium; 4University of Amsterdam, Pediatric Oncology, Amsterdam, Netherlands

10:00  PL7  CAMTA1, a 1p36 tumor suppressor candidate, activates differentiation
programmes in neuroblastoma cells
Kai-Oliver Henrich; Tobias Bauer; Volker Ehemann; Hedwig Deubzer; Sina Gogolin; Matthias
Fischer; Manfred Schwab; Frank Westermann
Germany

10:15 – 10:45  BREAK
10:45 – 12:00  Tuesday June 22nd
Hall A/B/C
Plenary session 2 – Biology
Chairs: Akira Nakagawara and Michelle Haber

10:45  PL8  Whole genome and transcriptome sequencing of ten stage IV primary neuroblastoma tumors: a TARGET project report
Olena Morozova 1; Edward F. Attiyeh 2; Ryan D. Morin 1; Martin Hirst 1; Timothee Cezard 1; Richard Moore 1; Cecelia Suragh 1; Nina Thiessen 1; Richard Varhol 1; Yongjun Zhao 1; Michael D. Hogarty 2; Shahab Asgharzadeh 2; Daniela S. Gerhard 2; Malcolm A. Smith 2; Javed Khan 2; Robert C. Seeger 2; John M. Maris 2; Marco A. Marra 1
1Canada; 2United States

11:00  PL9  Role of a novel inducible dependence receptor UNC5D in spontaneous regression of neuroblastoma: its functional cooperation with p53 and E2f1 for inducing programmed cell death
Yuyan Zhu; Yuanyuan Li; Seiki Haraguchi; Meng Yu; Miki Ohira; Atsuko Nakagawa; Eriko Isogai; Haruhiko Koseki; Yohko Nakamura; Hirofumi Arakawa; Akira Nakagawara
Japan

11:15  PL10  Metastatic neuroblastoma cancer stem cells display a mixed phenotype of tumor and niche origin required for survival
Loen Hansford 1; Olena Morozova 1; Tatiana Lipman 1; Kim Blakely 1; Miki Ohira 2; Paula Marrano 1; Paola Angelini 1; Jason Moffat 1; Carol Thiele 3; Paul Thorner 1; John Dick 1; Akira Nakagawara 2; Meredith Irwin 1; Marco Marra 1; David Kaplan 1
1Canada; 2Japan; 3United States

11:30  PL11  Cell-cell communication via ion fluxes in control of neuroblastoma cell cycle
Hiromi Hiyoshi 1; Shaimaa Abdelhady 1; Baldur Sveinbjörnsson 1; Lova Segerström 1; Loen Hansford 2; Meredith Irwin 2; David Kaplan 2; Per Kogner 1; John Inge Johnsen 1; Michael Andäng 1; Per Uhlén 1
1Sweden; 2Canada

11:45  PL12  PHOX2B is essential for peripheral sympathetic neuronal differentiation in the zebrafish
William Luther II; Rodney Stewart; John Kanki; A. Thomas Look; Rani E. George
United States

12:00 – 13:00 LUNCH
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Authors and Institutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>13:00 – 14:10</td>
<td>OR1</td>
<td>The KidsCancerKinome: Validation of Aurora kinases as potential drug targets in neuroblastoma and other pediatric tumors</td>
<td>Ellen Westerhout¹; Marcel Kool¹; Jan Molenaar¹; Peter Stroeken¹; Monique den Boer¹; Stephanie Segers¹; Steven Clifford²; Olivier Delattre³; Magdalena Benetkiewicz³; Claudia Lanvers⁴; Ron Pieters¹; Torsten Pietsch⁴; Marcel Holst⁴; Jane Renshaw²; Janet Shipley²; Massimo Serra⁶; Katie Scotlandi⁷; Birgit Geoerger⁷; Gilles Vassal³; Olivier Degrand³; Arnaud Verschuur¹; Rogier Versteeg¹; Huib Caron¹ ¹Netherlands; ²United Kingdom; ³France; ⁴Germany; ⁵Italy</td>
</tr>
<tr>
<td>13:10</td>
<td>OR2</td>
<td>Inhibition of Aurora-A as an approach to control N-Myc levels in neuroblastoma</td>
<td>Markus Brockmann¹; Louis Chesier²; Martin Eilers¹ ¹Germany; ²United Kingdom</td>
</tr>
<tr>
<td>13:20</td>
<td>OR3</td>
<td>Molecular analysis and therapeutic targeting of the PI3K/AKT/mTOR pathway in paediatric neuroblastoma</td>
<td>Paul Wood; David Ashley; Carleen Cullinane; Kathryn Kinross; Gretchen Poortinga; Kerry Ardley; Grant McArthur Australia</td>
</tr>
<tr>
<td>13:30</td>
<td>OR4</td>
<td>PI3K inhibitors prime neuroblastoma cells for chemotherapy in vitro and in vivo by shifting the balance towards pro-apoptotic Bcl-2 proteins and increased mitochondrial apoptosis</td>
<td>Ariane Bender¹; Daniela Opel¹; Ivonne Naumann¹; Roland Kappler¹; Lori Friedman²; Klaus-Michael Debatin¹; Simone Fulda¹ ¹Germany; ²United States</td>
</tr>
<tr>
<td>13:40</td>
<td>OR5</td>
<td>PLK1 is a novel target for high-risk neuroblastoma therapy</td>
<td>Sandra Ackermann; Felix Goeser; Johannes Schulte; Volker Ehemann; Angelika Eggert; Barbara Hero; Frank Berthold; Matthias Fischer Germany</td>
</tr>
<tr>
<td>13:50</td>
<td>OR6</td>
<td>MicroRNA-184 inhibits neuroblastoma cell proliferation and promotes apoptosis by targeting the serine/threonine kinase AKT2</td>
<td>Niamh H Foley; Isabella Bray; Derek M Murphy; Jacqueline Ryan; Amanda Tivnan; Patrick Buckley; Stallings Ray Ireland</td>
</tr>
<tr>
<td>14:00</td>
<td>OR7</td>
<td>Exploring a new therapy for neuroblastoma: silencing of doublecortin-like kinase using RNA-interference</td>
<td>Carla S. Verissimo; Jan J. Molenaar; John Meerman; Jordi C. Puigvert; Fieke Lamers; Maarten Rotman; Petra van Kulk-Romeijn; Erik H.J. Danen; Bob van de Water; Rogier Versteeg; Carlos P. Fitzsimons; Erno Vreugdenhil Netherlands</td>
</tr>
</tbody>
</table>

**13:00 – 14:10 Tuesday June 22nd**
**Hall A**
**Parallel session 1 – Targeting kinases**
**Chairs: Huib Caron and Pat Reynolds**

---

Page 106

Page 106

Page 106

Page 107

Page 107

Page 107

Page 107
13:00 – 14:10  Tuesday June 22\textsuperscript{nd}
Hall B
Special Clinical Session – Strategies for Progress
Chairs: Kate Matthay and Frank Berthold

SS2  Current and future strategies to improve outcomes in neuroblastoma: An update from the Children’s Oncology Group Neuroblastoma Disease Committee
John Maris, United States

SS3  Strategies to improve outcome and quality of life in patients with neuroblastoma: Activities of the SIOP European Neuroblastoma Group
Ruth Ladenstein, Austria

SS4  Recent achievements and future strategies of GPOH to improve outcome for children with neuroblastoma
Thorsten Simon, Germany

Invited discussants

This session focuses on current and future strategies for clinical studies with special emphasis on areas of priority for international collaboration. The session intends to be interactive, chaired by two experienced experts, including three main speakers, representing three collaborative clinical groups, COG, SIOPEN and GPOH. There will be invited discussants and an active auditorium is expected.

14:10 – 14:30  BREAK
13:00 – 14:10 Tuesday June 22nd
Hall C
Parallel session 2 – Tumour initiating stem cells
Chairs: Sylvain Baruchel and Johannes Schulte

13:00  OR8  HIF-2α maintains an undifferentiated state in neural crest-like human neuroblastoma tumor-initiating cells
Alexander Pietras 1; Loen M. Hansford 2; A. Sofie Johnsson 1; Esther Bridges 1; Jonas Sjölund 1; David Gisselsson 1; Matilda Rehn 1; Siv Beckman 3; Rosa Noguerà 4; Samuel Navarro 4; Jörg Cammenga 1; Erik Fredlund 1; David R. Kaplan 2; Sven Pählin 1
1Sweden; 2Canada; 3Switzerland; 4Spain

13:10  OR9  Identification of signaling pathways and drug candidates using primary neuroblastoma cancer stem cells by phosphoproteomics and transcriptome sequencing
Milijana Vojvodic; Olena Morozova; Kim Blakely; Natalie Grinshtein; Loen Hansford; Kristen Smith; Jiefie Tong; Paul Taylor; Meredith Irwin; Jason Moffat; Mike Moran; Marco Marra; David Kaplan
Canada

13:20  OR10  Induced stable neuroblastoma cancer stem cells
Naohiko Ikegaki; Paul Regan; Autumn Fox; Joshua Jacobs; Eric Rappaport; Xiao Tang
United States

13:30  OR11  Inhibition of global DNA methylation induces differentiation of human neuroblastoma tumor-initiating cells
A. Sofie Johnsson; Alexander Pietras; Caroline Wigerup; Sven Pahlman
Sweden

13:40  OR12  Exploiting the embryonic environment to reprogram cancer stem cells in neuroblastoma
Rachel Carter; Edwin Jesudason; Mike White; Violaine See; Paul Losty; Heather McDowell; Louis Chesler; Barry Pizer; Diana Moss
United Kingdom

13:50  OR13  Endocrine-gland vascular endothelial growth factor (EG-VEGF) in neuroblastoma tumor initiating cells
Elly Ngan 1; Cynthia Lau 1; Jana Woo 1; Wing-Keung Chan 1; Godfrey Chan 1; Yu Wang 1; David Kaplan 1; Paul Tam 1
1Hong Kong; 2Canada

14:00  OR14  Novel cardiac glycoside analogues selectively target neuroblastoma tumor initiating cells (TICs)
Paolo De Gouveia 1; Kristen Smith 1; Mayumi Fujitani 1; Murugesapillai Mylvaganam 1; Jamie La 1; Shaimaa Abdelhady 2; Michael Andang 2; David Uehling 1; Ahmed Mamai 1; Rima Al-awar 1; Clifford Lingwood 1; David R. Kaplan 1; Meredith S. Irwin 1
1Canada; 2Sweden

14:10 – 14:30 BREAK
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Authors</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:30</td>
<td>OR15</td>
<td>Anti-GD2 murine monoclonal antibody (MoAb) 3F8/Granulocyte-Macrophage colony stimulating factor (GM-CSF) plus 13-Cis-Retinoic acid (13-cis-RA) for consolidation of &gt;2nd complete remission/very good partial remission (CR/VGPR) of neuroblastoma</td>
<td>Brian Kushner; Kim Kramer; Shakeel Modak; Karima Yataghene; Nai-Kong Cheung</td>
<td>United States</td>
</tr>
<tr>
<td>14:40</td>
<td>OR16</td>
<td>Combined administration of in vitro expanded V_{9}V_{2+} T cells and bisphosphonate zoledronic acid as preclinical immunotherapeutic approach for neuroblastoma</td>
<td>Laura Emionite; Michele Cilli; Paola Bocca; Lizzia Raffaghello; Vito Pistoia; Ignazia Prigione</td>
<td>Italy</td>
</tr>
<tr>
<td>14:50</td>
<td>OR17</td>
<td>Galectin-1 modulates immune response towards a state of tolerance in neuroblastoma</td>
<td>Rocio Soldati 1; Elisa Berger 1; Ana C Zenclussen 1; Gerhard Jorch 1; Mariana Salatino 2; Gabriel A Rabinovich 1; Stefan Fest 1 1Germany; 2Argentina</td>
<td></td>
</tr>
<tr>
<td>15:00</td>
<td>OR18</td>
<td>Lenalidomide activates anti-tumor functions of NK cells and overcomes immune suppression by IL-6 and TGFβ1</td>
<td>Yibing Xu; Jianping Sun; Hong-Wei Wu; Michael Sheard; Hung Tran; Zesheng Wan; Cathy Liu; Robert Seeger</td>
<td>United States</td>
</tr>
<tr>
<td>15:10</td>
<td>OR19</td>
<td>Treatment of high risk neuroblastoma with autologous T lymphocytes engineered to recognize GD2</td>
<td>Chrystal Louis; Martin Pule; Barbara Savoldo; G Doug Myers; Claudia Rossig; Heidi Russell; Teresita Lopez; Gianpietro Dotti; Enli Liu; Hao Liu; Adrian Gee; Eric Yvon; Cliona Rooney; Helen Heslop; Malcolm Brenner</td>
<td>United States</td>
</tr>
<tr>
<td>15:20</td>
<td>OR20</td>
<td>A novel lentiviral-transduced dendritic cell vaccine targeting the survivin antigen is effective against neuroblastoma</td>
<td>Elisa Berger; Holger N Lode; Ana C Zenclussen; Gerhard Jorch; Stefan Fest</td>
<td>Germany</td>
</tr>
<tr>
<td>15:30</td>
<td>OR21</td>
<td>Immunotherapy for neuroblastoma by GD2 specific chimeric antigen receptor</td>
<td>John Anderson; Simon Thomas; Noureddine Himoudi; Martin Pule</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>15:40</td>
<td>OR22</td>
<td>NKT cells co-localize with tumor-associated macrophages in neuroblastoma in an innate response to tumor-induced hypoxia</td>
<td>Daofeng Liu; Liping Song; Jie Wei; Leonid Metelitsa</td>
<td>United States</td>
</tr>
<tr>
<td>15:50</td>
<td>OR23</td>
<td>Bone marrow response evaluation with a quantitative device identifies prognostic groups in patients over 18 months</td>
<td>Inge M. Ambros; Ulrike Pötschger; Andrea Ziegler; Ditha Modritz; Helmut Gadner; Ruth Ladenstein; Peter F. Ambros</td>
<td>Austria</td>
</tr>
</tbody>
</table>
14:30 – 16:00  Tuesday June 22\textsuperscript{nd}
Hall B

Workshop 3 – Next generation sequencing techniques in neuroblastoma genomics
Organisers: Ingrid Öra and Tommy Martinsson
Chairs: Javed Khan and Tommy Martinsson

14:30  WS17  Next generation sequencing technologies to investigate the cancer genome & Insights into neuroblastoma biology and tumor progression models I learnt from massively parallel sequencing strategies
Javed Khan, United States  

14:45  WS18  Next generation sequencing of the (small) RNA transcriptome - from catalogisation to quantitative expression profiling
Johannes H. Schulte, Germany  

15:00  WS19  Next-Generation Sequencing to characterize somatic alterations in neuroblastoma samples
Olivier Delattre, France  

15:15  WS20  The Neuroblastoma-TARGET project: Plans for sequencing, validation and frequency scans
John Maris, United States  

15:30  WS21  Finding variant needles in the neuroblastoma haystack: the Ghent NGS approach
Jo Vandesompele, Belgium  

15:45  Discussion
14:30 – 16:00  Tuesday June 22nd
Hall C
Parallel session 4 – p53 and molecular targets
Chairs: Naohiko Ikegaki and Glenn Marshall

14:30  OR24  Cooperative induction of apoptosis through p53 signaling and mTOR inhibition in neuroblastoma  
Eveline Barbieri, Zaowen Chen, Eugene Kim, Danielle Patterson, Denae Sikorski, Jason Shohet  
United States  

14:40  OR25  Repressed p53 stress responses in normal perinatal cells provides a susceptibility to N-Myc oncogenesis as an initiating event in embryonal malignancy  
Eric Sekyere, Wayne Thomas, Hongjuan Cui, Joanna Keating, Jin-Biao Chen, Anna Raif, Belamy Cheung, Kacper Jankowski, Neil Davies, Bernard Chen, Margo van Bekkum, Tammy Ellis, Murray Norris, Michelle Haber, Eugene Kim, Jason Shohet, Brandon Wainwright, Han-Fei Ding, Glenn Marshall  
Australia; United States  

14:50  OR26  Common and distinct MYC target genes in embryonal tumors  
Christina Poehler, Daniel Muth, Stephan Gade, Giulio Fiaschetti, Oskar Smrzka, Tim Beissbarth, Dominik Sturm, Stefan Pfister, Alexandre Arcaro, Heinrich Kovar, Matthias Fischer, Manfred Schwab, Frank Westermann  
Germany; Switzerland; Austria  

15:00  OR27  Addiction of MYCN amplified neuroblastomas to B-MYB underscores a reciprocal regulatory loop  
Francesco Gualdrini, Arturo Sala, Arturo Sala  
United Kingdom  

15:10  OR28  Combined massively parallel sequencing and synthetic lethal screening identifies multiple druggable targets in neuroblastoma  
Qing-Rong Chen, David Azorsa, Young Song, Jun Wei, Tom Badgett, Xiang Guo, Peter Johansson, Xinyu Wen, Susan Yeh, Catherine House, John Maris, Javed Khan  
United States  

15:20  OR29  Tumor regression and curability of preclinical neuroblastoma models by the novel targeted camptothecin EZN-2208  
Fabio Pastorino, Monica Loi, Puja Sapra, Pamela Becherini, Michele Cilli, Laura Emionite, Domenico Ribatti, Lee M Greenberger, Ivan D Horak, Mirco Ponzoni  
Italy; United States  

15:30  OR30  Selective targeting of neuroblastoma tumor initiating cells by a telomerase inhibitor IMETELSTAT  
Tatiana Lipman, Mayumi Fujitani, Loen Hansford, Ian Clarke, Calvin Harley, Robert Tressler, David Malkin, Erin Walker, Peter Dirks, Baruchel Sylvain, David Kaplan, Uri Tabori  
Canada; United States  

15:40  OR31  ABCC transporters influence multiple aspects of neuroblastoma biology, as well as clinical outcome, independent of cytotoxic drug efflux  
Australia; Italy; United States; Germany  

15:50  OR32  EZH2 mediates epigenetic silencing of candidate neuroblastoma tumor suppressor gene Casz1  
Chunxi Wang, Chan-Wook Woo, Zhihui Liu, Jun Wei, Young Song, Lifeng Wang, Victor Marquez, Javed Khan, Kai Ge, Carol Thiele  
United States; Republic of Korea
14:30 – 16:00  Tuesday June 22nd
Room 403
ANR Nurses Special Session

Special programme for nurses in pediatric oncology and other interested.
Hosted by ANR 2010 Nurses Committee; Lisa Burström, Karin Enskär, Pernilla Pergert, Eva Turup
16:00 – 16:45 Tuesday June 22nd
Hall A
Selected poster – Biology 1
Chairs: Katarina Ejeskär and Miki Ohira

16:05 SEL1 Omics analysis and evolution for identification of candidate genes in progression of neuroblastoma
Eiso Hiyama; Naomi Kamai; Arata Kamimatsuse; Keiko Hiyama; Yukina Hirai; Tsutomu Masujima
Japan

16:10 SEL2 Impaired activation of the tumor suppressor p14ARF impedes its oncosuppressive impact in neuroblastoma
Daniel Dreidax; Sina Gogolin; Daniel Muth; Marc Zapatka; Jessica Berthold; Matthias Fischer;
Manfred Schwab; Frank Westermann
Germany

16:15 SEL3 The FRAGILOME Project to discover new biomarkers for neuroblastoma
Larissa Savelyeva; Anne Blumrich; Sarah Zahedi; Manfred Schwab
Germany

16:20 SEL4 Expression profiling in neuroblastoma identifies a fourth subgroup with high expression of ERBB3
Frida Abel 1; Daniel Dalevi 1; Katleen De Preter 2; Joelle Vermeulen 2; Raymond Stallings 3; Per
Kogner 1; John Maris 4; Staffan Nilsson 1
1Sweden; 2Belgium; 3Ireland; 4United States

16:25 SEL5 A SP1/MIZ1/MYCN ternary complex induces repression of TRKA and p75NTR neurotrophin receptors and affects neuroblastoma malignancy through inhibition of the cell apoptotic response to NGF
Emanuele Valli 1; Giuliano Della Valle 1; Nunzio Iraci 1; Antonio Porro 1; Daniel Diolaiti 1; Roberto
Bernardoni 1; Martina Eilers 2; Giovanni Perini 1
1Italy; 2Germany

16:30 SEL6 NCYM, a protein product of an antisense MYCN gene co-amplified with MYCN, targets MYCN for functional modulation and affects the prognosis of neuroblastoma
Suenaga Yusuke; Yoshiki Kaneko; Daisuke Matsumoto; Miki Ohira; Yoshiko Nakamura;
Akira Nakagawara
Japan

16:35 SEL7 Identification of a new fusion gene on 11q23 in neuroblastoma tumor samples
Jan Molenaar 1; Marli Ebus 1; Jan Koster 1; Arjan Lakeman 1; Johannes Schulte 2;
Angelika Eggert 1; Ingrid Orr 3; Ray Stallings 4; Huib Caron 4; Rogier Versteeg 1
1Netherlands; 2Germany; 3Sweden; 4Ireland

16:40 SEL8 Characterization of amplicon junction sequences in genomic regions surrounding the MYCN gene in neuroblastoma tumors; implications for clinical follow-up of high-risk patients
Hanna Kryh; Jonas Abrahamsson; Elsa Jegerås; Rose-Marie Sjöberg; Irene Devenney; Per Kogner;
Tommy Martinsson
Sweden
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Authors</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:05</td>
<td>SEL9</td>
<td>Causal inference, a novel approach to disentangle the effects of off-protocol therapy from the primary effects of interest in COG protocol P9462: Topotecan vs. Topotecan+cyclophosphamide in relapsed neuroblastoma</td>
<td>Wendy B. London; Christopher N. Frantz; Laura A. Campbell; Robert C. Seeger; Babette A. Brumback; Susan L. Cohn; Katherine K. Matthey; Robert P. Castleberry; Lisa Diller United States</td>
<td>136</td>
</tr>
<tr>
<td>16:10</td>
<td>SEL10</td>
<td>Persistence of disease in long-term survivors of high-risk neuroblastoma. Analysis of ENSG5 cooperative trial</td>
<td>Lucas Moreno 1; Sucheta Vaidya 2; Ross Pinkerton 1; Ian J Lewis 1; John Imeson 1; Caroline Ellershaw 1; David Machin 1; Andrew DJ Pearson 1 1United Kingdom; 2Australia</td>
<td>136</td>
</tr>
<tr>
<td>16:15</td>
<td>SEL11</td>
<td>High dose MIBG and haploidentical stem cell transplantation with cell therapy in therapy resistant neuroblastoma</td>
<td>Jacek Toporski; Dominik Turkiewicz; Jan Tennvall; Michael Garkavij; Josefina Dykes; Katarina Le Blanc; Stig Lenhoff; Gunnar Juliusson; Stefan Scheduling; Ingrid Øra; Albert N. Bekassy Sweden</td>
<td>136</td>
</tr>
<tr>
<td>16:20</td>
<td>SEL12</td>
<td>Combined radioimmunotherapy and anti-angiogenic therapy for resistant neuroblastoma</td>
<td>Shakeel Modak; Brian H. Kushner; Kim Kramer; Neeta Pandit-Taskar; Jorge A. Carrasquillo; Pat Zanonzico; Peter Smith-Jones; Steven Larson; Nai-Kong V. Cheung United States</td>
<td>137</td>
</tr>
<tr>
<td>16:25</td>
<td>SEL13</td>
<td>MYCN amplified neuroblastoma differ in clinical features at initial presentation</td>
<td>Barbara Hero 1; Thorsten Simon 1; Ruediger Spitz 1; Jessica Theissen 1; Frank Westermann 1; Holger Christiansen 1; Freimut H. Schilling 1; Felix Niggli 2; Boris De Carolis 1; Frank Berthold 1 1Germany; 2Switzerland</td>
<td>137</td>
</tr>
<tr>
<td>16:30</td>
<td>SEL14</td>
<td>Diffusion-weighted whole-body imaging with background body suppression (DWIBS) in pediatric oncology patients - a feasibility assessment</td>
<td>Throstur Finnbogason; Bo Ehnmark; Hans Jacobsson; Bo Nordell; Linda Guler; Per Kogner; Lena Douglas Sweden</td>
<td>137</td>
</tr>
<tr>
<td>16:35</td>
<td>SEL15</td>
<td>Analysis of toxicity and efficacy of high dose chemotherapy with Busulfan and Melphalan followed by stem cell transplantation in high risk neuroblastoma patients: a retrospective study of a large cohort in a single institution</td>
<td>Stéphanie Proust-Houdemont; Ellen Benhamou; Christelle Dufour; Gisèle Goma; Nathalie Gaspar; Véronique Minard-Colin; Cormac Owens; Olivier Hartmann; Dominique Valteau-Couanet France</td>
<td>137</td>
</tr>
<tr>
<td>16:40</td>
<td>SEL16</td>
<td>Natural history of infantile neuroblastoma under “wait and see” observation — current status of patients after long term follow up for 5 - 15 years</td>
<td>Akihiro Yoneda; Masami Inoue; Takaharu Oue; Yasuyuki Mitan; Keisuke Nose; Hiroshi Nakai; Hisayoshi Kawahara; Akio Kubota; Masanori Nishikawa; Masahiro Nakayama; Keisei Kawa Japan</td>
<td>138</td>
</tr>
</tbody>
</table>
16:00 – 16:45  Tuesday June 22nd
Hall C
Selected poster – Translational 1
Chairs: Ingrid Öra and Cai Weisong

16:00  SEL17  Polyamine inhibition blocks initiation and progression of neuroblastoma
Michelle Haber 1; Nicholas F. Evageliou 2; Annette Vu 2; Jayne Murray 1; Ngan Ching Cheng 1; Xueyuan Liu 1; Kelly-Ann Corrigan 2; David Ziegler 1; Glenn M. Marshall 1; Murray D. Norris 1; Michael D. Hogarty 2
1Australia; 2United States

16:05  SEL18  Blocking Galectin-1 function reduces growth of aggressive neuroblastoma cells in vitro and in vivo
Alexander Schramm 1; Sali Timah 1; Flora Cimmino 2; Achille Iolascon 2; Jan Koster 3; Rogier Versteeg 3; Johannes Schulte 1; Angelika Eggert 1
1Germany; 2Italy; 3Netherlands

16:10  SEL19  Bortezomib delays neuroblastoma tumor growth while impairing bone growth, testis development and fertility in a male xenograft mouse model
Emma Eriksson; John I. Johnsen; Per Kogner; Lars Savendahl
Sweden

16:15  SEL20  Evaluation of the effect of acetyl l-carnitine on experimental cisplatin ototoxicity and neurotoxicity
Dilek Gunes; Günay Kırkým; Efsun Kolatan; Enis Alpin Güneri; Candan Özoðul; Bülent Perbetçiðoðlu; Osman Yülmaz; Özlem Tüfekçi; Kamer Mutaföðlu; Zekîye Altun; Safiye Aktaþ; Züþeyde Erbayraktar; Nur Olgun
Turkey

16:20  SEL21  BTK expression is critical in neuroblastoma tumor initiating cells
Erika Currier; Sharon Illenye; Lee Dorf; Lee Honigberg; Giselle Sholler
United States

16:25  SEL22  Inflammatory prostaglandin E2 induces neuroblastoma cell proliferation and survival in an autocrine and/or paracrine manner
Agnes Rasmuson 1; Anna Kock 1; John Inge Johnsen 1; Ole Martin Fuskevåg 2; Lena-Maria Carlson 1; Jaione Simon SantaMaria 2; Per Kogner 1; Baldur Sveinbjörnsson 2
1Sweden; 2Norway

16:30  SEL23  Inhibition of Fatty Acid Synthase (FASN) as a potential therapy in neuroblastomas with MYCN amplification
Patrick Mayes; Sharon Diskin; Edward Attiyeh; John Maris
United States

16:35  SEL24  Neuroblastoma-monomonuclear phagocyte interactions promoting tumor growth are suppressed by lenalidomide
Yibing Xu; Jianping Sun; Hong-Wei Wu; Michael Sheard; Hung Tran; Zesheng Wan; Cathy Liu; Robert Seeger
United States
16:00 – 18:30 Tuesday June 22nd and Wednesday June 23rd
Poster session – All posters will be displayed throughout the meeting

Odd numbers/left aligned posters = Presenting authors present at posters Tuesday June 22nd 16:45 – 17:30
Even numbers/indented posters = Presenting authors present at posters Wednesday June 23rd 16:45 – 17:30

**Posters – Biology**

**POB1** Identification *in vitro* and *in vivo* of tumoral glial precursor cells in neuroblastoma
Sandra Acosta 1; Eva Rodriguez 1; Cinzia Lavarrino 1; Kathleen De Preter 2; Idoya Garcia 1; Gemma Mayol 1; Carmen de Torres 1; Jaume Mora 1
1Spain; 2Belgium

**POB2** Integrated analysis of DNA methylation, copy number and mRNA expression identifies novel candidate tumor suppressor genes in neuroblastoma
Leah Alcock; Patrick Buckley; Kenneth Bryan; Sudipto Das; Karen Watters; Raymond Stallings
Ireland

**POB3** Ganglioneuroblastoma, nodular subtype and MYCN amplification: the hospital for sick children experience
Paola Angelini; Paula Marrano; Paul Thorner; Meredith Irwin; Sylvain Baruchel
Canada

**POB4** Interleukin-6-mediated activation of the signal transduction and activator of transcription (stat)3 contributes to chemoresistance and tumor progression in neuroblastoma
Tasnim Ara; Rie Nakata; Nino Keshelava; Robert Seeger; Yves DeClerck
United States

**POB5** Differential gene and pathway expression and alternate splicing in high-risk MYCN amplified and non-amplified neuroblastomas. A report from the Neuroblastoma TARGET (Therapeutically Applicable Research to Generate Effective Treatments) Initiative
Shahab Asgharzadeh; Lingyun Ji; Richard Sposto; Yue-Xian Tu; Michael Hadjidaniel; Edward Attiyeh; Michael D. Hogarty; Julie Gastier-Foster; Jun Wei; Xiang Guo; Daniela Gerhard; Malcolm A. Smith; Javed Khan; John M. Maris; Robert C. Seeger
United States

**POB6** Immunogenicity of neuroblastoma - insights from experimental models
Shifra Ash; Nadir Askenasy; Isaac Yaniv
Israel

**POB7** PHOX2B-mediated regulation of ALK expression in neuroblastoma pathogenesis
Tiziana Bachetti; Daniela Di Paolo; Valentina Mirisola; Chiara Brignole; Marta Bellotti; Irene Cafà; Chiara Ferraris; Michele Fiore; Diego Fornasari; Simona Di Lascio; Roberto Chiarle; Silvia Borghini; Ulrich Pfeffer; Mirco Ponzoni; Ceccherini Isabella; Patrizia Perri
Italy

**POB8** Natural histone deacetylase inhibitor, sulforaphane, inhibits growth and survival of human neuroblastoma
Reza Bayat Mokhtari 1; Herman Yeger 1; Bikul Das 2; Libo Zhang 1; Sushil Kumar 1; Gideon Koren 1; Sylvain Baruchel 1
1Canada; 2United States

**POB9** Promising effects of the PI3K/mTOR inhibitor PI-103 with currently applied chemotherapeutic drugs on neuroblastoma cell lines
Odette Besancon; Godelieve Tytgat; René Leen; Huib Caron; André van Kuilenburg
Netherlands

**POB10** Expression of the B-cell-activating factor BAFF and its receptors in opsonoclonus-myoclonus associated neuroblastoma
Giovanna Bianchi 1; Verena Fühlhuber 2; Claudio Gambini 1; Massimo Conte 1; Vito Pistoia 1; Lizzia Raffaghello 1; Franz Blaes 1; Barbara Hero 2
1Italy; 2Germany
POB11  Role of ATP and myeloid-derived suppressor cells in neuroblastoma microenvironment
Giovanna Bianchi; Ignazia Prigione; Laura Emioniite; Michele Cilli; Patrizia Pellegatti; Francesco Di Virgilio; Ilaria Marigo; Francesca Simonato; Vincenzo Bronte; Vito Pistoia; Lizzia Raffaghello
Italy

POB12  Non-transcriptional role of MYC and genomic rearrangements in neuroblastoma
Anne Blumrich; Daniel Muth; Christina Poehler; Stephan Gade; Frank Westermann; Manfred Schwab
Germany

POB13  Two common fragile sites FRA2Ctel and FRA2Ccen map to the borders of MYCN amplicons in neuroblastoma
Anne Blumrich; Marc Zapatka; Manfred Schwab; Larissa Savelyeva
Germany

POB14  Expression and clinical relevance of melanoma-associated antigens in neuroblastoma
Paola Bocca 1; Ignazia Prigione 1; Barbara Carlini 1; Maria Valeria Corrias 1; Soldano Ferrone 2; Vito Pistoia 1; Fabio Morandi 1
1Italy; 2United States

POB15  Heterogeneous MYCN amplification - amplicon, genomic background and genome instability
Dominik Bogen 1; Inge M. Ambros 1; Gabriele Aman 1; Ekkehard Spuller 1; Jennie Erichsen 2; Bettina Brunner 1; Ruth Ladenstein 1; Tommy Martinsson 1; Peter F. Ambros 1
1Austria; 2Sweden

POB16  Immunocytotoxic study of bone marrow in neuroblastoma patients - Polish experience
Katarzyna Bolek-Marzec; Aleksandra Wieczorek; Walentyna Balwierz
Poland

POB17  CHIPaway: A tool for visualization and analysis of high-throughput microarray based immunoprecipitation data
Kenneth Bryan; Patrick G. Buckley; Derek M. Murphy; Sudipto Das; Raymond L. Stallings
Ireland

POB18  Genome-wide DNA methylation profiling reveals extensive and complex epigenetic alterations in neuroblastic tumors
Patrick Buckley 1; Sudipto Das 1; Kenneth Bryan 1; Raymond Stallings 1; Leah Alcock 1; Rogier Versteeg 2
1Ireland; 2Netherlands

POB19  Identification of epigenetically regulated genes that predict patient outcome in neuroblastoma
Helena Carén; Anna Djos; Maria Nethander; Rose-Marie Sjöberg; Per Kogner; Staffan Nilsson; Tommy Martinsson
Sweden

POB20  Factors affecting the outcome of the p53 mediated DNA damage response in neuroblastoma
Jane Carr-Wilkinson 1; Rebecca Griffiths 2; Rebecca Elston 2; Laura, D Gamble 2; John Lunec 2; Deborah A. Tweddle 2
1United Kingdom; 2United Kingdom

POB21  Characterization of ALK rearrangements in neuroblastoma
Alex Cazes; Valentina Boeva; Agnès Ribeiro; Emmanuel Barillot; Olivier Delattre; Isabelle Janoueix-Lerosey
France

POB22  Clusterin interacts with HSP60: implications in neuroblastoma development
Korn-Anong Chaiwatanasirikul; Arturo Sala
United Kingdom
POB23  Human mesenchymal stromal cells (hMSCs) enhanced migration and invasion of neuroblastoma cells via SDF-1/CXCR4 and SDF-1/CXCR7 axes

Godfrey Chi-Fung Chan; Ming Ma
Hong Kong

POB24  Alterations of NDP kinase A/ NM23-H1 deregulate c-myc transcription

Christina Chang; Kai-Hui Chan; Larry Paris; Lin-Jen Ma; Renn-Shiuan Wei; Choon-Yee Tan
Taiwan

POB25  Overexpression of β1,4-N-acetylgalactosaminyltransferase III suppresses the malignant phenotype of neuroblastoma cells via β1-integrin signaling

Mei-leng Che; Hsiu-Hao Chang; Min-Chuan Huang
Taiwan

POB26  The role of protein tyrosine-phosphatases in neuroblastoma

Owen Clark; Andrew Stoker
United Kingdom

POB27  Identification of hypoxia signatures in neuroblastoma cell lines by l1-l2 regularization and data filtering

Andrea Cornero; Paolo Fardin; Massimo Acquaviva; Annalisa Barla; Sofia Mosci; Lorenzo Rosasco; Alessandro Verri; Luigi Varesio
Italy

POB28  Autophagy and its regulation in neuroblastoma

Sonia Cournoyer 1; Tina V Imbriglio 1; Carine Nyalendo 1; Claire Barelli 1; Pierre Teira 1; Michel Duval 1; Gilles Vassal 1; Hervé Sartelet 1
1Canada; 2France

POB29  All-trans retinoic acid induced differentiation of SK-N-BE cells results in extensive DNA methylation alterations of gene promoter regions

Sudipto Das; Patrick Buckley; Kenneth Bryan; Karen Watters; Niamh Foley; Leah Alcock; Isabella Bray; Raymond Stallings
Ireland

POB30  Chemokines CXCR5-CXCL13 cross-talk between malignant neuroblastoma cells and schwannian stromal cells suggests a role in the inhibition of metastatic dissemination

Federica Del Grosso; Simona Coco; Paola Scaruffi; Sara Stigliani; Francesca Valdora; Roberto Benelli; Simona Boccardo; Sandra Salvi; Mauro Truini; Michela Croce; Silvano Ferrini; Gian Paolo Tonini
Italy

POB31  N-glycosylation of ALK as a potential target for disruption of prosurvival signaling pathways in neuroblastoma cell lines

Federica Del Grosso; Marilena De Mariano; Lorena Passoni; Laura Paleari; Gian Paolo Tonini; Luca Longo
Italy

POB32  Discovery of gene regulatory pathways implicated in neuroblastoma pathogenesis through integration of coding and non-coding gene expression and gene copy number data

Katleen De Preter 1; Annelies Fieuw 1; Candy Kumps 1; Pieter Mestdagh 1; Bram De Wilde 1; Alexander Schramm 2; Johannes Schülte 2; Rosa Noguera 2; Angelika Eggert 2; Jo Vandesompele 1; Frank Speleman 1
1Belgium; 2Germany; 3Spain

POB33  The calcium-sensing receptor gene is inactivated by genetic and epigenetic mechanisms in neuroblastic tumors and its overexpression reduces neuroblastoma proliferation in vitro and in vivo

Carmen de Torres; Carla Casalà; José Luis Ordóñez; Solange Miguel; Francina Munell; Patricia Galván; Eva Rodríguez; Gemma Mayol; Idoia García; Elisa Martí; Enrique de Alava; Cinzia Lavarino; Jaume Mora
Spain

POB34  3D miRNA mutation screening in neuroblastoma

Bram De Wilde 1; Steve Lefever 1; Pieter Mestdagh 1; Nathalie Vanderstraeten 1; Valerie Vanderstraeten 1; Joachim De Schrijver 1; Filip Pattyn 1; Katleen De Preter 1; Gert Van Peer 1; Rogier Versteeg 1; Ray Stallings 2; Wim Van Criekinge 1; Frank Speleman 1; Jo Vandesompele 1
1Belgium; 2Netherlands; 3Ireland
POB35  Bmi-1 promotes neuroblastoma cell proliferation by regulation of cyclin levels
Jane Ding; Ling Mao; Han-Fei Ding
United States

POB36  Prickle1: Possible tumour suppressive role in neuroblastoma
Cecilia Dyberg; Panagiotis Papachristou; Thomas Ringstedt; Per Kogner; John Inge Johnsen
Sweden

POB37  X-linked Inhibitor of Apoptosis (XIAP) as a new target for NB therapy
Georg Eschenburg; Patrick Hundsdorfer; Holger N Lode
Germany

POB38  Focal amplifications and deletions at miRNA loci in neuroblastoma
Annelies Fieuw 1; Candy Kumps 1; Steve Lefever 1; Nadine Van Roy 1; Pieter Mestdagh 1; Johannes Schulte 2; Alexander Schramm 2; Angelika Eggert 2; Rosa Noguera 3; Anne De Paepe 1; Jo Vandesompele 1; Frank Speleman 1; Kathleen De Preter 1
1Belgium; 2Germany; 3Spain

POB39  Involvement of delta-like 1 homolog (drosophila) in the development of chemoresistance in neuroblastoma
Marjorie Flahaut; Aurélie Coulon; Katya Nardou; Annick Mühlthaler-Mottet; Nicole Gross
Switzerland

POB40  Mechanisms of bHLH mediated neuronal differentiation
Abraham Fong; Yi Cao; Zizhen Yao; Stephen Tapscott
United States

POB41  Nf-kB and IRF1, but not MYCN, control the expression of MHC class I and endoplasmic reticulum aminopeptidases in human neuroblastoma cells
Matteo Forloni; Sonia Albin; Silvia Lorenzi; Loredana Cifaldi; Renata Boldrini; Giuseppe Giannini; Pier Giorgio Natali; Patrizio Giacomini; Doriana Fruci
Italy

POB42  Differential expression of PI3K-Akt pathway genes in neuroblastoma.
Susanne Fransson; Frida Abel; Helena Eriksson; Tommy Martinsson; Katarina Ejeskär
Sweden

POB43  The impact of MYCN on the response to MDM2-p53 antagonists in neuroblastoma
Laura D. Gamble; Deborah A. Tweddle; John Lunec
United Kingdom

POB44  The role of MDMX on the response to MDM2-p53 antagonists in neuroblastoma
Laura D. Gamble; Deborah A. Tweddle; John Lunec
United Kingdom

POB45  Distinctive expression patterns of MicroRNA in neuroblastoma tumors of opposite outcomes
Charles-Henry Gattolliat; Guillaume Meurice; Matthieu Bauer; Laetitia Thomas; Bastien Job; Catherine Richon; Valérie Combaret; Philippe Dessen; Vladimir Lazar; Pierre Busson; Dominique Valteau-Couanet; Sélia Douc-Rasy; Jean Bénard
France

POB46  Neuroblastoma differentiation signalling pathways
Dirk Geerts; Johan van Nes; Ingrid Revet; Gerda Huizenga; Nathalie Schilderink; Peter van Sluis; Jan Koster; Rogier Versteeg
Netherlands

POB47  The polyamine metabolism genes ornithine decarboxylase and antizyme 2 predict aggressive behavior in neuroblastomas with and without MYCN amplification
Dirk Geerts 1; Jan Koster 1; David Albert 2; Dana-Lynn Koomoa 2; David Feith 2; Anthony Pegg 2; Richard Volckmann 1; Huib Caron 1; Rogier Versteeg 1; Andre Bachmann 2
1Netherlands; 2United States
POB48  Functional pRB loss is involved in impaired drug induced DNA damage response in MYCN amplified neuroblastoma cells
Sina Gogolin; Daniel Dreidax; Gabriele Becker; Volker Ehemann; Manfred Schwab; Frank Westermann
Germany

POB49  Modulation of neuroblastoma cell sensitivity towards anticancer drugs by MyCN expression
Vladimir Gogvadze; Bjorn Kruspig; Erik Norberg; Sten Orrenius; Boris Zhivotovsky
Sweden

POB50  Epigenetic alterations in disseminated neuroblastoma: influence of TMS1 gene hypermethylation in relapse risk
Elena Grau; Adela Cariste; Yania Yanez; Francisco Martinez; Carmen Orellana; Silvestre Oltra; Rosa Noguera; Miguel Hernandez; Victoria Castel
Spain

POB51  Combining peptide vaccination with immunostimulatory monoclonal antibodies provides potent immunotherapy in neuroblastoma
Juliet Gray; Martin Glennie; Peter Johnson
United Kingdom

POB52  Expression QTL analysis of tumor susceptibility in a mouse model of neuroblastoma
Christopher Hackett; Pui Kwok; Song Young; Javed Khan; Balmain Allan; William A. Weiss
United States

POB53  Age-dependent genotypes in aggressive neuroblastoma: MYCN amplification represents a few-hit/early-age form
Fredrik Hedborg; Cihan Cetinkaya; Tommy Martinsson; Per Kogner; Jan Dumanski; Catarina Träger; Teresita Diaz deStåhl
Sweden

POB54  Modeling the neuroblastoma tumor initiating cell microenvironment in 3D culture
Anna Herland; Caroline Wigerup; Sofie Johansson; Sven Påhlman; Ana I Teixeira
Sweden

POB55  Epigenetically silenced microRNAs contribute to neuroblastoma pathogenesis
Jasmien Hoebeeck; Pieter Mestdagh; Filip Pattyn; Kathleen De Preter; Ali Rihani; Joëlle Vermeulen; Tom Van Maerken; Steve Lefever; Justine Nuytens; Nurten Yigit; Anne De Paepe; Frank Speleman; Jo Vandewoestyne
Belgium

POB56  The interaction between GRP75 and retinoic acid receptor-α/retinoid X receptor-α is essential for retinoic acid-induced neuronal differentiation of neuroblastoma cells
Wen-Ming Hsu; Yu-Yin Shih; Hsinyu Lee; Hsueh-Fen Juan; Yeh-Jung Tsay; Yung-Feng Liao
Taiwan

POB57  Neurod1 is involved in the development of neuroblastoma especially at initiation stage
Peng Huang; Satoshi Kishida; Hitoshi Ichikawa; Kenji Kadomatsu
Japan

POB58  An observation of chromosomal abnormalities and MYCN and AURKA gene changes in neuroblastoma patients
Nihal Inandiklioglu; Sema Yilmaz; Osman Demirhan; Can Acipayam; Ibrahim Bayram; Fatih Erbey; Atila Tanyeli
Turkey

POB59  Chromosomal instabilities in a neuroblastoma patient
Nihal Inandiklioglu; Sema Yilmaz; Osman Demirhan; Ibrahim Bayram; Can Acipayam; Fatih Erbey; Atila Tanyeli
Turkey

POB60  Constitutive Hen2 expression in neural crest cells could be a trigger of neuroblastoma development
Eriko Isogai; Miki Ohira; Seiki Haraguchi; Akira Nakagawara
Japan
POB61  A novel orphan receptor, NLRR3, induces neuronal differentiation and is negatively regulated by MYCN and Miz-1 in neuroblastoma
Akter Jesmin; Takatori Atsushi; Hossain Md. Shamim; Nakagawa Atsuko; Suenaga Yusuke; Islam Rafiqul; Nakagawara Akira
Japan

POB62  Comprehensive screen for genes involved in tumorigenesis and tumor-initiating cell formation in MYCN Tg mice
Satoshi Kishida; Dong Liang Cao; Hitoshi Ichikawa; Kenji Kadomatsu
Japan

POB63  Common pathways in neuroblastoma and early-onset breast cancer
Kenneth Kompass; Katherine Matthay; John Maris; John Witte
United States

POB64  Neuropeptide Y in neuroblastoma - Interactions with BDNF and effect on cell survival
Anna Kuan-Celarier; Congyi Lu; Lindsay Everhart; Jeffrey Toretsky; Joanna Kitlinska
United States

POB65  Involvement of the CXCL12/CXCR4/CXCR7 axis in the malignant progression of human neuroblastoma
Julie Liberman; Marjorie Flahaut; Annick Mühlethaler-Mottet; Aurélie Coulon; Jean-Marc Joseph; Nicole Gross
Switzerland

POB66  Mycn metabolic programs enforce glucose more than glutamine addiction in neuroblastoma cells
Xueyuan Liu; Annette Vu; Michelle Gross; Stephen R. Master; Michael D. Hogarty
United States

POB67  Haploinsufficiency of candidate tumor suppressor gene CASZ1 blocks embryonic stem cell neurogenesis
Zhihui Liu; Carol Thiele
United States

POB68  Screening of ALK mutations and abnormalities in neuroblastoma cell lines and Italian neuroblastoma cases
Luca Longo; Marilena De Mariano; Federica Del Grosso; Lorena Passoni; Raffaella Defferrari; Katia Mazzocco; Aurora Castellano; Roberto Luksch; Alberto Garaventa; Gian Paolo Tonini
Italy

POB69  Ephrin/Eph signaling in neuroblastoma tumor initiating cells
Vanessa Lundin; Ana Teixeira
Sweden

POB70  TRIM16 acts as a tumour suppressor via inhibitory effects on cytoplasmic vimentin and nuclear E2F1 in neuroblastoma cells
Glenn Marshall; Jessica Bell; Jessica Koach; Owen Tan; Patrick Kim; Alena Malyukova; Wayne Thomas; Eric Sekyere; Tao Liu; Anne Cunningham; Vivienne Tobias; Murray Norris; Michelle Haber; Maria Kavallaris; Belamy Cheung
Australia

POB71  High-risk neuroblastoma without MYCN amplification - 11q-deletion tumors reveal a poor prognostic chromosome instability phenotype with later onset
Tommy Martinsson; Helena Carén; Hanna Kryh; Rose-Marie Sjöberg; Jennie Erichsen; Maria Netherand; Catarina Träger; Jonas Abrahamsson; Staffan Nilsson; Per Kogner
Sweden

POB72  Appearance of the novel activating F1174S ALK mutation in neuroblastoma correlates with disease progression, aggressive tumour behavior and unresponsiveness to therapy
Tommy Martinsson; Jonas Abrahamsson; Therese Eriksson; Helena Carén; Magnus Hansson; Per Kogner; Sattu Kamaraj; Joel Weinmar; Kristina Ruuth; Ruth Palmer; Bengt Hallberg
Sweden
<table>
<thead>
<tr>
<th>Programme</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>POB73</td>
<td>An integrative genomics screen uncovers ncRNA T-UCR functions in neuroblastoma tumours</td>
<td>166</td>
</tr>
<tr>
<td></td>
<td>Pieter Mestdagh 1; Erik Fredlund 2; Filip Pattyn 1; Ali Rihani 1; Tom Van Maerken 1; Joëlle Vermeulen 1; Candy Kumps 1; Bjorn Menten 1; Katleen De Preter 1; Alexander Schramm 2; Johannes Schulte 3; Rosa Noguera 4; Gurdrug Schleiermacher 4; Isabelle Janoueix-Lerosey 4; Geneviève Laureys 4; Rob Powel 5; David Nittner 5; Jean-Christophe Marine 1; Markus Ringnér 2; Frank Speleman 1; Jo Vandesompele 1</td>
<td>166</td>
</tr>
<tr>
<td></td>
<td>1Belgium; 2Sweden; 3Germany; 4Spain; 5France; 6United Kingdom</td>
<td></td>
</tr>
<tr>
<td>POB74</td>
<td>Mir-17-92 is a master regulator of TGFβ-pathway activity in neuroblastoma</td>
<td>166</td>
</tr>
<tr>
<td></td>
<td>Pieter Mestdagh 1; Anna-Karin Boström 2; Francis Impens 1; Erik Fredlund 2; Pasqualino De Antonellis 3; Kris von Stedingk 2; Bart Ghersiure 1; Gert Van Peer 1; Chiara Medaglia 4; Stefanie Schlief 2; Johannes Schulte 4; Alexander Schramm 4; Massimo Zollo 4; Kris Gevaert 1; Hakan Axelson 2; Frank Speleman 1; Jo Vandesompele 1</td>
<td>166</td>
</tr>
<tr>
<td></td>
<td>1Belgium; 2Sweden; 3Italy; 4Germany</td>
<td></td>
</tr>
<tr>
<td>POB75</td>
<td>Phosphoproteomic and expression analyses of a MYCN-amplified neuroblastoma cell line</td>
<td>166</td>
</tr>
<tr>
<td></td>
<td>Kelly Mikan; Kolla Kristjansdottir; Alexandre Chlenski; Kelly Regan; Stephen X. Skapek; Samuel L. Volchenboum</td>
<td>166</td>
</tr>
<tr>
<td></td>
<td>United States</td>
<td></td>
</tr>
<tr>
<td>POB76</td>
<td>Analysis of cellular mediators of oncogenic signaling originating from activated ALK in neuroblastoma cells</td>
<td>166</td>
</tr>
<tr>
<td></td>
<td>Izumi Miyake; Reiko Kamata; Hitoyasu Futami; Ryuichi Sakai</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Japan</td>
<td></td>
</tr>
<tr>
<td>POB77</td>
<td>Development and characterization of ALK dependent cellular models</td>
<td>167</td>
</tr>
<tr>
<td></td>
<td>Julie Mollet; Sarah Dubray; Virginie Raynal; Sophie Thomas; Heather Etchevers; Olivier Delattre; Isabelle Janoueix-Lerosey</td>
<td>167</td>
</tr>
<tr>
<td></td>
<td>France</td>
<td></td>
</tr>
<tr>
<td>POB78</td>
<td>Nucleotide excision repair and in vivo neuroblastoma chemoresistance to irinotecan</td>
<td>167</td>
</tr>
<tr>
<td></td>
<td>Fabienne Munier; Marie Regairaz; Cathy Philippe; Marion Legentil; Vladimir Lazar; Gilles Vassal</td>
<td>167</td>
</tr>
<tr>
<td></td>
<td>France</td>
<td></td>
</tr>
<tr>
<td>POB79</td>
<td>A role of human Sgo1 on the growth of human neuroblastoma cells</td>
<td>167</td>
</tr>
<tr>
<td></td>
<td>Yuko Murakami-Tonami; Satoshi Kishida; Kenji Kadomatsu</td>
<td>167</td>
</tr>
<tr>
<td></td>
<td>Japan</td>
<td></td>
</tr>
<tr>
<td>POB80</td>
<td>NF-kB signaling in neuroblastoma</td>
<td>167</td>
</tr>
<tr>
<td></td>
<td>Natalia Nowakowska; Rogier Versteeg; Dirk Geerts</td>
<td>167</td>
</tr>
<tr>
<td></td>
<td>Netherlands</td>
<td></td>
</tr>
<tr>
<td>POB81</td>
<td>Activation of the transcription factor FOXO3/FKHRL1 by doxorubicin and etoposide induces reactive oxygen species production and programmed cell death in neuroblastoma cells</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>Petra Oxbexer; Judith Hagenbuchner; Martin Hermann; Andrey Kuznetsov; Michael Ausserlechner</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>Austria</td>
<td></td>
</tr>
<tr>
<td>POB82</td>
<td>Histone deacetylase 10 contributes to the regulation of autophagy in neuroblastoma</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>Ina Oehme; Jan-Peter Linke; Barbara C. Böck; Till Milde; Marco Lodrini; Matthias Fischer; Wilfried Roth; Sylvia Kaden; Hermann-Josef Gröne; Hedwig E. Deubzer; Olaf Witt</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>Germany</td>
<td></td>
</tr>
<tr>
<td>POB83</td>
<td>Aberrant activation of ALK kinase by a short form ALK protein in neuroblastoma</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>Jun Okubo; Junko Takita; Riki Nishimura; Kentaro Ohki; Motohiro Kato; Yuyan Chen; Masashi Sanada; Akira Kikuchi; Takashi Igarashi; Yasuhide Hayashi; Seishi Ogawa</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>Japan</td>
<td></td>
</tr>
<tr>
<td>POB84</td>
<td>MYCN sensitizes human neuroblastoma cells to apoptosis by HIPK2 activation through a DNA damage response</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>Marialaura Petroni 1; Veronica Veschi 1; Andrea Prodosmo 1; Cinzia Rinaldo 1; Isabella Massimi 1; Maurizio Carbonari 1; Carlo Dominici 1; Heather McDowell 1; Christian Rinaldi 1; Isabella Screpanti 1; Luigi Frati 1; Armando Bartolazzi 1; Alberto Gulino 1; Silvia Soddu 1; Giuseppe Giannini 1</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>1Italy; 2United Kingdom</td>
<td></td>
</tr>
</tbody>
</table>
POB85  Expression of TWEAK/Fn14 in neuroblastoma; implications in apoptotic resistance and survival  
Ingvild Pettersen 1; Ninib Baryawno 2; Agnes Rasmussen 2; Wenche Bakkeland 1; Svetlana Zyкова 1; Jan-Olof Winberg 1; Ugo Moens 1; Per Kogner 2; John Inge Johnsen 1; Baldur Sveinbjörnsson 2  
1Norway; 2Sweden

POB86  Green Tea Catechins inhibit neuroblastoma growth in vitro and in vivo  
Izabela Pietrowska; Olesya Chayka; Sandra Cantilena; Arturo Sala  
United Kingdom

POB87  N-myc gene expression: Impact on leukocyte infiltration in 3D neuroblastoma spheroids  
Maura Puppo; Florinda Battaglia; Claudio Gambini; Andrea Gregorio; Paolo Fardin; Luigi Varessio  
Italy

POB88  Use of a microgravity culture system to assess biological behavior in neuroblastoma cell lines  
Robert Redden; Radhika Iyer; Laura Urbanski; Jane Minturn; Garrett Brodeur; Edward Doolin  
United States

POB89  A biological link between p53 and MYCN/MYC expression in neuroblastoma  
Paul Lucas Regan; Robby Edo; Naohiko Ikegaki; Eric Rappaport; Xiao Tang  
United States

POB90  Mitochondria-related destabilization of MYC family proteins in neuroblastoma cells by OSU-03012, FCCP and Salinomycin  
Paul Lucas Regan; Autumn Fox; Jaime Torres; Joshua Jacobs; Makoto Horiuchi; Takayuki Itoh; Xiao Tang; Naohiko Ikegaki  
United States

POB91  Nutlin-3 induced miRNA expression changes in neuroblastoma cells  
Ali Rihani; Tom Van Maerken; Nurten Yigit; Justine Nuytens; Pieter Mestdagh; Frank Speleman  
Belgium

POB92  Oncolytic effects of Sindbis virus on human neuroblastoma cells  
Eriko Saito; Tomoro Hishiki; Kengo Saito; Ayako Takenouchi; Takeshi Saito; Keita Terui; Yoshiharu Sato; Katsunori Kouchi; Hiroshi Shirasawa; Hideo Yoshida  
Japan

POB93  A novel whole genome amplification approach is useful to perform aCGH in microdissected Schwannian Stromal and neuroblastic components of ganglioneuroblastomas  
Paola Scaruffi 1; Simona Coco 1; Francesca Valdora 1; Sara Stigliani 1; Yan Zhang 2; Pengchin Chen 2; John Smutko 2; Gian Paolo Tonini 1  
1Italy; 2United States

POB94  Analysis of expression and inhibition of the Sonic Hedgehog signaling pathway in neuroblastoma  
Paula Schiapparelli 1; Paula Lazcoz 1; Tommy Martinsson 2; John Inge Johnsen 2; Per Kogner 2; Javier S Castresana 1  
1Spain; 2Sweden

POB95  Anaplastic Lymphoma Kinase (ALK) activates the small GTPase Rap1 via the Rap1-specific GEF C3 in neuroblastoma  
Christina Schonherr 1; Hai-Ling Yang 2; Marc Vigny 2; Ruth H. Palmer 1; Bengt Hallberg 1  
1Sweden; 2China; 2France

POB96  Deep sequencing of the small-RNA transcriptome reveals differential expression of microRNAs in high-risk versus low-risk neuroblastoma  
Johannes Schulte 1; Tobias Marschall 1; Marcel Martin 1; Philipp Rosenstiel 1; Pieter Mestdagh 2; Stefanie Schierf 1; Theresa Thor 1; Jo Vandesompele 2; Angelika Eggert 1; Stefan Schreiber 1; Sven Rahmann 1; Alexander Schramm 1  
1Germany; 2Belgium

Page 169

Page 169

Page 169

Page 169

Page 170

Page 170

Page 170

Page 171

Page 171

Page 171

Page 171
POB97  Prognostic significance of NKp30 spliceoforms in neuroblastoma
Michaela Semeraro-Kunz; Nicolas F. Delahaye; Veronique Minard-Colin; Clara Loecher; Sylvie Rusakiewicz; Laurence Zitvogel; Dominique Valteau-Couanet
France

Page 172

POB98  Galectin-3 binding protein/90 kDa Mac-2 binding protein stimulates interleukin-6 expression in the neuroblastoma microenvironment
Ayaka Silverman; Yasushi Fukaya; Hiroyuki Shimada; Susan Groshen; Robert Seeger; Yves DeClerck
United States

Page 172

POB99  TrkAll isoform expression is associated with aggressive behavior in human neuroblastomas
Anisha M. Simpson; Jennifer E. Light; Jane E. Minturn; Ruth Ho; Radhika Iyer; Carly R. Varela; Jennifer L. Mangino; Huaqing Zhao; Venkatadri Kolla; Garrett M. Brodeur
United States

Page 172

POB100  Evaluation of MCPIP expression patterns and their impact on the survival of neuroblastoma cell lines
Anna Skalniak; Danuta Mizgalska; Oksana Kovtonyuk; Jolanta Jura; Hanna Rokita
Poland

Page 172

POB101  NLRR1, a direct target gene of MYCN, modulates aggressive growth of neuroblastoma by selectively enhancing EGF and IGF signals through the components of lipid rafts
Atsushi Takatori; MD. Shamim Hossain; Jesmin Akter; Atsushi Ogura; Yohko Nakamura; Akira Nakagawara
Japan

Page 173

POB102  Aurora A kinase is a possible target of OSU-03012 to destabilize MYC family proteins
Yutaka Tamura 1; Naohiko Ikegaki 2
1Japan; 2United States

Page 173

POB103  Expression and function of RET in neuroblastoma cell lines
Laura H Tetri; Ruth Ho; Anisha M Simpson; Radhika Iyer; Jane E Minturn; Garrett M Brodeur
United States

Page 173

POB104  Irrespective of ALK mutational status, neuroblastoma tumors are sensitive to Akt inhibitor perifosine
Carol Thiele; Zhijie Li
United States

Page 173

POB105  Enhanced effect of IFN-γ on the induced-apoptosis of neuroblastoma cells by cytotoxic drugs
Haixia Tong; Jinhua Zhang
China

Page 174

POB106  JAGGED1 antagonizes NOTCH2 mediated cell migration
Tim van Groningen; Marloes Broekmans; Nurdan Akogul; Rogier Versteeg; Johan van Nes
Netherlands

Page 174

POB107  A migration signature in neuroblastoma cell lines and tumours identifies YAP1 as a regulator of cell migration
Johan van Nes; Mireille Indemans; Nurdan Akogul; Jan Koster; Rogier Versteeg
Netherlands

Page 174

POB108  Functional microRNA library screen to identify synthetically lethal interactions in neuroblastoma
Gert Van Peer; Pieter Mestdagh; Frank Speleman; Jo Vandesompele
Belgium

Page 174

POB109  Galectin-3 protects MYCN single copy neuroblastoma cells from apoptosis: a mechanism impaired by MYCN
Veronica Veschi 1; Marialaura Petroni 1; Isabella Massimi 1; Carlo Dominici 1; Heather P. McDowell 2; Isabella Speranti 1; Luigi Frati 1; Armando Bartolazzi 1; Alberto Gulino 1; Giuseppe Giannini 1
1Italy; 2United Kingdom

Page 175
POB110 Genome-wide analysis of favorable-stage neuroblastoma reveals discrete patterns of gene expression and alternative splicing between MYCN amplified and non-amplified tumors
Samuel L. Volchenboum; Susan L. Cohn; Rani E. George
United States

POB111 JAG2 induction in hypoxic tumor cells alters Notch signaling and enhances endothelial cell tube formation
Kristoffer von Stedingk; Alexander Pietras; David Lindgren; Sven Pahlman; Axelson Hakan
Sweden

POB112 Transcriptome analysis of chromosome 1p in neuroblastoma
Wenchao Wang; Yanan Kuang; Robert Distel; Rani E. George
United States

POB113 Expressional alterations in ultra-conserved non-coding RNA resulting from changes in MYCN levels and in response to ATRA-induced differentiation
Karen Watters; Kenneth Bryan; Niamh Foley; Ray Stallings
Ireland

POB114 ALK signaling in neuroblastoma
Ellen Westerhout; Peter Stroeken; Huib Caron; Rogier Versteeg
Netherlands

POB115 Regulation of differentiation by estrogen receptors in neuroblastoma cells
Ulrica Westermark; Jakob Lovén; Inga Müller; Marie Arsenian Henriksson
Sweden

POB116 Role of p53 and p73 in neuroblastoma chemosensitivity and neuroblastoma tumor initiating cells (TIC)
Jennifer Wolter; David Malkin; Meredith Irwin
Canada

POB117 Identification of proteomic changes associated with differentiated neuroblastoma using an in vitro differentiation system and an optimized proteomics platform based on 18O peptide labeling
Chen Yin; Wen-Ming Hsu; Yeou-Guang Tsay
Taiwan

POB118 Role of Caspase 8 and Caspase 3 in TRAIL-induced apoptosis of neuroblastoma cells
Jinhua Zhang; Haixia Tong
China

POB119 Creation of a CHD5 knockout (KO) mouse model
Tiangan Zhuang; Venkatadri Kolla; Tobias Raabe; Hiroshi Koyama; Mayumi Higashi; Peter S. White;
Garrett M. Brodeur
United States

Posters – Translational

POT1 Prognostic significance of tumor and microenvironment gene expression for children with metastatic MYCN non-amplified neuroblastoma
Shahab Asgharzadeh; Jill Salo; Lingyun Ji; Cathy Wei Yao Liu; Roger Pique-Regi; Andre Oberthuer;
Matthias Fischer; Yue-Xian Tu; Leonid Metelitsa; Wendy London; Hiroyuki Shimada; Frank Berthold;
Richard Sposto; Robert C. Seeger
1United States; 2Germany

POT2 Genomic characterization and targeted resequencing of high-risk neuroblastoma (the neuroblastoma TARGET)
Edward F. Attiyeh; Michael D. Hogarty; Yaël P. Mossé; Sharon J. Diskin; Hakon Hakonarson; Shahab Asgharzadeh; Richard Sposto; Wendy B. London; Julie M. Gastier-Foster; Daniela S. Gerhard;
Malcolm A. Smith; Jinghui Zhang; Javed Khan; Robert C. Seeger; John M. Maris
United States
POT3  Identification of miRNAs contributing to neuroblastoma chemoresistance

Duncan Ayers 1; Pieter Mestdagh 2; Ali Rihani 3; Alexander Schramm 4; Martin Michaelis 4; Jindrich Cinatl Jr. 3; Angelika Eggert 4; Philip J Day 1; Frank Speleman 2; Tom Van Maerken 2; Jo Vandesompele 2

1United Kingdom; 2Belgium; 3Germany

POT4  Gene expression signatures of mutant ALK in neuroblastoma

Anna Azarova; Kenneth Ross; James Christensen; Kimberley Stegmaier; Rani E. George

United States

POT5  Cytomegalovirus infection in neuroblastoma, high prevalence in tumors and reduced growth in vivo and in vitro using HCMV targeted therapies

Ninib Baryawno; Nina Wolmer-Solberg; Dieter Fuchs; Lonneke Verboon; Afsar Rahbar; Lova Segerstrom; Baldur Sveinbjornsson; John-Inge Johnsen; Per Kogner; Cecilia Soderberg-Naucke

Sweden

POT6  Inhibition of lipoxygenases promotes retinoic acid induced cell death in neuroblastoma

Emma Bell; Frida Ponthan; Huw Thomas; Kieran O’Toole; Penny, E Lovat; John Lunec; Deborah, A Tweddle; Christopher, P.F, Redfern

United Kingdom

POT7  Prognosis approach of one segmental chromosome aberration in neuroblastoma

Ana P. Berbegall; Eva Villamon; Marta Piqueras; Irene Tadeo; Adela Cafiete; Samuel Navarro; Victoria Castel; Rosa Noguera

Spain

POT8  Relationship between ALK expression and genetic predictive factors in neuroblastoma

Arnaud Berthier; Marta Piqueras; Eva Villamon; Ana P. Berbegall; Irene Tadeo; Victoria Castel; Samuel Navarro; Rosa Noguera

Spain

POT9  Potential role of mesenchymal stromal cells in experimental neuroblastoma treatment

Giovanna Bianchi; Laura Emionite; Fabio Morandi; Daga Antonio; Michele Cilli; Lizza Raffaghello; Vito Pistoia

Italy

POT10  Combinatory effect of 5-AZA-cytidin and Octreotide on neuroblastoma cell proliferation and apoptosis

Payman Björklund; Anna Oskarsson; Per Hellman

Sweden

POT11  ALK and PHOX2B mutations in neuroblastic tumours with highly suspected predisposition: Rare events and unexpected clinical features

Franck Bourdeaut; Agnes Ribeiro; Marion Gauthier-Villars; Jean Michon; Yves Perel; Gudrun Schleiermacher; Jeanne Amiel; Gaelle Pierron; Isabelle Janoueix-Lerosey; Olivier Delattre

France

POT12  TLR9 expression and functionality in neuroblastoma delineate a novel prognostic marker and therapeutic target

Chiara Briglione; Danilo Marimpietri; Daniela Di Paolo; Fabio Morandi; Fabio Pastorino; Alessia Zorzoli; Gabriella Pagnan; Monica Loi; Irene Caffa; Giuseppe Erminio; Riccardo Haupt; Claudio Gambini; Patrizia Perri; Vito Pistoia; Mirco Ponzoni

Italy

POT13  Arguments for the intravenous application of high dose ascorbic acid for treatment of neuroblastoma

Gernot Bruchelt; Beate Deubzer; Florian Mayer; Zyrafete Kuci; Marena Niewisch; Rupert Handgretinger

Germany

POT14  Molecular imaging of MYCN-amplified neuroblastoma tumorigenesis in orthotopic xenograft and transgenic TH-MYCN murine models

Erika Cantelli 1; Antonio Fasci 1; Carmelo Quarta 1; Laura Mezzanotte 1; Salvatore Serravalle 1; Korinne Di Leo 1; Cristina Nanni 1; Stefano Fanti 1; Aldo Roda 1; William A Weiss 2; Patrizia Hrelia 1; Andrea Pession 1; Roberto Tonelli 1

1Italy; 2United States
POT15  Microarray-based pathway analysis leads to the identification of potential molecular mechanisms underlying gamma-secretase inhibitor-induced neuronal differentiation of neuroblastoma cells
Hsiu-Hao Chang; Wen-Ming Hsu; Hsueh-Fen Juan; Yung-Feng Liao; Min-Chuan Huang; Hsinyu Lee; Fon-Jou Hsieh; Kai-Hsin Lin
Taiwan

POT16  IL-21-based immunotherapy of neuroblastoma in combination with lymphodepleting antibodies
Michela Croce; Anna Maria Oreno; Antonella Brizzolara; Valentina Rigo; Barbara Carlini; Maria Valeria Corrias; Silvano Ferrini
Italy

POT17  Characterisation of tumour progression, vascularisation and response to chemotherapy in transgenic mouse models of Neuroblastoma (TH-MYC and TH-MYCN/p53ER<sup>tam</sup>) using magnetic resonance imaging
Elizabeth Cullis<sup>1</sup>; Yann Jamin<sup>2</sup>; Lynsey Vaughan<sup>2</sup>; Sergey Popov<sup>2</sup>; Dow-Mu Koh<sup>1</sup>; Andrew Pearson<sup>1</sup>; Louis Chesler<sup>1</sup>; Simon Robinson<sup>1</sup>
<sup>1</sup>United Kingdom; <sup>2</sup>United Kingdom

POT18  Development and characterisation of bioluminescent orthotopic and metastatic neuroblastoma models
Estelle Daudigeos-Dubus; Valérie Rouffiac; Ingrid Leguerney; Yann Monnet; Paule Opolon; Olivia Bawa; Birgit Geoerger; Gilles Vassal
France

POT19  The DNA-binding protein, YB-1 is a direct N-Myc target and influences repair and resistance in neuroblastoma cell lines
Stephanie Degen; Steffi Kuhfitting-Kulle; Johannes H. Schulte; Frank Westermann; Alexander Schramm; Angelika Eggert; Kathy Astrahantseff
Germany

POT20  Comparative interactomics, transcriptomics and proteomics studies of CDK inhibitors roscovitine and CR8 effects on human neuroblastoma SH-SY5Y cells converge to a central role of Myc
Claire Delehouze; Nadège Loaec; Nathalie Desban; Laurent MeiJjer
France

POT21  ALK and pALK protein levels in NBL cell lines correlate with ALK mutation status and responsiveness to ALK inhibition
Floor Duijkers; Jose Gaal; Pieter Admiraal; Rob Pieters; Jules Meijerink; Ronald Krijger, de; Max Noesel
Netherlands

POT22  Development of a DNA methylation array normalization method for analyzing demethylating treatment effects in paired neuroblastoma cell lines
Floor Duijkers; Maarten Iterson, van; Jules Meijerink; Pieter Admiraal; Rob Pieters; Judith Boer, de; Renee Menezes, de; Max Noesel, van
Netherlands

POT23  Expression of chemokine CCL21 and its receptor CCR7 in in neuroblastoma
Josiah Dungwa; Urmila Uparkar; David Bates; Pramila Ramani
United Kingdom

POT24  Cancelled

POT25  Hypoxia gene signature as a prognostic factor in neuroblastoma patients
Paolo Fardin<sup>1</sup>; Andrea Cornero<sup>1</sup>; Massimo Acquaviva<sup>1</sup>; Annalisa Barla<sup>1</sup>; Sofia Mosci<sup>1</sup>; Rogier Versteeg<sup>2</sup>; Jan J. Molenaar<sup>2</sup>; Ingrid Ora<sup>2</sup>; Huib N. Caron<sup>2</sup>; Luigi Varesio<sup>1</sup>
<sup>1</sup>Italy; <sup>2</sup>Netherlands

POT26  Analysis of cytotoxic drugs that selectively target cells with MYC overexpression
Anna Frenzel; Ami Albihn; Hanna Zirath; Marina Vita; Marie Arsenian Henriksson
Sweden
POT27 Expression of the neuron-specific protein CHD5 is an independent marker of outcome in neuroblastoma
Idoia Garcia 1; Gemma Mayol 1; Eva Rodriguez 1; Mariona Suñol 1; Timothy Gershon 1; José Ríos 1; Nai-Kong Cheung 2; Carmen de Torres 1; Mark Kieran 1; Jaume Mora 1; Cinzia Lavanno 1
1Spain; 2United States

POT28 Sphingosine-1-phosphate signaling is a mechanism of fenretinide resistance and provides a novel therapeutic target
Matthew V Ghent; Taylor Chen; Youngleem Kim; Ana Jakimenko; Min Kang; C. Patrick Reynolds
United States

POT29 Omega-3 fatty acid supplementation delays the progression of neuroblastoma in vivo
Helena Gleissman 1; Lova Segerström 1; Mats Hamberg 1; Frida Ponthan 1; Magnus Lindskog 1;
John Inge Johnsen 1; Per Kogner 1
1Sweden; 2United Kingdom

POT30 ‘BH3 profiles’ identify neuroblastomas with exquisite ABT-737/chemotherapy in vivo and Bim signaling is a critical determinant sensitivity
Kelly C. Goldsmith; Michelle Gross; Xueyuan Liu; Susan K. Peirce; Chengyu Prince; Annette Vu;
Niel Chen; C. Patrick Reynolds; Michael D. Hogarty
United States

POT31 Cepharanthine reverses multidrug resistance and sensitizes neuroblastoma cells to vincristine-induced cell death
Regina Graham; John Thompson; James Guest; Keith Webster; Steven Vanni
United States

POT32 ATM deletion is a frequent event in neuroblastoma
F Gumy-Pause 1; H Oztasahn 1; M Khoshbeen-Boudal 1; B Pardo 1; DR Betts 2; P Maillet 1;
MD Hogarty 2; EF Attiyeh 2; AP Sappino 1
1Switzerland; 2Ireland; 3United States

POT33 Computer vision in neuroblastoma: computer-aided prognosis
Metin Gurcan; Olcay Sertel; Hiroyuki Shimada
United States

POT34 Analysis of aggressive human and mouse ALK neuroblastoma mutations
Bengt Hallberg; Sattu Kamaraj; Christina Schönhen; Kristina Ruuth; Cecilia Axelsson; Ruth Palmer
Sweden

POT35 Rapamycin upregulates osteoprotegerin and increases time to pathologic fracture in a mouse neuroblastoma bone metastasis model
Joseph Hartwich; Adrienne Myers; Cathy Ng; Andrew M Davidoff
United States

POT36 The addition of HIF inhibition potentiates the effects of angiogenesis inhibition in mouse neuroblastoma xenografts
Joseph Hartwich; Yunyu Spence; Cathy Ng; Christopher Morton; Andrew M Davidoff
United States

POT37 Screening at 18 months of age using the new serum marker for reducing the mortality of neuroblastoma: Simulation using Japanese population based cohort study
Eiso Hiyama; Arata Kamimatsuse; Naomi Kamei; Tsutomu Masujima; Keiko Hiyama; Megu Ohtaki
Japan

POT38 Effect Phosphoinositide-3-Kinase (PI3K) and mTOR dual inhibitors in Human Neuroblastomas
Ruth Ho; Jane Minturn; Valerie Brown; Radhika Iyer; Cecilia Sheen; Jessica Hulitt; Anisha Simpson;
Carly Varela; Jennifer Mangino; Venkatadri Kolla; Garrett Brodeur
United States

POT39 Analysis of molecular interactions between the GD2 ganglioside-specific mouse monoclonal antibody 14G2a and GD2-mimicking peptides
Irena Horwacik; Aleksandra Kowalczyk; Małgorzata Bzowska; Dominik Czaplicki; Hanna Rokita
Poland
POT40 NLRR2 is a novel regulator of neuroblastoma cell death via ER stress
Shamim Hossain; Atsushi Takatori; Jesmin Akter; Kamrul Hasan; Akira Nakagawara
Japan

POT41 Positive feedback loop of Mycn-nlrr1-egf/egfr signals in aggressive neuroblastomas
to accelerate cell growth
Shamim Hossain; Atsushi Takatori; Jesmin Akter; Yusuke Suenaga; Toshinori Ozaki; Akira Nakagawara
Japan

POT42 Application of chicken anti-human midkine IgY antibody to neuroblastoma diagnosis
Shinya Ikematsu; Shoma Tsubota; Chika Yamashiro; Satoshi Kishida; Yukio Yuzawa; Akira Nakagawara; Kenji Kadomatsu
Japan

POT43 Expression and gene status of HER2 in neuroblastic tumors
Ewa Izycka-Swieszewska; Agnieszka Wozniak; Jacek Kot; Wieslawa Grajikowska; Elzbieta Drozynska; Anna Balcerska; Danuta Perek; Bozena Dembowska; Janusz Limon
Poland; Belgium

POT44 Ki-67 proliferation index is marker of poor prognosis in neuroblastoma especially
in patients aged over 18 months
Ewa Izycka-Swieszewska; Beata Stefania Lipska; Elzbieta Drozynska; Anna Balcerska; Danuta Perek; Wieslawa Grajikowska; Bozena Dembowska; Teresa Klepacka; Wojciech Wozniak; Alicja Chybicka; Janusz Limon
Poland

POT45 MLPA (Multiplex Ligation-dependent Probe Amplification) and FISH comparison/validation
for genetic characterization of neuroblastoma
Marta Jeison; Gili Halevy-Berko; Galina Feinberg-Gorenstein; Svetlana Itskovitch; Shifra Ash; Jack Mardouck; Drorit Luria; Jerry Stein; Batia Stark; Smadar Avigad; Isaac Yaniv
Israel

POT46 Prolonged low-dose administration of the cyclooxygenase-2 inhibitor celecoxib
enhances the antitumor activity of irinotecan against neuroblastoma xenografts
Setsuko Kaneko; Michio Kaneko
Japan

POT47 CHD5 is part of the nucleosome remodeling and histone deacetylase (NuRD) complex
in neuroblastoma (NB) cell lines
Venkatadri Kolla; Tiangang Zhuang; Hiroshi Koyama; Koumudi Naraparaju; Mayumi Higashi; Gerd A. Blobel; Peter S. White; Garrett M. Brodeur
United States

POT48 Mechanisms of CHD5 inactivation in neuroblastomas
Hiroshi Koyama; Tiangang Zhuang; Jennifer E. Light; Venkatadri Kolla; Mayumi Higashi; Wendy B. London; Garrett M. Brodeur
United States

POT49 Neuroblastoma cell lines, phenotype and susceptibility towards natural killer cells
Kathelijne CJM Kraal; MM Ostaijen ten Dam; LM Ball; MJD van Tol; RM Egeler
Netherlands

POT50 UCHL1-Upregulation correlates with reduction of vital neuroblastoma cells
by fenretinide and doxorubicin treatment
Sandra Kuehnel; Grigore Cernaianu; Kai Stuehler; Helmut Meyer; Albrecht Bufe; Manfred Koeller; Barbara Sîtek; Ralf-Bodo Troebs
Germany

POT51 Low dose metronomic (LDM) administration of oral to potecan and pazopanib
as an effective preclinical antiangiogenic therapy in neuroblastoma
Sushil Kumar; Reza Mokhtari; Bing Wu; Libo Zhang; Shan Mann; Robert Kerbel; Herman Yeger; Sylvain Baruchel
Canada
POT52 Clinical significance of TRK family gene expression in neuroblastomas
Jennifer E. Light; Hiroshi Koyama; Eli Gordin; Jane E. Minturn; Radhika Iyer; Ruth Ho; Anisha M. Simpson; Venkatadri Kolla; Patrick W. McGrady; Wendy B. London; Garrett M. Brodeur
United States

POT53 Cancelled

POT54 Induction of miR-183 via an epigenetic mechanism suppresses neuroblastoma malignancy
Marco Lodrini; Johannes H. Schulte; Mirco Castoldi; Ina Oehme; Martina Muckenthaler; Olaf Witt; Hedwig E. Deubzer
Germany

POT55 Telomere elongation and chromosomal instability in non-MYCN amplified clinically aggressive neuroblastoma
Gisela Lundberg ¹; John-Kalle Länsberg ¹; Alexander Pietras ¹; Sven Påhlman ¹; Attila Frigyesi ¹; Victoria Castel ²; Samuel Navarro ²; Marta Piqueras ²; Rosa Noguera, ²; Tommy Martinsson ¹; David Gisselsson ¹
¹Sweden; ²Spain

POT56 Nanoparticle (NP) delivery of the Trk inhibitor lestaurtinib in neuroblastomas
Jennifer Mangino; Radhika Iyer; Michael Chorny; Ivan Alferiev; Jane Minturn; Ruth Ho; Anisha Simpson; Carly Varela; Audrey Evans; Robert Levy; Garrett Brodeur
United States

POT57 Bio-molecular and histo-pathological characterization of Neuroblastoma in adolescent and young adults (AYA). Italian experience with 33 cases
Katia Mazzocco; Raffaella Defferrari; Claudio Gambini; Angela Rita Sementa; Alberto Garaventa; Gian Paolo Tonini; Massimo Conte
Italy

POT58 Inhibition of PARP-1 enhances the efficacy of [131I]MIBG/Topotecan combinations in vitro
Anthony G McCluskey ¹; Annette Sorensen ¹; Mathias Tesson ²; Robert J Mairs ²; Marie Boyd ¹
¹United Kingdom; ²United Kingdom

POT59 CDK inhibitors Roscovitine and CR8 trigger Mcl-1 Down-regulation and apoptotic cell death in neuroblastoma cells
Laurent Meijer; Claire Delehouze; Nadège Loaec
France

POT60 Preclinical testing of novel kinase inhibitors in high-risk neuroblastoma
Ebba Palmberg; Linda Rickardson; Malin Wickstrom; Magnus Lindskog; John Inge Johnsen; Per Kogner
Sweden

POT61 Balance of pro- versus anti-angiogenic splice isoforms of vascular endothelial growth factor as a regulator of neuroblastoma growth
Maria Peiris ¹; David Owen Bates ¹; Pramila Ramani ²
¹United Kingdom; ²United Kingdom

POT62 Predictive consequences of risk stratification neuroblastoma patients using FISH on TMAP
Marta Piqueras; Samuel Navarro; Adela Cañete; Victoria Castel; Rosa Noguera
Spain

POT63 The effect of COX-2 expression on celecoxib sensitivity and tumour growth in neuroblastoma
Frida Ponthan; Emma Bell; C.P.F Redfern
United Kingdom

POT64 Targeting neuroblastoma and neuroblastoma tumour initiating cells with the oncolytic viruses myxoma and vesicular stomatitis virus
Nicole Redding ¹; Loen Hansford ¹; David Kaplan ¹; Grant McFadden ²; John Bell ¹; Steve Robbins ¹; Paul Beaudry ¹
¹Canada; ²United States
POT65 Survival pathways of high-risk neuroblastoma identified by functional genomics

Lauren Richard; James Annis; Carla Grandori; Julie Park
United States

POT66 Anti-angiogenic activity of the selective VEGFR-1,-2, -3 inhibitor Axitinib (AG-013736) in human neuroblastoma xenografts

Jochen Rössler 1; Yann Monnet 2; François Farace 2; Paule Opolon 2; Estelle Daudigeos-Dubus 2; Fabienne Munier 2; Gilles Vassal 2; Birgit Geoerger 2
1Germany; 2France

POT67 Relationship between ploidy and genetic instability of neuroblastoma in children below 18 months of age

Irene Tadeo; Amparo Ruiz-Sauri; Marta Piqueras; Eva Villamon; Ana P. Berbegall; Adela Cañete; Victoria Castel; Samuel Navarro; Rosa Noguera
Spain

POT68 Bi-directional regulation of the wild type of ALK in neuroblastoma: Its high expression in stage 4s tumors and transcriptional activation by MYCN and Sp1

Atsushi Takatori; Heggo Asmaa; Ajijur Rahman; Kamrul Hasan; Daisuke Takagi; Yasutoshi Tatsumi; Miki Ohira; Shamim Hossain; Jesmin Akter; Atsuko Nakagawa; Akira Nakagawara
Japan

POT69 CD133 regulates signal transduction pathways and prevents differentiation via RET suppression in neuroblastoma cells

Hisanori Takenobu; Osamu Shimozato; Hidemasa Ochiai; Yohko Yamaguchi; Miki Ohira; Akira Nakagawara; Takehiko Kamijo
Japan

POT70 Dendritic cell-based immunotherapy using sendai virus vector - a preclinical efficacy study against neuroblastoma: An advanced report

Sakura Tanaka; Tatsuro Tajiri; Yoshikazu Yonemitsu; Kyosuke Tatsuta; Ryota Souzaki; Yasuhide Ueda; Yuki Koga; Aiko Suminoe; Toshihiro Hara; Mamoru Hasegawa; Tomoaki Taguchi
Japan

POT71 DD3, a large non-coding RNA against the pro-apoptotic BMCC1 gene, is a candidate target for treating neuroblastoma

Yasutoshi Tatsumi; Tomoaki Yokoyama; Myat Lin Ooo; Ryo Takano; Daisuke Takagi; Miki Ohira; Akira Nakagawara
Japan

POT72 The novel PDK1 inhibitor OSU03012 and the dual PI3K/mTOR inhibitor PI103 target high-risk neuroblastoma in vitro and in vivo

Lova Segerström; Nibib Baryawno; Baldur Sveinbjörnsson; Lotta Elfman; Per Kogner; John Ingé Johnsen
Sweden

POT73 NK cells engineered to express the chimeric receptor scFv(ch14.18)-zeta specifically lyse GD2 expressing neuroectodermal tumors

Diana Seidel 1; Nicole Huebener 2; Tina Mueller 1; Doerthe Pferdmenges 1; Anastasia Shibina 2; Torsten Tonn 1; Ulrike Koehl 1; Ruth Esser 1; Winfried S. Wels 1; Holger N. Lode 3
1Germany; 2United States

POT74 Synergistic inhibition of neuroblastoma tumor development by targeting ornithine decarboxylase and topoisomerase II

Giselle Sholler; Erika Currier; Andre Bachmann
United States

POT75 The genetic and clinical implications of MYCN gain in neuroblastoma

Ryota Souzaki; Tatsuro Tajiri; Risa Teshiba; Yoshiaki Kinoshita; Sakura Tanaka; Tomoaki Taguchi
Japan

POT76 A new syngeneic MYCN-overexpressing neuroblastoma mouse model and MYCN-DNA vaccine

Alexander Stermann 1; Nicole Huebener 2; Diana Seidel 2; Anastasia Shibina 2; Stefan Fest 1; Holger N. Lode 3
1Germany; 2United States
POT77  Deregulation of rho/ras rnd neuronal differentiation pathways is associated with fatal outcome in high-risk disseminated neuroblastoma
Sara Stigliani 1; Simona Coco 1; Stefano Moretti 2; André Oberthuer 3; Jessica Theissen 3; Matthias Fischer 3; Francesca Valdora 1; Fabio Gallo 1; Carla De Vecchi 1; Alberto Garaventa 1; Frank Berthold 4; Stefano Bonassi 1; Gian Paolo Tonini 1; Paola Scaruffi 1
1Italy; 2France; 3Germany

POT78  Tumor cell detection in autologous stem cell harvests in patients with high risk neuroblastoma
J Slutterheim 1; F Vree 1; B Hero 2; L Zappeij - Kannegieter 1; C Voermans 1; R Schumacher-Kuckelkorn 2; U Koehl 3; J H Schulte 2; F Niggli 2; M C Fruhwald 2; M van Nooten 1; C M Niemeyer 2; U Bode 2; F H Schilling 2; C Schultz 2; N Graf 2; M Nathrath 3; I Schmid 2; H N Caron 1; C E van der Schoot 1; G A M Tjytga 1
1Netherlands; 2Germany; 3Switzerland

POT79  Functional analysis of the p53 pathway in neuroblastoma cells using the small-molecule MDM2 antagonist nutlin-3
Tom Van Maerken 1; Ali Rihani 1; Daniel Dreidax 2; Sarah De Clercq 1; Nurten Yigit 1; Jean-Christophe Marine 1; Frank Westermann 1; Anne De Paeppe 1; Frank Speleman 1; Jo Vandeweysepele 1
1Belgium; 2Germany

POT80  Detection of microRNAs in bone marrow from children with high-risk neuroblastoma predicts survival; a UK CCLG study.
Virginie Viprey 1; Maria Corrias 1; Walter Gregory 3; Penelope Brock 1; Susan Burchill 1
1United Kingdom; 2Italy; 3United Kingdom

POT81  SKP2-mediated neuroblastoma dedifferentiation is triggered by MYCN through CDK4 induction
Frank Westermann 1; Daniel Muth 1; Daniel Dreidax 1; Christina Pöhler 1; Sina Gogolin 1; Matthias Fischer 1; Kai Henrich 1; Volker Ehemann 1; Paul Gillespie 1; Manfred Schwab 1
1Germany; 2United States

POT82  DHA is converted to hydroperoxides and potentiates the cytotoxic effect of chemotherapeutics in neuroblastoma
Malin Wickström 1; Helena Gleissman 1; Rong Yang 2; Kimberly Martinod 2; Charles N. Serhan 2; John Inge Johnsen 1; Per Kogner 1
1Sweden; 2United States

POT83  Exploiting cell cycle aberrations in neuroblastoma by targeting checkpoint kinase chk1 using AZD7762 in neuroblastoma with p14<sup>ARF</sup>/MDM2/p53 defects
Hong Xu; Xiao Wei; Irene Cheung; Nai-Kong Cheung
United States

POT84  Preoperative analysis of 11q loss of heterozygosity using circulating tumor-released DNAin serum: A novel diagnostic tool for therapy stratification of neuroblastoma
Shigeki Yagyu; Tomoko Ichera; Takahiro Gotoh; Misuru Miyachi; Yoshiki Katsumi; Ken Kikuchi; Kunihiko Tsuchiya; Shinya Osone; Hiroshi Kuroda; Hajime Hosoi
Japan

POT85  Neuroblastoma tumor initiating cells express CD22 making them susceptible to HA22 anti-CD22 immunotoxin induced cell death
Shuang Yan 1; Zhijie Li 1; Amy Mckee 1; Maryalice Stetler-Stevenson 1; Loen Hansford 2; David Kaplan 2; Alan Wayne 1; Ira Pastan 1; Carol J Thiele 1
1United States; 2Canada

POT86  Genotype-guided neuroblastoma therapy, CP751,871 or Rapamycin
Libo Zhang; Herman Yeger; Reza Mohktari; Paula Marrano; Paul Thorner; David Kaplan; Sylvain Baruchel
Canada
Posters – Clinical

POC1 Neuroblastoma in children—experience in Croatia

*Mirna Anicic,* Ljubica Rajic; Ranka Femenic; Ernest Bilic; Josip Konja

*Croatia*

POC2 Phase I study of single agent perifosine for recurrent pediatric solid tumors

Oren Becher; Yasmin Khakoo; Shakeel Modak; David Lyden; Stephen Giltheeney; Jill Kolesar; Tanya Trippett; Eric Holland; Brian Kushner; Nai-Kong Cheung; Kim Kramer; Sofia Haque; Camelia Sima; Ira Dunkel

*United States*

POC3 Does the amount of bone marrow disease determine the outcome of patients with stage 4 neuroblastoma?

*Frank Berthold,* Roswitha Schumacher-Kuckelkorn; Barbara Hero; Thorsten Simon

*Germany*

POC4 Outcome of metastatic neuroblastoma treated with multi-modality approach including murine antiganglioside-2 monoclonal antibody (3F8)

Godfrey Chi-Fung Chan; Matthew Ming-Kong Shing; Rever Chak-Ho Li; Chun-Wing Luk; Siu-Cheung Ling; Chi-Kong Li; Shau-Yin Ha; Paul Kong-Hang Tam

*China*

POC5 Plasma fractionated total metanephrines for biochemical diagnosis of neuroblastoma

Laura Crosazzo Franscini; Eric Grouzmann; Mohamed Faouzi; Maja Beck Popovic

*Switzerland*

POC6 Apoptotic and adjuvant effects of triterpene-containing Viscum album L. extracts in neuroblastoma

*Catharina Delebinski,* Kristin Kemnitz-Hassanin; Ulrike Schmidt; Sebastian Jäger; Holger Lode; Georg Seifert

*Germany*

POC7 Phase I study of vincristine, irinotecan, and 131I-MIBG for patients with relapsed or refractory neuroblastoma: A new approaches to neuroblastoma therapy consortium study

*Steven DuBois* 1; Louis Chesler 2; Susan Groshen 1; Randall Hawkins 1; Hollie Jackson 1; Heike Daldrup-Link 1; Greg Yanik 1; Clinton Stewart 1; Yael Mosse 1; John Maris 1; Judith Villablanca 1; Katherine Matthy 1

1*United States; 2*United Kingdom*

POC8 Five-day courses of irinotecan in Chinese patients with refractory neuroblastoma

Qiushi Fan; Jinhua Zhang; Hongyu Zhao; Huawei Zou

*China*

POC9 Gefitinib (GFB) and Irinotecan (IRN) for children with high-risk (HR) neuroblastoma

Wayne Furman; Lisa McGregor; Clinton Stewart; Mihaela Onciu; Sandy Kovach; Andrew Davidoff; Victor Santana

*United States*

POC10 Does tumor histology after induction therapy predict outcome in patients with high-risk neuroblastoma?

Rani E. George; Antonio Perez-Atayde; Xiaopan Yao; Wendy B. London; Robert C. Shamberger; Lisa Diller

*United States*

POC11 Aromatic hydrocarbon receptor down-regulates MYCN expression and promotes neuronal differentiation of neuroblastoma

Wen-Ming Hsu; Pei-Yi Wu; Hsueh-Fen Juan; Hsinyu Lee

*Taiwan*

POC12 Results of treatment strategy of stage 4 infantile neuroblastoma based on born metastasis and MYCN amplification

Tomoko Iehara; Minoru Hamazaki; Takeo Tanaka; Hajime Hosoi; Tatsuro Tajiri; Michio Kaneko; Tohru Sugimoto; Tadashi Sawada

*Japan*
Meredith S. Irwin; Arlene Naranjo; Edward F. Attiyeh; Robert Seeger; Shahab Asgharzadeh; Richard Sposto; Lingyun Ji; Gregory Yanik; Yael P. Mosse; John M. Maris; Julie R. Park; Wendy London; Susan Kreissman; Michael D. Hogarty
1Canada; 2United States

POC14 False negative studies of neuroblastoma metastatic to the central nervous system (CNS)
Kim Kramer; Brian H Kushner; Shakeel Modak; Yasmin Khakoo; Neeta Pandit-Taskar; Hilda Stambuk; Mark M Souweidane; Nai-Kong Cheung
United States

POC15 Evolution of treatment strategies and risk stratification in management of neuroblastoma over two decades at tertiary cancer centre in India
Purna Kurkure; Tushar Vora; Brijesh Arora; Sripad Banavali; sajid Qureshi; Siddharth Laskar; Maryann Muckaden; V Seethalaxmi; Mukta Ramadwar; Sangeeta Desai; Seema Medhi; MGR Rajan; Akash Nahar; Gaurav Bahl; Seema Gulia
India

POC16 High-dose cyclophosphamide (Cy)-irinotecan (CPT-11)-vincristine (VCR) (HD-CCV) for primary refractory neuroblastoma
Brian Kushner; Kim Kramer; Shakeel Modak; Karima Yatahene; Nai-Kong Cheung
United States

POC17 A proposal for antibody based immunotherapy combined with haploidentical stem cell transplantation for high risk neuroblastoma
Peter Lang; Matthias Pleiffer; Ruth Ladenstein; Holger Lode; Ingo Müller; Tobias Feuchtinger; Philipp Schwarze; Rupert Handgretinger
1Germany; 2Austria

POC18 Illness experience and factors that constitute resilience in families with a neuroblastoma child
Ya-Ling Lee; Tzu-Chun Chen; Wen-Ming Hsu
Taiwan

POC19 The impact of a multidisciplinary team approach in the case management of neuroblastoma
Yen-Lin Liu; Wen-Ming Hsu; Hsiu-Hao Chang; Dong-Tsann Lin; Kai-Hsin Lin; Shiann-Tarng Jou; Meng-Yao Lu; Yung-Li Yang; Kai-Yuan Tzen; Steven Hsin-Feng Peng; Shiu-Feng Huang; Ya-Ling Lee
Taiwan

POC20 Second stem cell transplantation for relapsed high-risk neuroblastoma in Japan
Kimikazu Matsumoto; Koji Kato; Committee The Stem Cell Transplantation Japan
Japan

POC21 Role of minimal access surgery in children affected by neuroblastoma
Girolamo Mattioli; Stefano Avanzini; Piero Buffa; Alberto Michelazzi; Alberto Garaventa; Massimo Conte; Vincenzo Jasonni
Italy

POC22 Role of nursing in the implementation of chimeric anti-GD2 antibody with immunotherapy (ANBL0032) into clinical practice
Denise Mills; Anne Marie Maloney; Ann Chang
Canada

POC23 Phase I trial of Lestaurtinib for children with refractory neuroblastoma: A new approaches to neuroblastoma therapy (NANT) study
Jane E Minturn; Audrey E Evans; Judith G Villalba; Gregory A Yanik; Julie R Park; Susan Groshen; Edward T Hellriegel; Debra Bensen-Kennedy; Katherine K Matthey; Garrett M Brodeur; John M Maris
United States
POC24 Arsenic trioxide as radiosensitizer for 131I-MIBG therapy: Results of a pilot phase II study
Shakeel Modak; Neeta Pandit-Taskar; Jorge Carrasquillo; Brian H. Kushner; Kim Kramer; Pat Zanzonico; Peter Smith-Jones; Steven Larson; Nai-Kong V. Cheung
United States

POC25 Comparison of I-123 and I-131 mIBG scans in predicting survival in patients with stage 4 neuroblastoma
Arlene Naranjo; Marguerite T. Parisi; Barry L. Shulkin; Wendy B. London; Katherine K. Matthy; Susan G. Kreissman; Gregory A. Yanik
United States

POC26 Transverse myelopathy in neuroblastoma patients. Retrospective comparison of initial chemotherapy (CT) and neurosurgery (NS)
Catherina Annika Niemann; Barbara Hero; Boris De Carolis; Frank Berthold; Thorsten Simon
Germany

POC27 Decision of treatment reduction in selected children aged less than 18 months with a neuroblastoma without MYCN amplification and a numerical genomic profile
Charline Normand; Gudrun Schleiermacher; Gaelle Pierron; Agnès Ribeiro; Isabelle Janoueix-Lerosey; Pascale Philippe-Chomette; Thierry Van den Abbeele; Sabine Sarnacki; Y. Manach; Olivier Delattre; Jean Michon
France

POC28 Retinoids (RA) relieve EZH2-mediated epigenetic suppression of neuroblastoma differentiation
Doo-Yi Oh 1; Chan-Wook Woo 2; Chunxi Wang 1; Javed Khan 1; Carol J Thiele 1
1United States; 2Republic of Korea

POC29 Development of an automated quantitative method for scoring Metaiodobenzylguanidine (mIBG) scans in patients with neuroblastoma
Navin Pinto 1; Hiroyuki Abe 1; Daniel Appelbaum 1; Takeshi Hara 2; Yonglin Pu 1; Kunio Doi 1; Susan L. Cohn 1; Samuel L. Volchenboum 1
1United States; 2Japan

POC30 Is retroperitoneal lymphadenectomy for high risk abdominal neuroblastoma relevant
Sajid Qureshi; Purna Kurkure; Seethalakshmi Vishwanathan; Mukta Ramadwar; Sidharth Laskar
India

POC31 Concurrent ipsilateral nephrectomy versus kidney-sparing surgery in high-risk, intra-abdominal neuroblastoma
Amanda Roberts; Ahmed Nasr; Meredith Irwin; J. Ted Gerstle
Canada

POC32 Pilot study of high-dose chemotherapy using a novel preparative regimen with Busulfan, Melphalan, and Topotecan (TBM) followed by autologous hematopoietic stem cell transplant in high-risk neuroblastoma and other advanced stage and recurrent tumors
Joseph Rosenthal; Anna Pawlowska; Ellen Bolotin; Hossameldin Naeem; Andrew Dagis; Dajun Qian; Clarke Anderson
United States

POC33 Immunocytological GD2 negativity in treated and untreated neuroblastoma patients with bone marrow metastases
Roswitha Schumacher-Kuckelkorn; Barbara Hero; Anke Gradehandt; Thorsten Simon; Frank Berthold
Germany

POC34 Hematopoietic stem cell transplantation for high risk neuroblastoma in children
Larisa Shellkhova
Russian Federation

POC35 Follow-up study of survivors of childhood neuroblastoma - Report from a single institute in Japan
Hiroyuki Shichino; Motoaki Chin; Hirotugu Okuma; Eri Nishikawa; Maiko Hirai; Maiko Kato; Hiroshi Yagasaki; Tatsuhiro Urakami; Naokata Sumitomo; Yasuji Inamo; Hideo Mugishima
Japan
POC36  Retrospective analysis of treatment results of high risk neuroblastoma
Egor Shorikov; Olga Lemesheva; Tatiana Popova; Igor Vyatkin; Grigory Tsaur; Alexander Popov; Leonid Saveliev; Larisa Fechina
Russian Federation

POC37  Comparison of anti-GD2-antibody ch14.18 and 13-cis-retinoic acid as consolidation therapy for high-risk neuroblastoma. Results of the German NB97 trial
Thorsten Simon; Barbara Hero; Rupert Handgretinger; Martin Schrappe; Thomas Klingebiel; Michael C Fruehwald; Guenther Henze; Frank Berthold
Germany

POC38  Metachronous neuroblastoma in an infant with constitutional unbalanced translocation t(2;16)(p23;p13.3) involving ALK
Shui Yen Soh; Dimitri Stavropoulos; Sarah Bowdin; Paul Thorner; Sylvain Baruchel; David Malkin; M. Stephen Meyn; Meredith Irwin
Canada

POC39  Neuroblastomas with non-avid I$^{131}$MIBG scan and negative urinary catecholamine secretion: A single institute’s experience
Shui Yen Soh; Sylvain Baruchel; Meredith Irwin
Canada

POC40  Efficacy of tandem high-dose chemotherapy and autologous stem cell rescue in patients with high-risk neuroblastoma: a preliminary report of NB 2004 study at Samsung Medical Center
Ki Woong Sung; Heewon Cheuh; Soo Hyun Lee; Keon Hee Yoo; Hong Hoe Koo; Juyoun Kim; Eun Joo Cho; Kun Soo Lee
Republic of Korea

POC41  Measurement of tyrosine hydroxylase transcripts in bone marrow using biopsied tissue instead of aspirate for neuroblastoma
Ki Woong Sung; Seung-Tae Lee; Yeon Lim Suh; Young-Hyeh Ko; Chang-Seok Ki; Hee-Jin Kim; Jong-Won Kim; Sun-Hee Kim; Heewon Chueh; Soo Hyun Lee; Keon Hee Yoo; Hong Hoe Koo
Republic of Korea

POC42  Reduced-intensity allogeneic stem cell transplantation in children with neuroblastoma who have failed a prior tandem autologous stem cell transplantation
Ki Woong Sung; Heewon Cheuh; Soo Hyun Lee; Keon Hee Yoo; Hong Hoe Koo; Juyoun Kim
Republic of Korea

POC43  Neuroblastoma detected after ending of mass screening at 6 months of age in Japan
Tatsuro Tajiri; Ryota Souzaki; Yoshiaki Kinoshita; Sakura Tanaka; Yuhki Koga; Aiko Suminoe; Toshiro Hara; Tomoaki Taguchi
Japan

POC44  Whole-body diffusion-weighted MR imaging is useful to detect bone/bone marrow metastasis of neuroblastoma and monitor response to therapy
Yoshiyuki Takahashi; Nobuhiro Nishio; Hideki Muramatsu; Akira Shimada; Asahito Hama; Masafumi Ito; Kenichiro Kaneko; Hisami Ando; Hisroshi Fukatsu; Seiji Kojima
Japan

POC45  Identification of a therapy-sensitive subtype and stratification of progressive risk in advanced neuroblastomas
Takeo Tanaka; Yohko Kyo; Kunihiko Hayashi; Tomoko Iehara; Hajime Hosoi; Tohru Sugimoto; Minoru Hamasaki; Michio Kaneko; Tadashi Sawada
Japan

POC46  $^{18}$F-FDOPA PET scan is still useful in the presence of $^{123}$I-MIBG and $^{18}$F-FDG for neuroblastoma imaging
Kai-Yuan Tzen; Meng-Yao Lu; Hsiu-Hao Chang; Wen-Ming Hsu; Tsai-Yueh Luo; Lie-Hang Shen
Taiwan

POC47  Molecular imaging with $^{18}$F-FDOPA PET in the early detection of new metastatic neuroblastoma in bone marrow
Kai-Yuan Tzen; Meng-Yao Lu; Hsiu-Hao Chang; Wen-Ming Hsu
Taiwan
POC48  Efficacy of Treosulfan as a single agent in newly diagnosed neuroblastoma stage IV patients
Boyarshinov Vasiliy; Dolgopolov Igor; Pimenov Roman; Mentkevich George
Russian Federation

Page 213

POC49  Irinotecan/Temodal therapy as salvage treatment for children with neuroblastoma - single centre experience
Aleksandra Wieczorek; Walentyna Balwierz
Poland

Page 213

POC50  Minimal disease detection in non-metastatic neuroblastoma patients
Yania Yañez; Elena Grau; Silvestre Oltra; Adela Carhete; Francisco Martinez; Carmen Orellana; Rosa Noguera; Samuel Navarro; Victoria Castel
Spain

Page 213

POC51  Clinical report on the treatment of children in the late stage of neuroblastoma using chemotherapy combined with Zhongluo 3
Jinhua Zhang; Suning Chen; Fei Yu
China

Page 214

Posters – Late Breakers

POLB1  Effect of retinoic acid and chemotherapeutic agents on ultrastructural localization of Myc-N in neuroblastoma
Safiye Aktas; Zekiye Altun; Candan Ozogul; Nur Olgun; Dilek Gunes
Turkey

Page 214

POLB2  Betulinic acid affects metastasis related genes in neuroblastoma cells
Zekiye Altun; Safiye Aktas; Dilek Gunes; Nur Olgun
Turkey

Page 214

POLB3  Human neuroblastoma microenvironment supports T-cell activation in tumor associated lymphocytes
Lena-Maria Carlson; Anna DeGeer; Baldur Sveinbjörnsson; Abiel Orrego; Tommy Martinsson; Per Kogner; Jelena Levitskaya
Sweden

Page 215

POLB4  Expectant management of congenital adrenal neuroblastoma
Dennis A Cozzi; Amalia Schiavetti; Ermelinda Mele; Silvia Ceccanti; Simone Frediani; Anna Clerico; Carlo Dominici
Italy

Page 215

POLB5  Allicin increases metastasis related genes in neuroblastoma
Dilek Gunes; Safiye Aktas; Zekiye Altun; Nur Olgun
Turkey

Page 215

POLB6  Diagnostics and treatment of children with localized and locally advanced thoracoabdominal neuroblastoma
Anatoly Kazantsev; Andrey Ryabov; Polad Kerimov; Andrey Volobuev; Mikhail Rubansky
Russian Federation

Page 215

POLB7  Modeling the p53-Mdm2 core module in neuroblastoma
Florian Lamprecht; Daniel Dreidax; Sina Gogolin; Christina Pöhler; Manfred Schwab; Frank Westermann; Thomas Höfer
Germany

Page 216

POLB8  Biological characteristics of neuroblastoma in children of Belarus
Inna Proleskovskaya; Alena Valochnik; Natallia Savva
Belarus

Page 216

POLB9  Treatment results for neuroblastoma in children of Belarus
Inna Proleskovskaya; Tatyana Savich; Dmitriy Kochubinsky; Natallia Savva; Olga Aleinikova
Belarus

Page 216
POLB10  Autochthonous TNF alpha as an inducer of immune resistance and survival of neuroblastoma

Elian Rakhmanaliev 1; Jinxia Ma 1; Victor Levitsky 1; Murray Norris 2; Jelena Levitskaya 1

1United States; 2Australia

POLB11  Identifying lesions in translational control of gene expression in neuroblastoma by mRNA polysomal profiling and information-intensive computational integration

Angela Re; Erik Dassi; Viktoryia Sidarovich; Toma Tebaldi; Paola Scaruffi; Gian Paolo Tonini; Alessandro Quattrone

Italy

POLB12  An integrative bioinformatics approach in neuroblastoma identifies converging alterations in protein networks related to mitotic spindle assembly and splicing

Ooi Wen Fong; Angela Re; Natalia Arseni; Valentina Canella; Giulia Guarguaglini; Patrizia Lavia; Paola Scaruffi; Gian Paolo Tonini; Alessandro Quattrone

Italy

POLB13  Induction of human embryonic stem cells into sympathoadrenal cells

Michael D Hadjidaniel; Shahab Asgharzadeh

United States
18:00 – 18:15  Tuesday June 22nd
Hall A
Concluding remarks by Garrett Brodeur and Akira Nakagawara
Programme
Wednesday June 23rd
Wednesday June 23rd

08:00 – 09:00  Wednesday June 23rd
Hall A/B
The Road to Stockholm and Beyond 3
Chairs: Manfred Schwab and Frank Berthold

PL13 Infections linked to human cancers
Harald zur Hausen, Germany

Harald zur Hausen was born on March 11, 1936 in Gelsenkirchen-Buer, Germany. He studied Medicine at the Universities of Bonn, Hamburg and Düsseldorf and received his M.D. in 1960. After his internship he worked as postdoc at the Institute of Microbiology in Düsseldorf, subsequently in the Virus Laboratories of the Children’s Hospital in Philadelphia where he was later appointed as Assistant Professor. After a period of 3 years as a senior scientist at the Institute of Virology of the University of Würzburg, he was appointed in 1972 as Chairman and Professor of Virology at the University of Erlangen-Nürnberg. In 1977 he moved to a similar position to the University of Freiburg. From 1983 until 2003 he was appointed as Scientific Director of the Deutsches Krebsforschungszentrum (German Cancer Research Center) in Heidelberg. He retired from this position in 2003.

He had and has a number of special appointments, among them being the Chairman of several Scientific Price-Committees, between 1989-1991 Chairman of the Association of National Research Centers (Großforschungseinrichtungen) in Germany and from 1993 to 1996 President of the Organization of European Cancer Centers (OECI).

Zur Hausen received a number of national and international awards, among them the Robert-Koch-Price, the Charles S. Mott Price of the General Motors Cancer Research Foundation, the Federation of the European Cancer Societies Clinical Research Award, the Paul-Ehrlich-Ludwig Darmstätter-Price, the Jung-Price, Hamburg, the Charles Rudolphe Brupbacher Price, Zürich, the Prince Mahidol Award, Bangkok, the Raymond Bourgine Award, Paris, the Coley-Award, New York, the Life Science Achievement Award of the American Association for Cancer Research, San Diego, and the Nobel-Prize for Medicine, 2008. He received honorary doctorates from the Universities of Chicago, USA, Umeå, Sweden, Prague, Czech Republic, Salford, UK, Helsinki, Finland, Erlangen-Nürnberg and Würzburg, both Germany, Buenos Aires, Argentina and Ferrara, Italy.

He is an elected member of various academies (LEOPOLDINA, Heidelberg Academy of Sciences, Polish Academy of Sciences, Venezuela National Academy of Medicine, American Philosophical Society, Institute of Medicine of the National Academy of Sciences (USA), Foreign member of the US National Academy of Sciences and research organizations (EMBO, HUGO), and became an Honorary Member of a number of biomedical scientific societies. A large number of Special Lectures and Visiting Professorships, Memberships in Editorial Boards and active involvements in the organization of international meetings complement his curriculum.

From 2000 until 2009 zur Hausen was Editor-in-Chief of the International Journal of Cancer and from 2003 until 2009 Vice-President of the German National Academy for Natural Sciences and Medicine LEOPOLDINA in Halle.

Since 2006 he has been a Member of the Board of Directors of the International Union against Cancer (UICC), and a Member of the National Science Tansfer and Development Agency in Bangkok, Thailand.

PL14 The Yin and Yang functions of the Myc oncoprotein in cancer development and as targets for therapy
Marie Arsenian Henriksson, Sweden

Marie Arsenian Henriksson, BSc Uppsala University 1985, PhD Karolinska Institutet 1993, Postdoc Hannover Medical School, Germany 1993–1996, Associate Professor Karolinska Institutet 2001. Present position: Department Head MTC (Department of Microbiology, Tumor and Cell Biology) Karolinska Institutet.
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Authors</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00</td>
<td>PL15</td>
<td>Identification of selective inhibitors of neuroblastoma stem cells - Targeting the kinome</td>
<td>Natalie Grinshtein; Kristen Smith; David Uehling; Michael Prakesh; Methvin Isaac; Meredith Irwin; Alessandro Datti; Jeff Wrana; Rima Al-awar; David Kaplan</td>
<td>Canada</td>
</tr>
<tr>
<td>09:15</td>
<td>PL16</td>
<td>A new Aurora kinase inhibitor (CCT241736) regulates Mycn protein expression and prevents neuroblastoma growth in vitro and in vivo</td>
<td>Lynsey Vaughan; Elizabeth Cullis; Karen Barker; Yann Jamin; Spiros Linardopoulos; Vassilios Bavetsias; Butrus Atrash; Julian Blagg; Andrew Pearson; Simon Robinson; Louis Chesler</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>09:30</td>
<td>PL17</td>
<td>Mycn as a critical target of PI3K/mTOR inhibitors in neuroblastoma; paracrine effects on tumor vasculature</td>
<td>Yvan Chanthery; Chris Hackett; Melissa Itsara; Matt Grimmer; Louis Chesler; Katherine Matthay; William Weiss</td>
<td>United States; United Kingdom</td>
</tr>
<tr>
<td>09:45</td>
<td>PL18</td>
<td>Exploitation of ALK as a therapeutic target in neuroblastoma</td>
<td>Andrew Wood; Marci Laudenslager; Elizabeth Haglund; Joshua Courtright; Jeffrey Plegaria; Erica Carpenter; Sharon Diskin; Edward Attiyeh; Kristina Cole; Yana Toporovskaya; Bruce Pawel; Huaqing Zhao; Junghui Zhang; Patrick Reynolds; Patrick McGrady; Wendy London; Michele McTigue; Tami Marrone; Christensen James; John Maris; Yael Mosse</td>
<td>United States</td>
</tr>
<tr>
<td>10:00</td>
<td>PL19</td>
<td>An RNAi screen of the protein kinome identifies CHK1 as a therapeutic target in neuroblastoma</td>
<td>Kristina Cole; Jonathan Huggins; Michael Laquaglia; Chase Hulderman; Edward Attiyeh; Cynthia Winter; Sharon Diskin; Kristopher Bosse; Patrick Mayes; Jayanti Jagganathan; Geoffrey Norris; Yael Mosse; John Maris</td>
<td>United States</td>
</tr>
</tbody>
</table>

10:15 – 10:45 BREAK
10:45 – 12:00 Wednesday June 23rd
Hall A/B
Plenary session 4 – Translational
Chairs: John Maris and Gudrun Schleiermacher

10:45  PL20  Recruitment of histone deacetylase 2 by N-Myc and c-Myc to a transrepressor complex is a general therapeutic target in Myc-driven cancer
Glenn Marshall 1; Samuele Gherardi 2; Zilan Neiron 1; Toby Trahair 1; Pei Liu 1; Kacper Jankowski 1;
Nunzio Iraci 2; Michelle Haber 1; Murray Norris 1; Fabio Stossi 3; Benita Katzenellenbogen 2; Andrew
Biankin 1; Giovanni Perini 2; Tao Liu 1
1Australia; 2Italy; 3United States

11:00  PL21  Genome-wide mapping of MYCN binding sites in neuroblastoma reveals e-box motif frequencies and associations with regions of DNA hypermethylation
Derek Murphy; Patrick Buckley; Kenneth Bryan; Sudipto Das; Leah Alcock; Niamh Foley; Suzanne
Prenter; Isabella Bray; Karen Watters; Higgins Desmond; Raymond L. Stallings
Ireland

11:15  PL22  Accurate prediction of neuroblastoma outcome based on miRNA expression profiles
Stefanie Schlierf 1; Johannes Schulte 1; Benjamin Schowe 1; Pieter Mesdagh 2; Lars Kaderali 1;
Prabhav Kalaghatgi 1; Joelle Vermeulen 2; Bent Brockmeyer 1; Kristian Pajtler 1; Frank Speleman 2;
Katharina Morik 1; Angelika Eggert 1; Jo Vandesompele 2; Alexander Schramm 1
1Germany; 2Belgium

11:30  PL23  Individual survival time prediction from gene-expression and/or global genomic data of neuroblastoma patients using CASPAR
Andre Oberthuer; Prabhav Kalaghatgi; Yvonne Kahlert; Barbara Hero; Frank Berthold; Benedikt
Bros; Roland Eils; Matthias Fischer; Lars Kaderali
Germany

11:45  PL24  Identification of multiple protein disrupting mutations in stage 4 neuroblastoma using next generation sequencing transcriptome analysis
Thomas Badgett; Xiang Guo; Jun S Wei; Young K Song; Peter Johansson; Xinyu Wen; Qingrong
Chen; Catherine Tolman; Susan Yeh; Javed Khan
United States

12:00 – 13:00 LUNCH
### Programme 57

**Wednesday June 23**  
**Hall A**

**Parallel session 5 – Genomics, clinical correlation**  
**Chairs:** Tommy Martinsson and Wendy London

<table>
<thead>
<tr>
<th>Time</th>
<th>Title</th>
<th>Authors</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>13:00</td>
<td>Discovering epistatic genetic interactions associated with high-risk neuroblastoma</td>
<td>Mario Capasso; Kristopher Bosse; Sharon Diskin; Yael Mosse; Achille Iolascon, Marcella Devolo, John Maris</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1Italy; 2United States</td>
<td>116</td>
</tr>
<tr>
<td>13:10</td>
<td>Accumulation of segmental alterations determines progression in neuroblastoma</td>
<td>Gudrun Schliermann; Isabelle Janoueix-Lerosey; Agnes Ribeiro; Jerzy Klijianienko; Jerome Couturier; Gaelle Pierron; Veronique Mosseri; Alexander Valent; Nathalie Auger; Dominique Plantaz; Herve Rubie; Dominique Valatte-Couanet; Franck Bourdeaut; Valerie Combaret; Christophe Bergeron; Jean Michon; Olivier Delatitre</td>
<td>France</td>
</tr>
<tr>
<td>13:20</td>
<td>Improved outcome prediction of children with neuroblastoma using a miRNA signature</td>
<td>Pieter Mestdagh; Katleen De Preter; Joëlle Vermeulen; Arlene Naranjo; Isabella Bray; Victoria Castel; Caifu Chen; Angelika Eggert; Michael D Hogarty; Wendy B London; Rosa Noguera; Alexander Schramm; Johannes Schulte; Raymond Stallings; Rogier Versteeg; Geneviève Laureys; Nadine Van Roy; Frank Speleman; Jo Vandesompele</td>
<td>Belgium; United States; Ireland; Spain; Germany; Netherlands</td>
</tr>
<tr>
<td>13:30</td>
<td>Gene expression-based classification improves risk estimation of neuroblastoma patients</td>
<td>Andre Oberthuer; Barbara Hero; Frank Berthold; Dilatruz Juraeva; Andreas Faldum; Yvonne Kahlert; Shahab Ashgarzadeh; Robert Seeger; Paola Scaruffi; Gian Paolo Tonini; Isabelle Janoueix-Lerosey; Olivier Delatitre; Gudrun Schliermann; Jo Vandesompele; Joëlle Vermeulen; Frank Speleman; Rosa Noguera; Marta Piqueras; Jean Bénard; Alexander Valent; Smadar Avigad; Isaac Yaniv; Axel Weber; Holger Christiansen; Richard G. Grundy; Katharina Schardt; Manfred Schwab; Roland Eils; Patrick Warnat; Lars Kaderali; Thorsten Simon; Boris DeCarolis; Jessica Theissen; Frank Westermann; Benedikt Brors; Matthias Fischer</td>
<td>Germany; United States; Italy; France; Belgium; Spain; Israel; United Kingdom</td>
</tr>
<tr>
<td>13:40</td>
<td>Genomic portrait of tumor progression using next-generation sequencing</td>
<td>Jun Wei; Peter Johansson; Xiang Guo; Tom Badgett; Young Song; Xinyu Wen; Catherine House; Susan Yeh; Javed Khan</td>
<td>United States</td>
</tr>
<tr>
<td>13:50</td>
<td>A multi-local technique for risk evaluation of patients with neuroblastoma</td>
<td>Inge M. Ambros; Bettina Brunner; Clare Bedwell; Klaus Beiske; Jean Bénard; Nick Bown; Valerie Combaret; Jerome Couturier; Raffaella Defferrari; Nicole Gross; Marta Jeison; John Lunec 2; Barbara Marques; Tommy Martinsson; Katia Mazzocco; Rosa Noguera; Gudrun Schliermann; Frank Speleman; Ray Stallings; Gian Paolo Tonini; Deborah A Tweddle; Alexander Valent; Ales Vicha; Nadine Van Roy; Eva Villamon; Andrea Ziegler; Günther Schreier; Gerhard Aigner; Mario Drobsics; Ruth Ladenstein; Gabriele Amann; Jan Schouten</td>
<td>Austria; United Kingdom; Norway; France; Italy; Switzerland; Israel; Portugal; Sweden; Spain; Belgium; Ireland; United Kingdom; Czech Republic; Netherlands</td>
</tr>
<tr>
<td>14:00</td>
<td>Detecting the cutting edges. Highly sensitive and absolute specific detection of MYCN amplified neuroblastoma cells by amplicon-fusion-site (AFS) PCR</td>
<td>Axel Weber; Sylvia Taube; Sven Starke; Eckhard Bergmann; Nina Merete Christiansen; Holger Christiansen</td>
<td>Germany</td>
</tr>
</tbody>
</table>

**14:10 – 14:30 BREAK**
### Parallel session 6 – Differentiation and epigenetics

**Chairs:** Sven Påhlman and Godfrey C.F. Chan

<table>
<thead>
<tr>
<th>Time</th>
<th>Session ID</th>
<th>Title</th>
<th>Authors</th>
<th>Country(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13:00</td>
<td>OR40</td>
<td>The homeobox transcription factor HoxC9, a key regulator of development, suppresses tumourigenicity of neuroblastoma</td>
<td>Hayriye Kocak; Sandra Ackermann; Barbara Hero; Yvonne Kahlert; Jessica Theissen; Volker Ehemann; Frank Westermann; Margarete Odenthal; André Oberthur; Frank Berthold; Matthias Fischer</td>
<td>Germany</td>
</tr>
<tr>
<td>13:10</td>
<td>OR41</td>
<td>Identification of miRNAs implicated in neuronal development and neuroblastoma oncogenesis using fetal neuroblast miRNA profiles</td>
<td>Sara De Brouwer; Pieter Mestdagh; Nicky D’Haene; Johannes Schulte; Angelika Eggerts; Alexander Schramm; Rosa Noguera; Claire Hoyoux; Geneviève Laureys; Jo Vandesompele; Katleen De Preter; Frank Speleman</td>
<td>Belgium, Germany, Spain</td>
</tr>
<tr>
<td>13:20</td>
<td>OR42</td>
<td>Neuroblastoma Phox2b variants stimulate proliferation and de-differentiation of immature sympathetic neurons</td>
<td>Tobias Reiff; Konstantina Tsravinia; Afsaneh Majdazari; Mirko Schmidt; del Pino Isabel; Hermann Rohrer</td>
<td>Germany</td>
</tr>
<tr>
<td>13:30</td>
<td>OR43</td>
<td>Multidrug resistance-associated protein 4 regulates cAMP-dependent differentiation in neuroblastoma and represents a target for therapeutic inhibition</td>
<td>Murray Norris; Marcia Munoz; Claudia Flemming; Fujiko Watt; Anasuya Vishvanath; Michelle Henderson; Antonio Porro; Glenn Marshall; Giovanni Perini; Michelle Haber</td>
<td>Australia, Italy</td>
</tr>
<tr>
<td>13:40</td>
<td>OR44</td>
<td>Versatile <em>in vivo</em> roles for caspase-8 in neuroblastoma tumorigenesis</td>
<td>Tal Teitz; Madoka Inoue; Marcus Valentine; Kejin Zhu; Manrong Jiang; Jerold E. Rehg; Razqallah Hakem; William A. Weiss; Jill M. Lahti</td>
<td>United States, Canada</td>
</tr>
<tr>
<td>13:50</td>
<td>OR45</td>
<td>Histone deacetylase 8 in neuroblastoma tumorigenesis</td>
<td>Ina Oehme; Hedwig E. Deubzer; Dennis Wegener; Diana Pickert; Jan-Peter Linke; Barbara Hero; Annette Kopp-Schneider; Frank Westermann; Scott M. Ulrich; Andreas von Deimling; Matthias Fischer; Olaf Witt</td>
<td>Germany, United States</td>
</tr>
<tr>
<td>14:00</td>
<td>OR46</td>
<td>Genome-wide DNA methylation profiling reveals extensive and complex epigenetic alterations in neuroblastic tumors</td>
<td>Patrick Buckley; Sudipto Das; Kenneth Bryan; Karen Watters; Leah Alcock; Rogier Versteeg</td>
<td>Ireland, Netherlands</td>
</tr>
</tbody>
</table>

**14:10 – 14:30** **BREAK**
Workshop 4 – Future directions in targeted therapy for neuroblastoma
Organisers and chairs: John Inge Johnsen and Carol Thiele

13:00 – 14:30 Wednesday June 23rd
Hall C

Introduction

13:05  WS22  Perspectives in immunotherapy of neuroblastoma
       Holger Lode, Germany

13:17  WS23  Second generation GD2-targeted immunotherapy and future perspectives
       Alice Yu, United States

13:29  Discussion neuroblastoma immunotherapy

13:33  WS24  Targeting Signal Transduction Pathways – Taking aktion!
       Carol Thiele, United States

13:47  WS25  Targeting apoptosis pathways in neuroblastoma
       Simone Fulda, Germany

14:01  WS26  Omega-3 fatty acids in cancer: The protectors of good and the killers of evil?
       Helena Gleissman, Sweden

14:15  WS27  Optimizing drug development for neuroblastoma by close integration with adult oncology
       Pat Reynolds, United States

14:28  Closing remarks

14:30 – 14:45 BREAK
13:00 – 15:30  Wednesday June 23rd
Room 403
Charities special session

Meeting’s for charities representatives and other interested
Hosted by the Swedish Children’s Cancer Foundation.
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Authors/node</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:30</td>
<td>OR47</td>
<td>SIRT1 enhances N-Myc protein stability in a positive feedback loop which converts N-Myc expression from a low to high level</td>
<td>Glenn Marshall 1; Pei Liu 1; Samuele Gherardi 2; Chris Scarlett 1; Antonio Bedalov 3; Ning Xu 1; Nunzio Iraci 2; Margo van Bekkum 1; Eric Sekyere 1; Kacper Jankowski 1; Toby Trahair 1; Michelle Haber 1; Murray Norris 1; Andrew Biankin 1; Giovanni Perini 2; Tao Liu 1 1Australia; 2Italy; 3United States</td>
</tr>
<tr>
<td>14:40</td>
<td>OR48</td>
<td>Identification of therapeutical targets for MYCN-amplified neuroblastoma by functional genomics</td>
<td>Masafumi Toyoshima; Julie Park; Carla Grandori 1Australia</td>
</tr>
<tr>
<td>14:50</td>
<td>OR49</td>
<td>MYCN transcriptionally controls the expression of the Nijmegen Brekage Syndrome gene product p95 nibrin/NBS1</td>
<td>MariaLaura Petroni; Massimiliano Mellone; Sonia Albini; Veronica Veschi; Isabella Massimi; Isabella Sorepanti; Luigi Frati; Dorianna Fruci; Beatrice Cardinali; Alberto Gulino; Giuseppe Giannini 1Italy</td>
</tr>
<tr>
<td>15:00</td>
<td>OR50</td>
<td>Bmi1 is a MYCN target gene and regulates tumorigenesis via repression of KIF1B and TSLC1 in neuroblastoma</td>
<td>Hidemasa Ochiai; Hisanori Takenobu; Atsuko Nakagawa; Yoko Yamaguchi; Miki Ohira; Yuri Okimoto; Yoichi Kohno; Akira Nakagawara; Takehiko Kamijo 1Japan</td>
</tr>
<tr>
<td>15:10</td>
<td>OR51</td>
<td>The interplay between Mycn, microRNAs and estrogen receptor-α during differentiation of the post-migratory sympathetic nervous system</td>
<td>Jakob Lovén 1; Nikolay Zinin 1; Therese Wahlström 1; Inga Müller 1; Petter Brodin 1; Erik Fredlund 1; Ulf Ribacke 2; Andor Pivarcsi 1; Sven Pålhlman 1; Marie Henriksson 1 1Sweden; 2United States</td>
</tr>
<tr>
<td>15:20</td>
<td>OR52</td>
<td>Targeting MYCN by modulation of the fate of its mRNA: a new potential therapeutic approach for neuroblastoma</td>
<td>Viktoryia Sidarovich; Valentina Adami; Pamela Gatto; Gian Paolo Tonini; Alessandro Quattrone 1Italy</td>
</tr>
<tr>
<td>15:30</td>
<td>OR53</td>
<td>Bortezomib and HDAC inhibitor PCI-24781 show synergistic activity in neuroblastoma in vitro and in vivo models, inducing ROS and depressing MYCN</td>
<td>Erika Currier; Sharon Illenye; Jennifer Libous; Jeffrey Bond; Pamela Lescault; Giselle Sholler 1United States</td>
</tr>
<tr>
<td>15:40</td>
<td>OR54</td>
<td>Re-activation of CLUSTERIN by epigenetic drugs as a therapeutic approach for MYCN tumourigenesis</td>
<td>Daisy Corvetta 1; Olesya Chayka 1; Samuele Gherardi 2; Emanuele Valli 2; Sandra Cantilena 1; Izabela Piotrowska 1; Giovanni Perini 2; Arturo Sala 1 1United Kingdom; 2Italy</td>
</tr>
<tr>
<td>15:50</td>
<td>OR55</td>
<td>Identification of small molecules inhibiting Myc oncoprotein function</td>
<td>Karin Ridderstråle; Qinzi Yan; Siti Mariam Zakaria; Per Hydbring; Lars-Gunnar Larsson 1Sweden</td>
</tr>
</tbody>
</table>
Parallel session 8 – Novel clinical strategies and follow up
Chairs: Barbara Hero and Geneviéve Laureys

14:30

OR56 The role of dietary restriction in the mechanisms of differential cellular protection: a strategy to enhance the efficacy of chemotherapy in the treatment of neuroblastoma
Giovanna Bianchi, David Lee, Fernando Safdie, Laura Emionite, Vito Pistoia, Valter Longo, Lizzia Raffaghello
Italy; United States

14:40

OR57 Fenretinide (4-HPR) orally formulated in Lym-X-Sorb™(LXS) lipid matrix or as an intravenous emulsion increased 4-HPR systemic exposure in patients with Recurrent or Resistant Neuroblastoma. A new approaches to neuroblastoma therapy (NANT) consortium trial
Min H. Kang, Araz Marachelian, Judith G. Villablanca, John M. Maris, Matthew M. Ames, Joel M. Reid, Katherine K. Matthay, C. Patrick Reynolds, Barry J. Maurer
United States

14:50

OR58 Phase II trial of meta-iodobenzylguanidine (mIBG) with intensive chemotherapy and Autologous Stem Cell Transplant (ASCT) for high risk neuroblastoma. A New Approaches to Neuroblastoma Therapy (NANT) study
Gregory Yanik, Brian Weiss, John Maris, Judy Villablanca, Barry Shulkin, Araz Marachelian, Howard Katzenstein, Raymond Hutchinson, Ken Koral, David Hubers, Daphne Haas-Kogan, Susan Groshen, Rajen Mody, Adi Lewinson, Shelli Anuszkiewicz, Beth Hasenauer, Katherine Matthay
United States

15:00

OR59 A phase IIa trial of ultratrace (no-carrier added) iobenguane I-131 (MIBG): A New Approaches to Neuroblastoma Therapy (NANT) study
Katherine Matthay, Brian Weiss, Judith G. Villablanca, John Maris, Greg Yanik, Susan Groshen, Hollie Jackson, Randall Hawkins, Fariba Goodarzian, Ashok Panigrahy, Steven Dubois, James Stubbs, John Barrett, John Babich, Alexander Towbin, Norman LaFrance
United States

15:10

OR60 Characteristics of relapsing localized neuroblastoma: A preliminary report of the second SIOPEN study (LNESG2, localized neuroblastoma European study group 2)
Maja Beck Popovic, Emma Garcia, Nicole Gross, Valérie Combaret, Peter Ambros, Klaus Beiske, Alessandro Jenkner, Anne-Sophie Défachelles, Adela Cañete, Bénédicte Brichard, Walentina Balwierz, Vassilios Papadakis, Shifra Ash, Ellen Ruud, Ruth Ladenstein, Ingrid Ora, Keith Holmes, Bruno De Bernardi, Jean Michon, Véronique Mosseri
Switzerland; France; Austria; Norway; Italy; Spain; Belgium; Poland; Greece; Israel; Sweden; United Kingdom

15:20

OR61 Outcome for stage 3 neuroblastoma: A report from the Children’s Oncology Group
United States; Australia

15:30

OR62 Do relapsed high-risk neuroblastoma patients have a second chance? Results of the German neuroblastoma trials
Thorsten Simon, Frank Berthold, Arndt Borkhardt, Bernhard Kremens, Boris De Carolis, Barbara Hero
Germany

15:40

OR63 Anti-GD2 murine monoclonal antibody (MoAb) 3F8 for consolidation of first complete/very good partial remission of high risk stage 4 neuroblastoma
Nai-Kong Cheung, Brian H. Kushner, Kim Kramer, Shakeel Modak, Suzanne L. Wolden, Michael P. La Quaglia
United States
OR64  Changes over three decades in the prognostic influence of age in patients with neuroblastoma: A report from the International Neuroblastoma Risk Group Project
Veronica Moroz 1; David Machin 1; Andreas Faldum 2; Barbara Hero 2; Tomoko Iehara 3; Veronique Mosseri 4; Ruth Ladenstein 5; Bruno De Bernardi 6; Hervé Rubie 6; Frank Berthold 6; Katherine K. Matthay 7; Tom Monclair 8; Peter F. Ambros 8; Andrew D.J. Pearson 1; Susan L. Cohn 7; Wendy B. London 7

1United Kingdom; 2Germany; 3Japan; 4France; 5Austria; 6Italy; 7United States; 8Norway
14:45 – 15:55  Wednesday June 23rd
Hall C
Parallel session 9 – ALK
Chair: Isabelle Janouieux-Lerosey and Gian Paolo Tonini

14:45
OR65  Skewed distribution and oncogenic properties of ALK hotspot mutations in neuroblastoma
Candy Kumps 1; Sara De Brouwer 1; Piotr Zabrocki 1; Michaël Porcu 1; Ellen Westerhout 2; Arjan Lakeman 2; Jo Vandesompele 1; Jasmien Hoebeeck 1; Tom Van Maerken 1; Anne De Paepe 1; Geneviève Laureys 1; Johannes Schulte 2; Alexander Schramm 2; Joëlle Vermeulen 1; Nadine Van Roy 1; Klaus Beiske 4; Marleen Renard 4; Rosa Noguera 4; Olivier Delattre 6; Isabelle Janouieux-Lerosey 6; Per Kogner 7; Tommy Martinsson 7; Akira Nakagawara 8; Miki Ohira 8; Hub Caron 1; Karín Verstraeten 1; Ann De Bondt 1; Jan Cools 1; Jorge Vialard 1; Angelika Eggert 3; Rogier Versteeg 2; Katleen De Preter 1; Frank Speleman 1
1Belgium; 2Netherlands; 3Germany; 4Norway; 5Spain; 6France; 7Sweden; 8Japan

14:55
OR66  High ALK receptor tyrosine kinase expression precedes ALK mutation as a determining factor of an unfavorable phenotype in primary neuroblastoma
Johannes Schulte 1; Bent Brockmeyer 1; Hagen Bachmann 1; Sandra Nowacki 1; Benedikt Brors 1; Yvonne Kahlert 1; Andre Oberthur 1; Katleen de Preter 2; Kristian Pajtler 1; Jessica Theissens 1; Frank Westermann 1; Jo Vandesompele 2; Frank Berthold 1; Barbara Hero 1; Angelika Eggert 1; Alexander Schramm 2; Matthias Fischer 1
1Germany; 2Belgium

15:05
OR67  Risk stratification of neuroblastoma by genomic signature including ALK abnormality
Miki Ohira; Yohko Nakamura; Toshio Kojima; Junko Takita; Motohiro Kato; Seishi Ogawa; Shigeyuki Oba; Shin Ishii; Takehiko Kamiyo; Akira Nakagawara
Japan

15:15
OR68  Analysis of human ALK neuroblastoma mutations in Drosophila melanogaster
Therese Eriksson; Christina Schönherr; Kristina Ruuth; Bengt Hallberg; Ruth Palmer
Sweden

15:25
OR69  Role of ALK and its ligands in neuroblastoma
Fabienne Munier; Marie Regairaz; Céline Renauleaud; Estelle Daudigeos-Dubus; M. Luis Mir; Birgit Geoerger; Gilles Vassal
France

15:35
OR70  Effects of selective ALK inhibitors to neuroblastoma
Junko Takita; Jun Okhobo; Riki Nishimura; Kentaro Ohki; Naoki Uchisaka; Yuyan Chen; Masashi Sanada; Akira Kikuchi; Takashi Igarashi; Yasuhide Hayashi; Seishi Ogawa
Japan

15:45
OR71  Therapeutic targeting of ALK on neuroblastoma cells by systemic delivery of GD₂-targeted liposomes entrapping small interfering RNA
Daniela Di Paolo; Chiara Ambrogio; Fabio Pastorino; Chiara Brignole; Roberta Carosio; Monica Loi; Gabriella Pagnan; Michele Cilli; Domenico Ribatti; Roberto Chiarle; Mirco Ponzoni; Patrizia Perri
Italy
16:05 SEL25 Identification and molecular characterization of human neuroblastoma tumor-initiating cells
Aurelie Coulon; Marjorie Flahaut; Annick Mühlethaler-Mottet; Julie Liberman; Gregor Kiowski; Lukas Sommer; Nicole Gross Switzerland

16:10 SEL26 Synergy of targeted GMCSF and IL2 to tumor microenvironments is mediated by an adaptive anti-neuroblastoma immune response
Lode, Holger; Bleeke, Matthias; Reisfeld, Ralph; Siebert, Nicolai
1University of Greifswald, Pediatric Hematology and Oncology, Greifswald, Germany; 2TSRI, Immunology, La Jolla, United States

16:15 SEL27 Opposite roles of distinct caspase-10 isoforms in death receptor apoptotic pathway
Annick Mühlethaler-Mottet; Katia Balmas Bourloud; Katya Nardou; Nicole Gross Switzerland

16:20 SEL28 The tumor suppressor candidate gene APITD1/CENP-S on chromosome 1p36 is involved in chromosome segregation and DNA damage repair
Cecilia Krona; Hanna Kryh; Samantha Zeitlin; Dan Foltz; Don Cleveland; Katarina Ejeskär; Rose-Marie Sjöberg; Helena Carén; Tommy Martinsson
1Sweden; 2United States

16:25 SEL29 Conditional MYCN knockdown using shRNAs encoded by lentivirus vectors
Jørn Remi Henriksen; Bjørn Helge Haug; Jochen Buchner; Cecilia Løkke; Trond Flægstad; Christer Einvik Norway

16:30 SEL30 Integration of genome-wide ChIP-data of MYCN/MYC and histone marks with gene expression
Filip Pattyn; Christina Pöhler; Daniel Muth; Stephan Gade; Tim Beißbarth; Frank Speleman; Manfred Schwab; Jo Vandesompele; Frank Westermann
1Belgium; 2Germany

16:35 SEL31 RUNX3, mapped to chromosome 1p36, is a tumor suppressor functionally regulating p53 and MYCN in neuroblastoma
Tomoki Yokochi; Wei Gao; Fan Yu; Chizu Yamada; Toshinori Ozaki; Miki Ohira; Yohko Nakamura; Ken-ichi Inoue; Yoshiaki Ito; Atsuko Nakagawa; Akira Nakagawara
1Japan; 2Singapore

16:40 SEL32 The p53 target Wig-1 is a novel regulator of N-Myc at the mRNA level
Anna Vilborg; Cinzia Bersani; Margareta Wilhelm; Weng-Onn Lui; Klas Wiman Sweden
<table>
<thead>
<tr>
<th>Time</th>
<th>Poster</th>
<th>Title</th>
<th>Authors</th>
<th>Countries/Institutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:05</td>
<td>SEL33</td>
<td>Neurocristopathy-associated Phox2b mutations cause Sox10 dysregulation and affects self-renewal, proliferation and differentiation of autonomic neural progenitors</td>
<td>Hideki Enomoto; Mayumi Nagashimada; Hiroshi Ohta; Teruhiko Wakayama; Kazuki Nakao</td>
<td>Japan</td>
</tr>
<tr>
<td>16:10</td>
<td>SEL34</td>
<td>In vivo analysis of human neuroblastoma cell lines in a human embryonic stem cell derived microenvironment - Impact of cues from the microenvironment</td>
<td>Jessica Cedervall; Seema Jamil; Isabell Hultman; Rouknuddin Ali; Lena Kanter; Abiel Orrego; Bengt Sandstedt; Baidur Sveinbjörnsson; John Inge Johnsen; Per Kogner; Lars Åhrlund-Richter</td>
<td>Sweden</td>
</tr>
<tr>
<td>16:15</td>
<td>SEL35</td>
<td>Tenascin-C+/Oct-4+ perivascular neuroblastoma cells serve as progenitors of tumor-derived endothelial cells</td>
<td>Annalisa Pezzolo; Federica Parodi; Danilo Marimpietri; Lizzia Raffagellho; Claudia Cocco; Angela Pistorio; Manuela Mosconi; Claudio Gambini; Michele Cilli; Silvia Deagilo; Fabio Malavasi; Vito Pistoa</td>
<td>Italy</td>
</tr>
<tr>
<td>16:20</td>
<td>SEL36</td>
<td>NVP-BEZ235 a dual PI3K/mTOR inhibitor destabilises Mycn in vitro and is growth inhibitory in the TH-MYCN murine neuroblastoma model</td>
<td>Lynsey Vaughan; Elizabeth Cullis; Karen Barker; Yann Jamin; Simon Robinson; Andrew Pearson; Carlos Garcia-Echeverria; Michel Maira; Louis Chesler</td>
<td>United Kingdom; Switzerland</td>
</tr>
<tr>
<td>16:25</td>
<td>SEL37</td>
<td>Development of novel therapeutic strategy for neuroblastoma: Reactivation of the p53 tumor suppressor function by small molecules</td>
<td>Elisabeth Hedström; Yao Shi; Mikhail Burmakin; Galina Selivanova</td>
<td>Sweden</td>
</tr>
<tr>
<td>16:30</td>
<td>SEL38</td>
<td>Modeling neuroblastomagenesis from neural crest stem cells in vitro and in vivo</td>
<td>Johannes Schulte; Anna Bohrer; Sven Lindner; Katleen de Preter; Frank Speleman; Jo Vandesompele; Jan Molenaar; Rogier Versteeg; Kristian Pajtler; Jochen Maurer; Hubert Schorle; Alexander Schramm; Angelika Eggert</td>
<td>Germany; Belgium; Netherlands</td>
</tr>
<tr>
<td>16:35</td>
<td>SEL39</td>
<td>FOXO3/FKHRL1 is activated in high-risk neuroblastoma and contributes to chemotherapy-resistance and angiogenesis</td>
<td>Kathrin Geiger; Judith Hagenbuchner; Martina Rupp; Christina Salvador; Bernhard Meister; Consolato Sergi; Petra Obexer; Michael Ausserlechner</td>
<td>Austria; Canada</td>
</tr>
<tr>
<td>16:40</td>
<td>SEL40</td>
<td>Segmental chromosome aberrations and ploidy in localized neuroblastomas without MYCN amplification – Report from the SIOP Europe Neuroblastoma (SIOPEN) Group on the LNESG I Trial</td>
<td>Inge M Ambros; Gian Paolo Tonini; Jerome Couturier; Klaus Beiske; Jean Benard; Maria Boavida; Nick Bown; Huib Caron; Valerie Combaret; Raffaella Defferrari; Nicole Gross; Marta Jeison; John Lunec; Tommy Martinsson; Katia Mazzocco; Rosa Noguera; Gudrun Schlieermacher; Alexandre Valen; Nadine Van Roy; Andrew DJ Pearson; Ruth Ladenstein; Veronique Mosseri; Bruno De Bernardi; Jean Michon; Peter F Ambros</td>
<td>Austria; Italy; France; Norway; Portugal; United Kingdom; Netherlands; Switzerland; Israel; Sweden; Spain; Belgium; United Kingdom</td>
</tr>
</tbody>
</table>
16:00 – 16:45 Wednesday June 23rd
Hall C
Selected poster – Translational 2
Chairs: Meredith Irwin and Rochelle Bagatell

16:05 SEL41 Segmental chromosome abnormalities and age over 36 months at diagnosis are associated with increased risk of relapse in localised unresectable neuroblastoma without MYCN amplification - A preliminary report from the SIOPEN Neuroblastoma (SIOPEN) Biology Group
R Defferrari; K Mazzocco; IM Ambros; PF Ambros; C Bedwell; C Beiske; J Benard; N Bown; V Castel; V Combaret; J Couturier; B De Bernardi; A Garaventa; N Gross; R Hant; J Kohler; M Jeason; R Ladenstein; J Lunec; B Marques; T Martinsson; R Noguera; S Parodi; H Rubie; G Schlieirmacher; F Speleman; A Valenti; N Van Roy; A Vicha; E Villamon; GP Tonini
for the SIOPEN Biology Group

16:10 SEL42 Drug-induced senescence in MYCN-amplified neuroblastoma - gene expression profiling and functional consequences
Sabine Taschner-Mandl 1; Agata Kowalska 1; Heidemarie Binder 1; Dietmar Rieder 1; Zlatko Trajanoski 1; Javed Khan 2; Frank Speleman 3; Inge M Ambros 1; Peter F Ambros 1
1 Austria; 2 United States; 3 Belgium

16:15 SEL43 Parvovirus H1 induces oncolytic effects on human neuroblastoma cells in vitro and in neuroblastoma xenograft-bearing nude rats
Jeannine Lacroix; Barbara Leuchs; Georgi Hristov; Junwei Li; Hedwig E. Deubzer; Jean Rommelaere; Olaf Witt; Jörg R. Schlehofer
Germany

16:20 SEL44 Targeting MYCN in neuroblastoma with small molecules in vitro and in vivo
Hanna Zirath; Lova Segerström; Anna Frenzel; Per Kogner; Marie Henriksson
Sweden

16:25 SEL45 Protein interactions of the PHOX2B variants identified in patients with neuroblastoma
Wenchao Wang; Quan Zhong; William Luther II; A. Thomas Look; David Hill; Marc Vidal; Rani E. George
United States

16:30 SEL46 Targeted therapeutics in chemotherapy-refractory neuroblastoma
W. Clay Gustafson 1; Benjamin Houseman 1; Louis Chesler 2; Melissa itsara 1; Kevan Shokat 3; William A Weiss 1
1 United States; 2 United Kingdom

16:35 SEL47 Validation of Survivin as a therapeutic target in neuroblastoma
Fieke Lamers; Linda Schild; Ida van der Ploeg; Marl Ebus; Jan Koster; Rogier Versteeg; Huib Caron; Jan Molenaar
Netherlands

16:40 SEL48 Rituximab is a novel neuroblastoma therapy with efficacy against neuroblastoma tumor initiating cells in vitro and in vivo
Paola Angelini; Loe Hansford; David Kaplan; Meredith Irwin
Canada
16:00 – 18:30  Wednesday June 23\textsuperscript{rd}
Poster session – All posters will be displayed throughout the meeting

Odd numbers/left aligned posters = Presenting authors present at posters Tuesday June 22\textsuperscript{nd} 16:45 – 17:30
Even numbers/indented posters = Presenting authors present at posters Wednesday June 23\textsuperscript{rd} 16:45 – 17:30
18:00 – 18:15  Wednesday June 23rd
Hall A
Concluding remarks by Robert Seeger and Angelika Eggert
<table>
<thead>
<tr>
<th>Time</th>
<th>Hall A</th>
<th>Hall B</th>
<th>Hall C</th>
<th>Poster Halls</th>
</tr>
</thead>
<tbody>
<tr>
<td>07:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>08:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>08:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>08:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>08:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>09:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>09:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>09:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>09:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19:45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Thursday June 24th

08:00 – 09:00 Thursday June 24th
Hall A/B
The Road to Stockholm and Beyond 4
Chairs: Olivier Delattre and Murray Norris

PL25  Modeling neuropsychiatric disorders in the mouse
Mario Capecchi, United States

Mario Capecchi graduated from George School and received his B.S. in chemistry and physics from Antioch College in 1961 and his Ph.D. in biophysics from Harvard University in 1967. He completed his thesis work under the guidance of Dr. James D. Watson, co-discoverer of the structure of DNA. Mario advanced quickly through the ranks at Harvard Medical School but he was looking for something different. He sought an environment that believed in long-term investment, one that would allow him to address the big questions. Dr. Capecchi began his research at the University of Utah, 1973. Thirty-four years later, on December 10, 2007, Dr. Capecchi received the highest honor in his field, the Nobel Prize, for his work in molecular biology. His pioneering work in gene targeting of mouse embryo-derived stem cells has set a new standard for research worldwide. This renowned discovery holds endless possibilities for development of treatments and ultimately cures for every known human disease. Dr. Capecchi believes we, as citizens of the world, we have many challenges that face us. Health issues and many diseases that plague us... but also global concerns such as war, equal opportunity for all people, the world economy and most importantly global warming. Millions of lives are in peril. We must begin now to address these issues and make a difference while we still can.

PL26  Genetic and developmental therapeutic studies in a transgenic mouse model for high-risk neuroblastoma
Bill Weiss, United States

William A. Weiss MD, PhD is a Professor of Neurology, Pediatrics, and Neurosurgery at UCSF, oversees child neurology at San Francisco General Hospital, codirects the pediatric malignancies program in UCSF’s cancer center, is associate editor of Cancer Research and of the NeuroOncology Journals, and advises brain tumor programs at the Children’s Hospital of Los Angeles, Mayo Clinic, Saint Jude Children’s Research Hospital, and the University of Calgary. Dr. Weiss’ lab has developed murine models of glioma, medulloblastoma and neuroblastoma based on recapitulating cardinal genetic abnormalities in transgenic mice and is using these mice to investigate both Mycn and EGFR/PI3K/mTOR signaling pathways. Dr. Weiss organized two past international meetings of scientists and physicians modeling neural tumors in the mouse. He received graduate degrees from Stanford University, completed residency training at Boston Children's and UCSF, and postdoctoral training with J. Michael Bishop, MD.
**Thursday June 24**

**Hall A/B**

**Plenary session 5 – Clinical**

**Chairs: Tom Monclair and Mattias Fischer**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Authors</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00</td>
<td>PL27</td>
<td>Widespread dysregulation of miRNAs by MYCN amplification and chromosomal imbalances in neuroblastoma: Association of miRNA expression with survival</td>
<td>Isabella Bray 1; Kenneth Bryan 1; Suzanne Prenter 1; Patrick G Buckley 1; Niamh H Foley 1; Derek M Murphy 1; Leah Alcock 1; Pieter Vandesompele 2; Frank Speleman 3; Wendy B London 4; Patrick W McGrady 5; Desmond G Higgins 1; Anne O'Meara 1; Kateleen De Preter 1; Maureen O'Sullivan 1; Raymond L Stallings 1</td>
<td>1Ireland; 2Belgium; 3United States</td>
</tr>
<tr>
<td>09:15</td>
<td>PL28</td>
<td>Evaluation of PHOX2B, tyrosine hydroxylase (TH), GD2 and ELAVL4 expression for minimal residual disease (MRD) detection in neuroblastoma patients</td>
<td>Alexander Druy; Grigory Tsaur; Alexander Popov; Tatyana Verzhbitskaya; Egor Shorikov; Leonid Saveliev; Larisa Fechina</td>
<td>Russian Federation</td>
</tr>
<tr>
<td>09:30</td>
<td>PL29</td>
<td>QRT-PCR for TH and Phox2B mRNA in peripheral blood and bone marrow from children with high risk neuroblastoma predicts overall survival; a SIOPEN molecular monitoring group study</td>
<td>Virginie Viprey 1; Maria Corrias 2; Andrei Tchirkov 3; Katrien Swerts 4; Ales Vicha 5; Sandro Dallorso 2; Walter Gregory 6; Roberto Luksch 7; Penelope Brock 8; Dominique Valteau-Couanet 9; Genevieve Laureys 4; Josef Malis 5; Vassilios Papadakis 7; Pavel Bician 8; Ruth Ladenstein 9; Susan Burchill 9</td>
<td>1United Kingdom; 2Italy; 3France; 4Belgium; 5Czech Republic; 6United Kingdom; 7Greece; 8Slovakia; 9Austria</td>
</tr>
<tr>
<td>09:45</td>
<td>PL30</td>
<td>Analyses of mIBG scoring as a prognostic indicator in patients with stage 4 neuroblastoma. A Children’s Oncology Group (A3973) report</td>
<td>Gregory Yanik; Marguerite Parisi; Barry Shulkin; Arlene Naranjo; Susan Kreissman; Wendy London; Judy Villablanca; Patrick McGrady; Katherine Matthay</td>
<td>United States</td>
</tr>
<tr>
<td>10:00</td>
<td>PL31</td>
<td>Characterization of neuroblastoma imaging studies using F-18-DOPA PET/CT</td>
<td>Kai-Yuan Tzen; Meng Yao Lu; Hsiu-Hao Chang; Kai-Hsin Lin; Shiann-Tarng Jou; Yung-Li Yang; Dong-Tsann Lin; Wen-Ming Hsu</td>
<td>Taiwan</td>
</tr>
</tbody>
</table>

**10:15 – 10:45 BREAK**
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
</table>
| 10:45 | PL32    | Clinical and biological features predictive of survival after relapse of neuroblastoma: A study from the International Neuroblastoma Risk Group (INRG) Database | Victoria Castel 1; Kate K Matthay 1; Tom Monclair 1; Andrew D Pearson 1; Susan L. Cohn 2; Wendy B London 2  
Spain; United States; Norway; United Kingdom |
| 11:00 | PL33    | Topotecan-vincristine-doxorubicin in metastatic neuroblastoma failing to respond to rapid COJEC. Preliminary results of a Siopen Group Study | Loredana Amoroso 1; Guy Makin 1; Ruth Ladenstein 1; Genevieve Laureys 1; Roberto Luksch 1; Victoria Castel 2; Peppy Brock 2; Caroline Thomas 4; Dominique Valteau-Couanet 4; Alberto Garaventa 1  
Italy; United Kingdom; Austria; Belgium; Spain; France |
| 11:15 | PL34    | Suppression of human anti-mouse antibody response by rituximab plus cyclophosphamide permits continuation of anti-GD2 immunotherapy | Shakeel Modak; Brian H. Kushner; Kim Kramer; Irene Y. Cheung; Nai-Kong V. Cheung  
United States |
| 11:30 | PL35    | Long term outcome: the price of treatment for surviving high-risk neuroblastoma | Barbara Hero; Simon Thorsten; Dagmar Dilloo; Bernhard Kremens; Lorenz Grigull; Hans-Gerhard Scheel-Walter; Frank Berthold  
Germany |
| 11:45 | PL36    | Long-term toxicity in survivors of ENSG5 trial for children with high-risk neuroblastoma | Lucas Moreno 1; Sucheta Vaidya 1; Ross Pinkerton 2; Ian J Lewis 1; John Imeson 1; Caroline Ellershaw 1; David Machin 1; Andrew DJ Pearson 1  
United Kingdom; Australia |
| 12:00 |         | LUNCH                                                                 |                                                                                            |

---

10:45 – 12:00 Thursday June 24
Hall A/B
Plenary session 6 – Clinical
Chairs: Ruth Ladenstein and Julie Park
13:00 – 14:30 Thursday June 24th
Hall A/B
Parallel session 10 – Genomics, candidate loci
Chairs: Peter Ambros and Frank Westermann

13:00 OR72 A regulatory BCL2 promoter polymorphism (-938C>A) is associated with outcome in neuroblastoma
Bent Brockmeyer; Hagen Bachmann; Annette Kunkele; Kristian Pajtler; Winfried Siffert; Angelika Eggert; Alexander Schramm; Johannes Schulte
Germany

13:10 OR73 Identification of critical domains that mediate the transcriptional and growth-inhibiting functions of the neuroblastoma tumor suppressor gene CASZ1
Ryan Virden; Zhihui Liu; Carol Thiele
United States

13:20 OR74 KIF1Bβ tumor suppressor, identified from the homozygous deletion at chromosome 1p36.2, interacts with YME1L1 metalloprotease to induce apoptosis through mitochondrial morphogenesis and cytochrome c release
Koji Ando; Kiyohiro Ando; Tomoki Yokochi; Sonja Kramer; Akira Mukai; Toshinori Ozaki; Akira Nakagawara
Japan

13:30 OR75 Coordinate expression of Let-7 family members in neuroblastoma and their dysregulation by DNA copy number loss
Fernandez Raquel; Bryan Kenneth; Patrick G Buckley; Isabella Bray; Leah Alcock; Raymond L Stallings
Ireland

13:40 OR76 A genome-wide association study (GWAS) of neuroblastoma
John Maris 1; Sharon Diskin 1; Kristopher Bosse 1; Le Nguyen 1; Robert Schnepf 1; Edward Attiyeh 1; Yael Mosse 1; Mario Capasso 2; Cynthia Winter 1; Maura Diamond 1; Marci Laudenslager 1; Kai Wang 1; Haitao Zhang 1; Cuiping Hou 1; Cecilia Kim 1; Joseph Glessner 1; Wendy London 1; Nazneen Rhaman 3; Hongzhe Li 1; Marcella Devoto 1; Hakon Hakonarson 1
1United States; 2Italy; 3United Kingdom

13:50 OR77 Acquired segmental copy number changes in relapsed neuroblastoma
David Cobrinik; Irene Y. Cheung; Nai-Kong V. Cheung
United States

14:00 OR78 Identification and characterization of somatic rearrangements in neuroblastoma cell lines using genome-wide massively parallel sequencing
Isabelle Janoueix-Lerosey; Valentina Boeva; Stéphanie Jouannet; Romain Daveau; Alex Cazes; Gudrun Schleiermacher; Valérie Combaret; Emmanuel Barillot; Olivier Delattre
France

14:10 OR79 Genome/transcriptome analysis of metastatic neuroblastoma, reveals an increase of structural aberrations and deregulation of rho/ras and telomerase pathways associated with poor patients outcome
Simona Coco 1; Jessica Theissen 2; Paola Scaruffi 1; Sara Stigliani 1; Stefano Moretti 3; André Oberthuer 2; Barbara Hero 2; Matthias Fischer 2; Stefano Bonassi 1; Fabio Gallo 1; Carla De Vecchi 1; Frank Berthold 2; Gian Paolo Tonini 1
1Italy; 2Germany; 3France

14:20 OR80 Irregular chromosome segregation by tripolar divisions; mechanisms for heterogeneity of karyotypes in neuroblastoma
Fumio Kasai 1; Hirofumi Kobayashi 1; Willem Rens 2; Malcolm A. Ferguson-Smith 3; Yasuhiro Kaneko 1
1Japan; 2United Kingdom; 3United Kingdom

14:30 – 14:45 BREAK
13:00 – 14:30 Thursday June 24th
Hall C
Parallel session 11 – Prognostic factors and markers
Chairs: Kurkure Purna and Dominique Valteau-Couanet

13:00 **OR81** Is subtotal resection sufficient for treatment of ganglioneuroma and localized ganglioneuroblastoma intermixed?
Boris De Carolis; Thorsten Simon; Ivo Leuschner; Dietrich von Schweinitz; Thomas Klingebiel; Rudolf Erttmann; Lothar Schweigerer; Peter Kaatsch; Frank Berthold; Barbara Hero
Germany

13:10 **OR82** Survival variability by race and ethnicity in neuroblastoma: A Children’s Oncology Group (COG) Study
Navin Pinto; Tara Henderson; Smita Bhatia; Wendy London; Patrick McGrady; Catherine Crotty; Can-Lan Sun; Susan L. Cohn
United States

13:20 **OR83** Stable incidence of neuroblastoma during 28 years in Sweden with significant sex differences and improved survival, in particular for children with high-risk disease with MYCN amplification
Catarina Träger; Åsa Vernby; Helena Caren; Hanna Kryh; Fredrik Hedborg; Tommy Martinsson; Göran Gustafsson; Per Kogner
Sweden

13:30 **OR84** Long term outcome and impact of biology within risk adapted treatment strategies: The Austrian neuroblastoma trial A-NB94
Ruth Ladenstein; Inge Ambros; Ulrike Poetschger; Christian Urban; Georg Ebetsberger; Bernhard Meister; Gabriele Aman; Ekkehart Spuller; Karin Dieckmann; Ernst Horcher; Bettina Brunner; Andrea Ziegler; Peter Ambros
Austria

13:40 **OR85** Exon-Level gene expression analyses of primary neuroblastoma improves risk prediction and identifies MYCN status as major determinant of alternative transcript use
Alexander Schramm 1; Benjamin Schowe 1; Tobias Marschall 1; Marcel Martin 1; Joelle Vermeulen 2; Jo Vandeseompele 2; Jessica Theissen 1; Barbara Hero 1; Theresa Thorn 1; Katharina Monik 1; Sven Rahmann 1; Angelika Eggert 1; Johannes Schulte 1
1Germany; 2Belgium

13:50 **OR86** High expression of KIF1Bβ-interacting protein MAP1A and its family member MAP1B significantly correlates with favourable prognosis of neuroblastoma
Sonja Kramer 1; Miki Ohira 1; Tomoki Yokochi 1; Koji Ando 1; Akira Mukai 1; Angelika Eggert 2; Akira Nakagawara 1
1Japan; 2Germany

14:00 **OR87** Determination of 17q gain in neuroblastoma patients by analysis of circulating DNA
Valerie Combaret; Stephanie Brejon; Isabelle Iacono; Gudrun Schleiermacher; Gaelle Pierron; Agnes Ribeiro; Christophe Bergeron; Aurelien Marabelle; Alain Puisieux
France

14:10 **OR88** Phox2B but not TH mRNA detected by QRT-PCR in peripheral blood stem cell harvest predicts time to relapse in randomised children with high risk neuroblastoma; a SIOPEN molecular monitoring group study
Sandro Dallorso 1; Maria Corrias 1; Virginie Viprey 2; Ales Vicha 3; Katrien Swerts 4; Andrei Tchirkov 5; Walter Gregory 2; Roberto Luksch 1; Penelope Brock 2; Josef Malis 3; Genevieve Laureys 4; Dominique Valteau-Couanet 5; Ruth Ladenstein 6; Susan Burchill 2
1Italy; 2United Kingdom; 3Czech Republic; 4Belgium; 5France; 6Austria

14:20 **OR89** Clinical utility of minimal residual disease marker panel during sequential phases of a multi-modality treatment of high-risk neuroblastoma
Irene Cheung; Brian Kushner; Kim Kramer; Shakeel Modak; Nai-Kong Cheung
United States

14:30 – 14:45 BREAK
14:45 – 15:30 Thursday June 24th
Hall A/B
Closing and Awards of ANR 2010
Towards ANR 2012!
Chairs: Michelle Haber and Susan L. Cohn
## Contents

<table>
<thead>
<tr>
<th>Neuroblastoma update course</th>
<th>C1 – C12</th>
<th>78</th>
</tr>
</thead>
<tbody>
<tr>
<td>Workshops</td>
<td>WS1 – WS27</td>
<td>81</td>
</tr>
<tr>
<td>Special sessions</td>
<td>SS1 – SS4</td>
<td>87</td>
</tr>
<tr>
<td>Plenary sessions</td>
<td>PL1 – PL36</td>
<td>88</td>
</tr>
<tr>
<td>Parallel sessions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 – Targeting kinases</td>
<td>OR1 – OR7</td>
<td>106</td>
</tr>
<tr>
<td>2 – Tumour initiating stem cells</td>
<td>OR8 – OR14</td>
<td>108</td>
</tr>
<tr>
<td>3 – Immunotherapy</td>
<td>OR15 – OR23</td>
<td>110</td>
</tr>
<tr>
<td>4 – p53 and molecular targets</td>
<td>OR24 – OR32</td>
<td>112</td>
</tr>
<tr>
<td>5 – Genomics, clinical correlation</td>
<td>OR33 – OR39</td>
<td>116</td>
</tr>
<tr>
<td>6 – Differentiation and epigenetics</td>
<td>OR40 – OR46</td>
<td>118</td>
</tr>
<tr>
<td>7 – Targeting MYCN</td>
<td>OR47 – OR55</td>
<td>120</td>
</tr>
<tr>
<td>8 – Novel clinical strategies</td>
<td>OR56 – OR64</td>
<td>123</td>
</tr>
<tr>
<td>9 – ALK</td>
<td>OR65 – OR71</td>
<td>126</td>
</tr>
<tr>
<td>10 – Genomics, candidate loci</td>
<td>OR72 – OR80</td>
<td>128</td>
</tr>
<tr>
<td>11 – Prognostic factors and markers</td>
<td>OR81 – OR89</td>
<td>130</td>
</tr>
</tbody>
</table>

### Selected posters

| Biology 1                          | SEL1 – SEL8 | 134 |
| Clinical                           | SEL9 – SEL16 | 136 |
| Translational 1                    | SEL17 – SEL24 | 138 |
| Biology 2 and 3                    | SEL25 – SEL40 | 140 |
| Translational 2                    | SEL41 – SEL48 | 144 |

### Posters

| Biology                          | POB1 – POB119 | 147 |
| Translational                   | POT1 – POT86 | 178 |
| Clinical                        | POC1 – POC51 | 201 |
| Late Breakers                   | POLB1 – POLB13 | 214 |

### Author index

218

### Keyword index

233
Neuroblastoma update course

C1 Using genome-wide strategies to discover new gene aberrations

Spelman, Frank
Belgium

What is needed to successfully combat cancer? For certain childhood cancers, such as neuroblastoma, much progress could be made if we were able to achieve high cure rates, e.g., above 80% for acute lymphoblastic leukemias. However, treatment with DNA damaging drugs can cause serious side effects due to the lack of specificity, an issue which is of particular importance when treating children. Also, treatment may ultimately fail due to intrinsic and acquired drug resistance. Recent successes in ‘targeted therapeutics’ have raised the hope that an increasing number of highly effective drugs specifically inhibit a genetic defect in cancer cells. Direct pre-treatment side effects will become available. Few cancer types however carry a common ‘driver’ mutation thus precluding a simple ‘one tumor - one target’ strategy. Many tumors carry complex genetic alterations impeding the definition of clear mechanistic principles to develop targeted therapeutic strategies. Moreover, even with a well-defined molecular target and highly efficient therapeutic compound, drug resistance occurs and therefore a strategy based on targeting multiple pathways or multiple vulnerable nodes in a key pathway (or both) is warranted. Present genome-wide technologies are allowing a deeper probing into the cancer genome with unprecedented speed for detecting DNA copy number alterations with resolution up to a few kb or less, genome wide expression profiling at the exon level and high throughput sequencing of entire cancer genomes, chromatin marks and methylation patterns. New RNA sequencing and open the perspective of achieving molecular portraits of individual cancer genomes, a prelude to personalized medicine in cancer treatment. In order to be able to rationally use this rapidly increasing amount of genetic information, we are in desperate need for equally performant assays that allow determining the functional relevance of mutations or differentially expressed genes. At the same time, we will need a systems biology approach to translate these results to the various levels of genomic information in order to track down connections and nodes of the complex regulatory networks that are perturbed in cancer. In this presentation I will give an overview of the current progress and the art of genome-wide technologies for studying cancer as well as emerging fields and future challenges.

C2 Biological and clinical relevance of ALK mutations

George, Fan Yi; Mosse, Yael
1Dana-Farber Cancer Institute, Harvard Medical School, Department of Pediatric Oncology, Boston, United States; 2Children’s Hospital of Philadelphia, Department of Oncology, Philadelphia, United States

No abstract available

C3 International Neuroblastoma Risk Group (INRG): Next steps

Pearson, A.D.J.; London, W.B.; Cohn, S.L.; and the INRG Task Force.
1Institute of Cancer Research, Royal Marsden Hospital, United Kingdom; 2Harvard Medical School, Children’s Hospital Boston, Boston, United States; 3University of Chicago, Section of Hematology / Oncology, Chicago, United States

The International Neuroblastoma Risk Group (INRG) classification system was developed to establish a consensus approach for pre-treatment risk classification. The statistical and clinical significance of 13 potential prognostic factors were analyzed in a cohort of 8,800 children diagnosed with neuroblastoma between 1990-2002 from North America and Australia, Europe, and Japan. Factors prognostic of event-free survival were identified using survival tree regression analyses. Stage, age, histologic category, grade of tumor differentiation, the status of the MYCN oncogene, chromosome 11q status, and DNA ploidy were the most highly significant independent factors and these were utilised in the classification system, published in 2009. The INRG is now being utilized in prospective co-operative clinical studies. Consensus documents on biological features, staging, and detection of minimal residual disease, MIBg scanning and entry and response criteria for phase II studies have been published or are in preparation. Twelve retrospective studies have been undertaken utilizing the INRG data base. The INRG has been a significant success, but the challenge is now to build on this system and develop a more precise classification system - the second INRG. Already the prognostic impact of the presence of or absence of segmental chromosomal alterations is being employed in therapeutic decisions. The next INRG will utilise extensively genomic data, both DNA and mRNA and possibly data from methylation, micro RNA and genome-wide association studies. Incorporating data relating to aberrations of the ALK pathway is also a focus. It is anticipated that inclusion of genomic data may allow some clinical data for example age not be needed to describe the population. The current INRG cohort has been analyzed and clinically and statistically significant complementarity between the absence of image-defined risk factors (IDRF), a key component of the new INRG staging system, and the likelihood of a successful minimally invasive surgery. The two areas that continue to be challenging for application of minimally invasive surgery are: resection of extensive, infiltrative retroperitoneal and thoracic masses, and thorough lymph node dissection. In the former situation, current minimally invasive techniques are neither delicate nor earlier return to normal activities, and improved cosmesis. These gains are balanced by persistent challenges, which include monoscopic vision, decreased manipulative degrees of freedom, and lack of tactile feedback. Surgery remains a key modality in diagnosis and treatment of patients with neuroblastoma. The practice of minimally invasive surgery in the treatment of neuroblastoma continues to evolve as technical capabilities improve and the overall role of surgery adapts to our current therapeutic strategy for this disease. As the importance of biological tumor characteristics in risk stratification has increased, it has become increasingly crucial to obtain adequate tumor tissue at initial presentation. Both image-guided needle biopsy and videoscopic incisional biopsy are useful techniques that can decrease the morbidity associated with tumor acquisition for histologic and molecular studies. A team approach among surgeons, radiologists, pathologists and oncologists is crucial to assure that accurate tissue is obtained and the appropriate studies are performed. Thoracoscopy and laparoscopy are likely to become more prevalent in definitive resection of primary neuroblastoma tumors in the coming years. These techniques are ideally suited to dissection of well-localized tumors that do not invade or encase adjacent vital structures. In fact, there is significant complementarity between the absence of image-defined risk factors (IDRF), a key component of the new INRG staging system, and the likelihood of a successful minimally invasive surgery. The two areas that continue to be challenging for application of minimally invasive surgery are: resection of extensive, infiltrative retroperitoneal and thoracic masses, and thorough lymph node dissection. In the former situation, current minimally invasive techniques are neither delicate nor efficient enough to accomplish these procedures. Although laparoscopy can be used successfully for retroperitoneal lymph node sampling, this technique is not generally used for resection of the bulky nodal disease that accompanies large infiltrative retroperitoneal tumors. The barriers to increased utilization of minimally invasive surgery for treatment of neuroblastoma are both technical and educational. If previous trends continue, it is likely that minimally invasive surgery will continue to improve in the coming years. It is important to assure that training programs focus on increasing the skills of residents and fellows to keep pace with these technical improvements.

C4 Using PET and MIBG to evaluate disease and response

Sharp, Susan
Cincinnati Children’s Hospital Medical Center, Department of Radiology, Cincinnati, United States

Functional imaging plays an important role in neuroblastoma assessment. I-123-MIBG remains the most frequently used functional imaging agent with a high sensitivity and specificity for neuroblastoma. I-123-MIBG is useful in depicting disease extent at diagnosis, following therapy response, and localizing residual/recurrent disease. Use of FDG PET is increasing and studies continue to clarify its role. FDG has been shown to be especially useful in situations where information in the absence of FDG activity in areas that should be considered when MIBG demonstrates less disease than suspected by conventional imaging or clinical symptoms. The spatial resolution of FDG PET/CT also aids in disease delineation, especially in the chest, abdomen, and pelvis.

C5 The evolving role of minimally invasive surgery in treatment of neuroblastoma

Nucktern, Jed
Texas Children’s Hospital/ Baylor College of Medicine, Pediatric Surgery United States

Minimally invasive surgery, both thoracoscopy and laparoscopy, have emerged as important therapeutic modalities over the past two decades. These techniques have many advantages over traditional open approaches including better visualization, decreased postoperative pain, earlier return to normal activities, and improved cosmesis. These gains are balanced by persistent challenges, which include monoscopic vision, decreased manipulative degrees of freedom, and lack of tactile feedback. Surgery remains a key modality in diagnosis and treatment of patients with neuroblastoma. The practice of minimally invasive surgery in the treatment of neuroblastoma continues to evolve as technical capabilities improve and the overall role of surgery adapts to our current therapeutic strategy for this disease. As the importance of biological tumor characteristics in risk stratification has increased, it has become increasingly crucial to obtain adequate tumor tissue at initial presentation. Both image-guided needle biopsy and videoscopic incisional biopsy are useful techniques that can decrease the morbidity associated with tumor acquisition for histologic and molecular studies. A team approach among surgeons, radiologists, pathologists and oncologists is crucial to assure that adequate tissue is obtained and the appropriate studies are performed. Thoracoscopy and laparoscopy are likely to become more prevalent in definitive resection of primary neuroblastoma tumors in the coming years. These techniques are ideally suited to dissection of well-localized tumors that do not invade or encase adjacent vital structures. In fact, there is significant complementarity between the absence of image-defined risk factors (IDRF), a key component of the new INRG staging system, and the likelihood of a successful minimally invasive surgery. The two areas that continue to be challenging for application of minimally invasive surgery are: resection of extensive, infiltrative retroperitoneal and thoracic masses, and thorough lymph node dissection. In the former situation, current minimally invasive techniques are neither delicate nor efficient enough to accomplish these procedures. Although laparoscopy can be used successfully for retroperitoneal lymph node sampling, this technique is not generally used for resection of the bulky nodal disease that accompanies large infiltrative retroperitoneal tumors. The barriers to increased utilization of minimally invasive surgery for treatment of neuroblastoma are both technical and educational. If previous trends continue, it is likely that minimally invasive surgery will continue to improve in the coming years. It is important to assure that training programs focus on increasing the skills of residents and fellows to keep pace with these technical improvements.

ANR 2010, June 21-24 2010
Neuroblastoma
treatment reduction, whereas for patients with an unfavourable profile, a neuroblastome in whom it can be deemed safe to propose controlled profile might be used to identify patients with low or intermediate-risk patients with low and intermediate-risk nB. Indeed, a favourable genomic markers and will be helpful for further treatment stratification, especially in genomic profile adds critical prognostic information to conventional clinical individual markers, is important to predict relapse in nB patients. the profile characterized by the presence of segmental alterations is the genetic and clinical markers with prognostic significance, a genomic account the genomic profile, but also previously described individual alterations in tumor progression. In multivariate analyses, taking into consideration the used treatment modalities (3 year overall survival > 95%). current after diagnosis in those patients, the outcome is excellent regardless of clinical regression is seen in one third of patients beyond the 1. year of life. 6. regression may be observed in patients with 1p aberration and with more than 40% long term survival despite intensive chemotherapy followed by mIBg, if the therapy is continued beyond the interim assessment. 7. Approximately 25 % of the patients present symptoms of clinical regression or objective treatment response. 8. Approximately 25 % of the patients present symptoms requiring chemotherapeutic intervention. 9. Approximately 25 % of the patients present symptoms due to the presence of segmental alterations is the strongest predictor of relapse, rather than individual genetic markers. Thus, the analysis of the overall genomic pattern, which probably unravels particular genomic instability mechanisms, rather than the analysis of individual markers, is important to predict relapse in NB patients. The genomic profile adds critical prognostic information to conventional clinical markers and will be helpful for further treatment stratification, especially in patients with low and intermediate-risk NB. Indeed, a favourable genomic profile might be used to identify patients with low or intermediate-risk neuroblastoma in whom it can be deemed safe to propose controlled treatment reduction, whereas for patients with an unfavourable profile, an upfront increase in treatment could be justified.
Late Outcomes after Treatment for Neuroblastoma

Diller, Lisa
Dana-Farber Cancer Institute/Children's Hospital, Pediatric Oncology, Boston MA, United States

Few data are available on the the long-term outcomes in children who have been treated for neuroblastoma. Reductions in therapy for low and intermediate risk neuroblastoma should be expected to be associated with fewer late effects. However, recent improvements in therapy for high-risk neuroblastoma along with more widespread use of stem cell transplantation should result in more survivors. Our goals were to determine in delineating the medical, social and psychological outcomes in survivors of aggressive chemoradiotherapy. This presentation will review available published data from the Childhood Cancer Survivor Study and other large cohort studies, as well as from small clinical case series of survivors. Late mortality risk and the causes of late mortality will be discussed. The risk of musculoskeletal and growth problems will be reviewed, as well as the risks of hearing loss, endocrinopathy, secondary leukemias and solid tumors. At the Dana-Farber Cancer Institute/Children's Hospital, we reviewed the long-term outcomes in 95 patients treated for high risk neuroblastoma in the time period 1994-2007 to determine hazard prevalence of hypothyroidism, insulin resistance, short stature, hearing loss, ovarian failure, cardiomyopathy, school problems and dental agenesis. These unpublished data will be reported. The role of radiation compared with other therapies in the induction of late effects will be discussed.

Clinical and biological features predictive of survival after relapse of neuroblastoma: A study from the International Neuroblastoma Risk Group (INRG) database

London, Wendy B
Children's Oncology Group Harvard Medical School, Boston, United States

Background: In NB, most patients (pts) who relapse eventually die. Prognostic factors are used to stratify treatment at diagnosis, but typically not at the time of relapse. Our goals were to determine research in delineating which factors were predictive of time to death post-relapse; b) if time from diagnosis until relapse/progression has a predictive role.

Methods: Retrospective analysis included INRG pts with first event of relapse, progressive disease, or secondary malignancy (excluding pts whose first event was death). Time from diagnosis until event ("time-to-first-event") was calculated and analyzed as <1 year (yr) vs. ≥1 yr. 5-yr estimates of overall survival (OS) vs. standard survival (SOS); time from first event until death or last contact, are presented (lifetable methods). Time-to-first-event was tested in a multivariable Cox model (adjusting for clinical and biologic factors; hazard ratios (HR) for increased risk of death post-relapse were calculated.

Results: From 8,800 INRG pts, 2,266 experienced a non-death first event. Median time to relapse was 13.2 months (mo) (range: 1 day to 11.4 yrs). The 5-yr OS after first event was 20%±1%. Time-to-first-event (HR=1.8), age >18 mo (HR=2.3), INSS stage 4 (HR=3.4), MYCN amplified (HR=2.8), diploidy (HR=1.6), high MKI (HR=2.0), undifferentiated grade (HR=1.6), and 1p aberration (HR=1.7) were significantly predictive of death after relapse (p<0.0001), but not 11q aberration. Compared to pts whose first event occurred <6 mo from diagnosis, pts who relapsed 6-<18 mo from diagnosis had increased risk of death, while relapses ≥18 mo from diagnosis had decreased risk of death. Shorter time-to-first-event was not independently predictive of death after adjustment for undifferentiated grade, high MKI, MYCN amplification, or diploidy.

Conclusions: Time to first relapse is a significant predictor of time to death after relapse; however, the risk of death is higher for pts who relapse within 6-<18 mo, but lower for pts who relapse ≥18 mo from diagnosis. Stratification of relapsed NB pts according to the timing of first relapse, age, stage, MYCN, and MKI, and diploidy is important in retrieval study designs.

Molecularly-targeted therapy in Neuroblastoma

Chester, Louise
The Institute of Cancer Research, Paediatric Oncology, Sutton, United Kingdom

Improvements in survival rates for children's cancers have stagnated within the last decade, and have been achieved in large part through intensified dosing of standard chemotherapeutic agents. This has resulted in incremental increases in drug toxicity, secondary malignancy and long-term disability for paediatric patients. Clearly, improved therapeutics and approaches are required. In theory, molecularly-targeted therapeutics that selectively inhibit the activity of a single molecule can be synthesized. Ideally the targeted molecule should play an essential role in the genesis or maintenance of the cancer of interest, such that partial or complete inhibition is cytotoxic to tumour cells and results in tumour regression in the absence of any secondary, "off-target" effects. In practice, very few molecules with such ideal characteristics have been identified and successfully "drugged" in adult cancer to date, and arguably no molecule with a function restricted to a paediatric cancer has been rationally targeted with a selective drug. Nevertheless, a variety of compelling targets with favourable therapeutic profiles have emerged lately, through the application of enhanced sequencing and coordinated "omics" approaches. In Neuroblastoma, discrete molecular targets exist and are being identified which are of substantial therapeutic interest by virtue of their unique functions, pharmaceutical accessibility and significant association with clinical outcome. Amplification of the MYCN gene is among the first known oncogenic mutations associated with a paediatric cancer, and has been used to stratify therapy for many years. Expression of the Mycn oncoprotein is largely restricted to tumour tissue and can initiate spontaneous tumours in transgenic mice, making it an ideal therapeutic target. Nevertheless no drug exists to date targeting this critical oncoprotein. Although attempts to inhibit the dimerisation function of this transcription factor have failed, recent publications point to indirect approaches that could stimulate destabilisation of Mycn oncoprotein, and would be predicted to impact progression of “MYC-addicted" tumours. Several potential therapeutics are in development that could address this mechanism. Somatic tumour and germline point mutations in the anaplastic lymphoma kinase (ALK) gene have been identified in a significant proportion of Neuroblastoma patients, implying a critical role for ALK in the initiation of this disease. Attempts are underway to establish the oncogenic role of ALK through genome analysis. Initial clinical evaluation of an ALK-targeted agent is in progress. Further efforts will likely be required to develop agents more specifically targeted to individual, etiologic ALK mutations as these are unravelled. Finally, aberrant expression of critical molecular signalling pathways occurs in discrete clinical subgroups of Neuroblastoma, (i.e.: WNT pathway in high-risk patients without MYCN gene amplification) providing additional opportunities for development of pathway-specific strategies. Taken together, these findings suggest additional approaches by which we could incorporate molecularly-targeted therapeutics into existing treatment strategies for discrete subgroups of high-risk neuroblastoma patients.
Workshops
WS1–WS27

WS1
Tumor initiating/stem cells, hypoxia and vascularization - what are the connections?

Pietras, Alexander1; Johansson, A Sofie2; Gisselsson, David2; Wigergup, Caroline3; Pihlman, Sven1
1 Lund University, University Hospital Malmö, Center for Molecular Pathology, Department of Laboratory Medicine, CREATE Health, Malmö, Sweden; 2 Lund University, Department of Clinical Genetics, Lund, Sweden

We have isolated tumor initiating/stem cells (TICs) from bone marrow of patients with aggressive neuroblastoma. We have further shown that TICs have high HIF-2α levels and express VEGF at normoxic (21%) oxygen levels. Knockdown of HIF-2α in the TICs, which have few or no large-scale genomic aberrations by SNP-array analysis, induced symmetric neuronal differentiation in vitro and to a greater extent in vivo in xenograft tumors. These NB TICs have stem cell characteristics, as epigenetic modification together with relevant growth factor combinations could drive these cells towards ganglionic, glial/Schwann and melanocytic lineages. Presumably due to the reduced VEGF expression, tumors of HIF-2α-silenced cells were poorly vascularized, widely necrotic and resembled the bulk of tumor cells in clinical NBs by expressing sympathetic neuronal markers including tyrosine hydroxylase, while control tumors were immature, well-vascularized and stroma-rich. We conclude that HIF-2α is required to maintain the self-renewed putative NB TICs toward a more differentiated cell phenotype and that NB TICs might initiate and support tumor vascularization. As similar data for human primary glioma stem cells (high HIF-2α levels, VEGF expression, perivascular localization, immature phenotype) were recently reported, important links between pseudo-hypoxic states, tumor aggressiveness, stem cell-like phenotypes and tumor vascularization appear to be common denominators of neurally derived tumors and possibly also of other malignancies.

WS2
Development of the autonomic nervous system: a molecular view

Rothe, Hermann
Max-Planck-Institute for Brain Research, Research Group Developmental Neurobiology, Frankfurt, Germany

The development of sympathetic neurons from neural crest progenitors is elicited by Bone Morphoprogenetic Proteins (BMPs), which are secreted from endothelial and smooth muscle cells of the dorsal aorta. BMPs induce a group of transcription factors, i.e. Ascl1, Phox2b, Phox2a, Gata3 and Ispnmt that, in turn, control the expression of terminal differentiation genes, e.g. TH, DBH. More recently it became apparent that reduced VEGF expression, tumors of HIF-2α-silenced cells were poorly vascularized, widely necrotic and resembled the bulk of tumor cells in clinical NBs by expressing sympathetic neuronal markers including tyrosine hydroxylase, while control tumors were immature, well-vascularized and stroma-rich. We conclude that HIF-2α is required to maintain the self-renewed putative NB TICs toward a more differentiated cell phenotype and that NB TICs might initiate and support tumor vascularization. As similar data for human primary glioma stem cells (high HIF-2α levels, VEGF expression, perivascular localization, immature phenotype) were recently reported, important links between pseudo-hypoxic states, tumor aggressiveness, stem cell-like phenotypes and tumor vascularization appear to be common denominators of neurally derived tumors and possibly also of other malignancies.

WS3
Tumor initiating cells from bone marrow of high-stage neuroblastoma patients

Kaplan, David
United States

No abstract available

WS4
TICs from MYCN amplified neuroblastomas

Molesan, John J; Etus, Marli E; de Preter, Katleen; Westerman, Bart; van Nes, Johan; Speleman, Frank; Caron, Hub N; Versteeg, Rogier
Department of Amsterdam, Department of Human Genetics and Department of Pediatric Oncology, Amsterdam, Netherlands

We have isolated Tumor Initiating Cells from several MYCN amplified neuroblastoma tumors and bone marrow samples with a success rate of about 30%. The isolation methods were described before and consisted of filter isolation of neural stem cell medium containing bFGF and EGF. For all TIC lines we have corresponding tumor samples and normal DNA available. Using array CGH we showed that TIC lines were related to the primary tumor samples. Some TIC lines seemed to be grossly identical to the primary tumors while other TIC lines and corresponding primary tumors did show genomic variability. Affymetrix mRNA expression analysis showed interesting characteristics of neural stem cells in TIC lines that were maintained in neural stem cell medium for a prolonged period. A strong increase in PROM1 positivity was seen. We were able to subculture all TICs at high growth rates in NMRI nu/nu mice. Growth characteristics of the xenografts were initially very diverse but upon maintained passageing from mouse to mouse we could create lines that showed less variation in growth in vivo. These new TIC models are of value for further neuroblastoma research.

WS5
The miRNAome of TICs in relation to tumor cells and fetal neuroblasts

de Preter, Katleen1; De Brouwer, Sara1; Hansford, Loen1; Mestdagh, Pieter1; D’Haene, Nicky1; Sermon, Karen1; Studer, Lorenz2; Vandesmopele, Jo1; Kaplan, David1; Speleman, Frank1
1 Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium; 2 University of Toronto, Hospital for Sick Children, Toronto, Ontario, Canada

Recent studies have demonstrated the important role of miRNAs in normal development, stem cells and cancer. Therefore, we decided to investigate the possible involvement of miRNA deregulation in neuroblastoma. To achieve this goal we have profiled a total of 450 miRNAs using a robust real time quantitative PCR platform (Mestdagh et al., 2008). In total we analyzed 100 tumors as well as 10 tumor initiating cell lines (TICs), 7 embryonal stem cell lines, one neuronal progenitor cell line and 7 fetal neural crest stem cells. Analysis of a stem cell activity score (deduced from published stem cell miRNA studies) of the profiled samples showed a markedly high activity score for the embryonal stem cell lines and the TIC cell lines, in keeping with their presumed stem cell phenotype. Moreover, differentially expressed miRNAs were found for each tumor cell type vs the tumor samples and the normal neuroblast samples highlighted a number of potentially important miRNAs contributing to the tumor (stem cell) phenotype which are currently under study. In conclusion, this study uncovered the deregulated miRNA landscape of neuroblastoma tumor cells and comparison with various normal cell types including embryonal stem cells and fetal neuroblasts has allowed to pinpoint several strong candidate miRNAs for further study.

WS6
Identification and molecular characterization of human neuroblastoma tumor-initiating cells

Coulon, Aurelie1; Flahaut, Marjorie1; Multhelaher-Mottet, Annick1; Liberman, Julie2; Klowski, Gregor2; Sommer, Lukas2; Gross, Nicole1; Paelstic Neuroanatomy Research Unit, University Hospital, CHUV, Lausanne, Switzerland; 1University of Zurich, Cell and Developmental Biology, Institute of Anatomy, Zurich, Switzerland

Background: Neuroblastoma (NB) displays a cellular heterogeneity within the tumor. There is increasing evidence that at the top of this observed tumor cell hierarchy, there is a sub-population of tumor-initiating cells (TICs), responsible for initiation and maintenance of the tumor. Candidates TICs have been isolated in a variety of adult solid tumors, representing a potential therapeutic target. However, this population has not yet been identified or characterized in NB. The most common extracranial childhood solid tumour originates from neural crest-derived malignant sympathoadrenal cells. We have identified cells within primary NB tissues and cell lines that express markers of neural crest stem cells and their derivatives, leading us to postulate the existence of TICs in NB tumour that recapitulate the properties of sympathetic precursor cells.

Method: We proposed a novel approach to identify and characterize NB TICs by prospectively identifying their self-renewal properties. From a very aggressive stage 4 NB sample, we selected self-renewing putative TICs by their sphere-forming capacity and analyzed their gene expression profiles by time-course micro-array analysis.

Results: Supervised and unsupervised analyses provided a list of sphere markers genes involved in embryogenesis and nervous system development (CD133, EDNRA/B, NOTCH1/3, GPR177…), and drug resistance (MDR1, ABCA1). To determine whether the sub-populations selected in spheres correspond to TICs, their tumorigenic potential was assayed in vivo using syngeneic NB tumor cell line xenografts. A 3D sphere assay demonstrated the presence of a subpopulation of TICs in 100% of primary NB tumors, leading us to conclude that NB is an ideal model system to study cancer stem cells.

Conclusion: In our study, we identified new NB-TICs specific markers and we characterized heterogeneous sphere sub-populations that will be individually analyzed by functional assays.
Vascular mimicry in human neuroblastoma: identification of the progenitors of tumor-derived endothelial cells

Ferrola, Vito; Pozz, Annalisa
Laboratory of Oncology, G. Gaslini Institute, Genoa, Italy

Vascular mimicry is a phenomenon whereby cancer cells are incorporated in vascular structures where they may acquire features typical of "professional" endothelial cells (EC). We previously demonstrated for the first time that primary NB-associated endothelial microvessels (EM) may be lined by tumor-derived endothelial cells (TEC) and, accordingly, TEC lined EM is formed in immunodeficient mice. Using human MYCN amplified and MYCN non-amplified neuroblastoma (NB) cell lines. In this study we have investigated whether the embryonal stem cell markers OCT-4, c-kit and CD44 could act as TEC progenitors. In both primary and metastatic NB cells as well as NB cell lines were found to express OCT-4. Tenascin-C (TNC), a huge protein of the extracellular matrix expressed by most tumor cells and involved in tumor growth, metastasis and angiogenesis was consistently expressed on NB cell surface and found to mark exclusively OCT-4+/TNC+ cells. The availability of a surface marker allowing positive selection of OCT-4+ NB cells made it possible to generate in vitro and in vivo NB angiogenic progenitors. These results will be discussed in the frame of the potential role of TEC in chemoresistance and tumor relapse.

WS9
Exploiting the embryonic environment to reprogram cancer stem cells in neuroblastoma

Carter, Rachel; Mullassey, Dhanya; Jesudason, Edwin; White, Mike; See, Violaine; Losty, Paul; McDowell, Heather; Chetler, Louis; Pizer, Barry; Moss, Diana
1School of Biomedical Sciences, Department of Human Anatomy & Cell Biology, Liverpool, United Kingdom; 2Academic Paediatric Surgery, Division of Child Health, Alder Hey Children’s NHS Foundation Trust, and School of Biological Sciences, Liverpool University, Liverpool, United Kingdom; 3AtrA was used to model OCT-4+/S100A6+ cells in vitro.

Identification in vitro and in vivo of tumoral gliar progenitors in neuroblastoma tumors

Mora, Jesus
Hospital Sant Joan de Déu, Developmental Tumour Biology Laboratory, Department of Oncology, Barcelona, Spain

Background: Neuroblastic tumors (NB) are derived from multipotential neural crest stem cells, and composed by a neuroblast component and Schwannian-like (glial) stroma. A correlation has been established between the degree of differentiation of the neuroblastic subtype, the amount of glia and patient outcome. However, the physiological relationship between these tumor neuronal cells and neuroblastic cells has not been clarified. We reported lineage specificity of membrane gD2 marker amongst the sorted cell subpopulation isolated from a MYcn amplified nB tumor. The I-type cell line sK-n-Be2c differentiated with 1µM ATRA was used to model gD2+/S100A6+ cells in vitro.

Results: In the neuroblastoma tumor samples analyzed (n=23), intense cell membrane gD2 expression was observed in all the cells morphologically identified as neuroblastic. Conversely, no specific staining was detected in primary ganglioneuroma or ganglioneuro- blastoma tumors. S100A6 expression was detected in the nuclear membrane of all the cells which make up the Schwannian stroma and also in the cells morphologically constituting the blood vessels. Moreover, S100A6 nuclear expression was observed in isolated, sparse undifferentiated neuroblasts. On the basis of these results, gD2 and S100A6 double immunostaining was performed in 14 primary non-treated diagnostic neuroblastoma samples and 9 post-treatment specimens. Concomitant expression of both markers was observed in a subpopulation of neuroblasts (<25% of the total) in 12 (85%) of the 14 samples obtained at diagnosis. In those tumors, the majority of the G2D2+/S100A6+ neuroblasts within the tumor were found isolated and surrounded by G2D2+/S100A6+ neuroblasts. Six (65%) of the 9 post-treatment neuroblastoma samples analyzed contained undifferentiated/poorly differentiated neuroblasts. In all of these samples, a variable percentage of neuroblastic cells displayed G2D2+/S100A6+. The presence of co-staining cells was, in average, higher than in diagnostic samples and distributed in non-necrotic areas where blood vessels were found. Surprisingly, G2D2+/S100A6+ double stained cells morphologically Schwannian-like, were also identified in primary tumors at diagnosis in the stromal bundles and in some of the blood vessels. A significant proportion of G2D2+/S100A6+ neuroblasts were distributed around the tumoral blood vessels, suggesting that G2D2+/S100A6+ neuroblasts are not distributed randomly, but either arise from or tend to populate near the stromal regions. All bone marrow specimens showed G2D2+/S100A6+ representing less than 10% of the total. By FACS analysis, the percentage of G2D2+ cells ranged from 15-89% of the total nucleated cells, and thus potentially are all tumor cells. On the other hand, 3-35% of these cells were S100A6+ positive. Double stained cells represented percentages ranging from 11-53% of cells, distributed both in a large and small subpopulations. A small percentage of CD133+ cells were identified, 0.1-0.7% of the total viable population. The proportion of CD133+ cells did not correspond to that of G2D2+/S100A6+. For one MYCN amplified tumor, all FACS-sorted G2D2+/S100A6+ differentially stained subpopulations showed MYCN amplification.

Conclusions: Our results show, in primary neuroblastoma tumor samples, the presence of a morphologically undistinguishable tumoral subpopulation of neuroblasts that has features of both neuronal and glial lineage. The clinical correlations, however, suggested that these co-expressing cells were most likely cells in the process of differentiation towards glial lineage. In vitro, we were able to recapitulate the differentiation stages which give rise to these bipotential cells, and demonstrate how some neuroblastic tumor cells give rise to other cellular components of the tumor.
WS10
Low dose metronomic anti-angiogenic (LDM) oral topotecan and pazopanib as a potential model for maintenance therapy neuroblastoma
Baruchel, Sylvain
Hospital for Sick Children, Hematology Oncology, Toronto, Canada
Background: Angiogenesis plays a critical role in neuroblastoma (NB) growth and metastasis. Low dose metronomic (LDM) chemotherapy, combining with VEGF pathway inhibitors is an emerging treatment strategy having increased efficacy with reduced toxicity. This strategy can potentially target tumor hypoxic zone which are more susceptible to develop drug resistance.
Objectives: Pharmacokinetic/ pharmacodynamic markers and efficacy of LDM topotecan (TP) with/pazopanib (PZ) in oral antihypertensive tyrosine kinase inhibitor (TKI) was evaluated in xenograft and metastatic and TICs NB mouse model.
Methods: In vitro IC50 was established using SK-N-BE(2) and SH-SY5Y cell lines. NOD-SCID mice model was used for both subcutaneous primary tumour and metastatic experiments. Mice were randomized into four groups: control group, LDM TP (1.0mg/Kg) PZ (30mg/kg and 150µg/kg) and the combination (1.0mg/Kg TP +150µg/kg PZ). For the localized tumor model, the animals were treated daily till 56 days; while for metastatic model, animals were treated until death. Micro vessel density, Angiogenesis in circulating Endothelial cells (CECs) and circulating Endothelial Progenitor cells (CEPs) were determined by flow cytometry. Pharmacokinetic studies were conducted to determine the plasma concentration-time profiles of both the drugs.
Results: IC50 of SK-N-BE(2) and SH-SY5Y cells was 125.0mg/ml and 4.0mg/ml respectively. Pazopanib did not induce cytotoxicity in NB cell up to 10µg/ml. In SK-N-BE(2) a statistically significant efficacy (tumor size the end of treatment) was observed for single agent (TP or PZ) and combination, combination (p=0.0002) > LDM TP (p=0.0008)> than PZ. In the SK-N-BE(2) the three treatment regimens significantly prolonged animal overall survival compared to control group. PZ TP and PZ+TP, LDM PZ+TP (P=0.001) was superior than single agent PZ or TP. No toxicity was observed in any of the groups. This was not correlated with CECs CEPs and circulating density. The Cmax of Pz in single agent and combination group was 130.5µg/ml and 125.6µg/ml respectively. PZ plasma concentration was superior than TP (p<0.05). The TP and PZ concentration in combination of LDM TP and PZ reduced the levels of viable CEP (P = 0.125) and CEC (P = 0.005) after 7 days treatment.
Conclusion: Daily LDM TP and PZ and combination are effective and safe regimens in allic neuroblastoma mouse models. The reduction in microvessel density and CEC/CEP levels supports the anti-angiogenic effect of these drugs.Correlation between HIF1α and response as well as TICs mouse model related studies are ongoing.

WS11
The neuroblastoma miRNA map, prioritization and functional evaluation of candidate miRNAs
Mestdagh, Pietert
Belgium
MicroRNAs are tiny regulators of coding gene expression. While a growing body of evidence implicates deregulated microRNA expression in human cancers, insights in global miRNA function remain limited. For neuroblastoma, a number of candidate miRNAs, including miR-34a, miR-19-92, miR-18a, miR-10b and miR-18a, have been established and, most likely, many others await identification. To streamline the selection of candidate miRNAs, we present the microRNA body map, an interactive compendium and mining tool of high-dimensional microRNA expression profiles and functional annotation inferred through integrative transcriptomics. This combination of miRNA expression and function greatly enhances miRNA prioritization and might prove to be a valuable tool in completing the neuroblastoma miRNA map. Following prioritization of candidate miRNAs, functional evaluation is crucial to understand their role in neuroblastoma biology. The miR-19-92 miRNAs are among the top candidates for neuroblastoma. However, insights in miR-19-92 function are limited. To fully elucidate miR-19-92 function in neuroblastoma, we measured protein-response upon miR-19-92 activation using quantitative mass spectrometry and found miR-19-92 miRNAs to be implicated in multiple hallmarks of the tumorigenic program. Most importantly, miR-17-92 was identified as a potent inhibitor of TGF-β signaling. By functionally testing both upstream and downstream of S Mad2, miR-17-92 activated all four target clamped down of TGF-β-signaling by downregulation of multiple key effectors along the signaling cascade as well as through direct inhibition of TGF-β-responsive genes. Of interest, several of the empirically identified miR-17-92 targets could also be inferred through the miRNA body map, demonstrating its great potential in miRNA prioritization.

WS12
MicroRNAs regulating neuroblastoma cell differentiation
Foley, Niamh1; Bray, Isabella2; Werner, Bryan3; Bryan, Kenneth3; Bernas, Tyra4; Prehn, Joche1; Stalling, Raymond1
1Royal College of Surgeons in Ireland, Cancer Genetics, Dublin, Ireland; 2Royal College of Surgeons in Ireland, Physiology, Dublin, Ireland
MicroRNAs are non-coding RNAs which function as complex negative regulators of post-transcriptional gene expression during normal development. Their dysregulation contributes to the pathogenesis of many cancers, including neuroblastoma. Here, we identify miRNAs that contributes to a major way the process of all-trans retinoic acid (ATRA) induced in vitro differentiation of neuroblastoma SK-N-BE cells. We demonstrate that ectopic over-expression of two of the most significantly up-regulated miRNAs in response to ATRA, miR-10a and miR-10b, results in neutre outgrowth, over-expression of neuronal differentiation markers GAP43 and TUBB3, and a decrease in both MYCN expression and cell growth, which are characteristic of ATRA induced differentiation. The reduction in MYCN is through a secondary effect, as neither miRNA is predicted to target the 3' UTR of the MYCN mRNA. Ectopic up-regulation of miR10a/b also directly decreases the abundance of a large set of mRNAs through their target’s 3' UTRs, as evidenced by statistically significant enrichment for miR-10a/b seed sites among down-regulated genes identified through expression profiling with microarrays. Gene ontology analysis of the down-regulated, putative direct targets of miR-10a/b indicate significant enrichment for genes involved with transcriptional regulation, explaining why miR-10a/b ectopic up-regulation also results in a major cascade of secondary transcriptional alterations, such as MYCN mRNA levels. Further, we demonstrate that miR-10a/b recapitulates many elements of an ATRA induced differentiated phenotype through direct targeting of the nuclear receptor co-repressor 2 (NCOZ2), a gene known to be up-regulated in ATRA induced differentiation. siRNA mediated knockdown of NCOZ2 by itself completely recapitulates the effects of miR-10a/b over-expression. Although the biological effects of miR-10a and miR-10b are indistinguishable using in vitro models, they have remarkably different patterns of expression in primary neuroblastoma tumors of differing genetic subtypes, with under-expression of miR-10a having a greater negative impact on patient survival in the 11q14-15 subgroups. We conclude that the -10a/b contributes to the process of in vitro neuroblastoma cell differentiation, producing a differentiated phenotype that is remarkably similar to that induced by ATRA, through direct targeting of NCOZ2. The antiproliferative effects of these miRNAs indicate that they could be of potential benefit for miRNA mediated therapeutic strategies if targeted delivery is achievable.

WS13
MYCN-regulated microRNAs repress estrogen receptor-α (ER) expression and neuronal differentiation in human neuroblastoma
Lovén, Jakob1; Westermark, Ulrica K1; Zinin, Nikolay1; Müller, Inga1; Pühman, Sver1; Argenen Henningson2; Karlinska Institutet, Department of Microbiology, Tumor and Cell Biology (MTC), Stockholm, Sweden; 2University of Lund, University Hospital MAS, Center for Molecular Pathology, Department of Laboratory Medicine, Malmö, Sweden
MYCN, a proto-oncogene normally expressed in the migrating neural crest, is in its amplified state a key factor in the genesis of human neuroblastoma (NB). However, the mechanisms by which MYCN-regulated NB progression are poorly understood. The impact of deregulated microRNA expression in NB has just begun to emerge reinforcing the importance of microRNA biology in NB-associated tumorigenesis. We have previously identified MYCN-regulated miRNA signature involving the activation and down-regulation of several microRNA genes from paralogous clusters. In line with previous reports, we showed that MYCN transcriptionally activates oncogenic microRNAs from the miR-17–92 cluster and its paralogs miR-10a–363 and miR-10b–25. Expression analysis in NB tumors confirmed increased levels of these microRNAs in MYCN-amplified samples. In particular, we demonstrated that miR-18a and miR-19a from the miR-17–92 cluster target and repress the expression of estrogen receptor-alpha (ESR1), a ligand-inducible transcription factor implicated in neuronal differentiation. Importantly, we demonstrated expression of ESR1 in human fetal sympathetic ganglia, suggesting a role for ESR1 during sympathetic nervous system development. Resorption of ESR1 in NB cells led to marked growth arrest and neuronal differentiation. Moreover, lentiviral-mediated reexpression of ESR1 in MYCN amplified NB cells resulted in severe growth retardation followed by robust morphological and biochemical differentiation. ESR1 represents a previously undescribed MYCN-regulated neural gene that constitutes a novel oncogenic circuitry in which the repression of ESR1 through MYCN-regulated microRNAs may play a fundamental role in NB tumorigenesis. We propose that MYCN amplification may disrupt estrogen-signaling sensitivity in primitive sympathetic cells through deregulation of ESR1, thereby preventing the normal induction of neuroblast differentiation. We are currently investigating putative interacting growth factors and downstream targets involved in the regulation of differentiation by ESR1 in NB cells.
WS14 Assessing the role of miRNAs in neuroblastoma biology – from expression profiling to functional analysis
Schulte, Johannes; Schlierf, Stefanie; Schramm, Alexander; Fogert, Angelika
University Children’s Hospital Essen, Pediatric Hematology/Oncology; Essen, Germany

miRNAs (miRNAs) constitute a family of small RNA species that regulate translation and stability of mRNA. Soon after their discovery, miRNAs were found to act as tumor suppressor genes by blocking the translation of their targets. As oncoproteins by inducing translation of tumor suppressor genes. Most interestingly, miRNAs can be therapeutically targeted using Agomirs to restore or Antagomirs to inhibit the translation of their targets. We have extensively analyzed miRNA expression in neuroblastoma using real-time PCR, microarray and next generation sequencing approaches. miRNA signatures predictive of clinical course have been identified, as well as miRNAs of functional importance in neuroblastoma biology. These miRNAs include the MYCN-regulated miRNA Cluster 17-92, the MYCN-activated miR-9 and miR-221, as well as the tumor suppressive miR-34a/b/c, miR-524 and miR-628. The main future challenges include exploring miRNA signatures as prognostic factors in a clinical setting and comparing these signatures to miRNA predictors. Furthermore, comprehensive identification of miRNA targets of microRNAs is an essential step towards miRNA targeted therapies. Strategies for target identification include bioinformatic analysis, proteomics and RNA-Immunoprecipitation. Finally, the physiological role of miRNAs as well as their role in tumor biology has to be explored in vivo using xenograft and knock-out mouse models.

WS15 Bioinformatic tools to integrate and analyse high throughput neuroblastoma data
Koster, Jan; van Sluis, Peter; Ora, Ingrid; Caron, Hubert; Molenaar, Jan; Versteeg, Roger
1Academic Medical Center, University of Amsterdam, Dept. of Human Genetics, Amsterdam, Netherlands; 2Academic Medical Center, University of Amsterdam, Dept. of Pediatric Oncology, Amsterdam, Netherlands

High throughput mRNA profiling is an efficient tool to identify expression levels for any gene in series of tumor and cell lines. Many tools have been developed for analysis of such data. However, most of them require specialist bioinformatic support or are time consuming or are not integrated in one user-friendly platform. We developed a tool for basic and advanced analysis of such data, called R2. This web-based tool enables swift and user-friendly analyses by any interested researcher. We have generated Affymetrix expression profiles of a series of 88 neuroblastoma tumors, as well as from a limited number of ganglioneuromas and ganglioneuroblastomas. The series is annotated for clinical and molecular parameters. R2 enables to analyse expression values for any gene, establish its prognostic value and find correlating expression patterns with other genes. It also permits global expression analysis and prognostic ordering for gene sets, which can be formed by KEGG pathways, functional groups, etc. Differentially expressed genes can efficiently be identified within annotated parameters, like age, stage, histology etc. Any resulting gene list can be analysed for pathway or gene ontology enrichment, or be visualized in heat maps. Finally, the R2 tool and database includes Affymetrix expression profiles of over 20,000 normal and tumor samples from other tissues, grouped per tumor or tissue type. This enables quick searches for expression profiles of specific genes over a wide range of tissues. The web-based R2 analysis tool and database will be made publicly available at the ANR.

WS16 Genomic and proteomic study of microRNAs in pediatric cancers
Wei, Jun; Chen, Qingrong; Johansson, Peter; Beckstead, Wesley; Song, Young; Cheuk, Adam; Khan, Jawed
National Cancer Institute, Pediatric Oncology Branch, Bethesda, United States

MicroRNAs (miRNAs) are an important class of gene expression regulators that play a critical role in cancer biology. To understand the importance of miRNAs in the tumorigenesis and tumor progression, we took a genomic approach to study the global expression of miRNAs in pediatric tumors by performing parallel microRNAs and mRNA profiling on 57 tumor xenografts and cell lines representing 10 different pediatric solid tumors using microarrays. We found that pediatric cancers express cancer-specific microRNAs. Of the fourteen microRNAs differentially expressed between rhabdomyosarcoma and neuroblastoma, 8 of them were validated in independent patient tumor samples. Exploration of the expression of microRNAs in relation with their host genes showed that the expression for 43 of 68 (63%) microRNAs located inside known coding genes was significantly correlated with that of their host genes. Among these microRNAs, 5 of 7 microRNAs in the OncornIR-1 cluster correlated significantly with their host gene MHKG1. In addition, the expression level of MHKG1 could predict the outcome of neuroblastoma patients independently from the current neuroblastoma risk-stratification in two independent patient cohorts, indicating a novel function of this microRNA cluster in neuroblastoma biology. With the advent of the massive parallel sequencing technologies which miRNA molecules can be directly sequenced and counted, we applied the next-generation sequencing technologies to characterize miRNA expression in high-risk neuroblastoma. We initially sequenced a small-RNA pool of four stage 4, MYCN-amplified neuroblastoma tumors. This sequencing run yielded 4.1 million high-quality reads. Sequence analysis revealed the expression levels of 525 known miRNAs (mature and minor miR*) and 6 novel miRNA genes. Two miRNAs, let-7i and let-7a, had extremely high expression levels, accounting for ~50% of total reads in each sample. These miRNAs have been implicated in other cancers with important roles in carcinogenesis. The high expression of let-7i and let-7a was confirmed by quantitative RT-PCR. We also validated six novel miRNAs. In parallel to the expression studies, we attempted to understand the function of an important microRNA, miR-34a, located on 1p36 which is commonly lost of heterozygosity in neuroblastoma and other tumors. Transactivation of miR-34a and its other family members (miR-34b and c) by p53 has been shown to be critical to p53 function. In addition, we and others have reported that miR-34a could directly target important oncoproteins such as MYCN and E2F3. Furthermore, miR-34a caused significant suppression of cell growth through increased apoptosis and growth arrest in tumor cells. Therefore, miR-34a is a bona fide tumor suppressor. In order to identify the downstream targets of miR-34a globally, we utilized a proteomic approach, namely Isotope coded Affinity tags (ICAT), to detect protein changes in neuroblastoma cells transfected with miR-34a. Consistent with the working mechanism of microRNAs, miR-34a has a small effect on the mRNA expression levels measured by ICAT. Among the affected proteins, 192 were down regulated and 143 were up regulated (≥2 fold). Gene ontology analysis showed that proteins involving in cell cycle, transcription and translation were significantly enriched among the down regulated genes; whereas proteins involved in apoptosis and development were over-represented in the up regulated gene list. Several important pathways such as caspase 3, NF-κB, and YY1 were clearly altered by miR-34a in a network analysis, indicating that these are the potential key downstream pathways that miR-34a regulates. Therefore, ICAT study of global protein changes provides an insight of the biology and therapeutic potential of miR-34a-based therapies.

WS17 Next generation sequencing technologies to investigate the cancer genome & insights into neuroblastoma biology and tumor progression models learn from massively parallel sequencing strategies
Khan, Jawed
United States

For the first half of my talk I will discuss the next generation sequencing (NGS) technologies for mapping the cancer genome. NGS technology directly identifies billions of nucleic acid species in parallel in a single experiment. Different from the Sanger method of traditional sequencing, the massively parallel DNA sequencing technology not only gene sequencing but also sequence information for each nucleic acid strand, but also determines the abundance of each nucleic acid species due its large capacity, resulting in a digital readout of levels for any sequence, even those at low levels beyond the detection of sensitivity of hybridization-based technologies. I will discuss this technology and its wide-ranging applications for both DNA and RNA studies. For DNA it is possible to sequence an entire cancer genome in one month, a staggeringly short time considering that it took 13 years to sequence a handful of human genomes by the Human Genome Project. There are also hybridization-based methods for pulling down the DNA from protein coding or a defined region (termed ‘genome-partitioning’) for targeted resequencing. With these methods it is possible to detect every single nucleotide variant, mutation, genomic rearrangement, profile the whole ‘methylome’, and determine copy number alteration at the base pair level of a given genome. For RNA studies it is possible to determine the gene expression level of every gene, identify every splice variants, novel transcripts, single nucleotide variants and mutations for the expressed genome. It will also identify: novel gene rearrangements that result in chimeric fusion gene products. Finally I will discuss how next and next-next generation sequencing has and will revolutionize the field of cancer genomics. Neuroblastoma is an extremely heterogeneous disease in which the outcome can range from spontaneous regression of the tumor to relentless progression leading to the death of the patients. In the second half of the talk I will discuss the application of next generation sequencing of neuroblastoma transcriptomes and genomes and its use for deciphering the biology of this enigmatic malignancy, identifying novel transcripts, splice variants, and single nucleotide variants and targets for therapy. I will also discuss its application for determining tumor progression models in this disease.
Next generation sequencing of the (small) RNA transcriptome - from catalogisation to quantitative expression profiling

Abstract Book 85

Next generation sequencing to characterize somatic alterations in neuroblastoma samples

Abstract Book 85

WS18

Next generation sequencing of the small RNA transcriptome - from catalogisation to quantitative expression profiling

Schulte, Johannes H; Eggert, Angelika; Schramm, Alexander
University Children's Hospital Essen, Department of Pediatric Hematology/Oncology, Essen, Germany

With next generation sequencing (NGS), the unbiased global analysis of transcript structure and abundance is now feasible. This allows an unprecedented view of the transcriptome, uncovering allele specific expression, posttranscriptional RNA modifications including RNA editing, splicing and point mutations/deletions, as well as single nucleotide variations (SNV), which include SNPs and somatic mutations. To achieve optimal transcriptome coverage, the whole RNA is fractionated to separately analyze intermediate and large RNA. In addition to the latter ones require diverse sequencing studies, including short reads, long reads and paired end sequencing reads, to uncover complex splice variants and potential fusion and read-through transcripts. Technical challenges include (i) mapping of the often ambiguous reads, (ii) the detection and quantification of postranscriptional editing events as well as splicing patterns, (iii) normalization of quantitative expression data, (iv) integration with genomic data and, in a final step, (v) presentation and interpretation of the respective data. We will discuss results of our most recent small RNA NGS study, as well as a general perspective for transcriptome sequencing in neuroblastoma.

WS19

Next-generation sequencing to characterize somatic alterations in neuroblastoma samples

Jahouhès, Jean-Charles; Sarré, Patricia; Boué, Valentina; Jouannet, Stéphanie; Daveau, Romain; Cazes, Alex; Schleiermacher, Gudrun; Combarèze, Valérie; Barillot, Emmanuel; Delattre, Olivier
1Inserm U830, Institut Curie, Paris, France; 2Inserm U900, Institut Curie, Université Paris-Sud, Villejuif, France; 3Commissariat à l’Énergie Atomique et aux Énergies Alternatives, Centre d’Etudes de Saclay, Gif-sur-Yvette, France; 4Centre de Recherche en Biologie Moléculaire et Cellulaire, Institut Curie, Paris, France; 5Institut Curie, Paris, France; 6Centre Léon Bérard, Laboratoire de Recherche Translactionnelle, Lyon, France

Background: The genetic alterations of neuroblastoma (NB) cell lines and tumors have been, up to now, characterized using conventional strategies including cytogenetic and molecular methods, providing a picture of genomic rearrangements at a quite low resolution. Next-Generation Sequencing technologies now offer the possibility to identify all somatic mutations of all classes in individual cancer genomes.

Methods: We use several strategies in order to characterize genomic alterations in NB samples including paired-end sequencing of mate-paired libraries, RNA-sequencing of a normalized random primed cDNA library and whole genome sequencing (30x coverage).

Results: For two cell lines, almost 60 million pairs and for five tumors, almost 50 million paired-end reads were obtained from the mate-paired libraries and aligned against the reference genome. Various criteria were applied in order to identify aberrant links with the highest relevance, including both inter and intra-chromosomal rearrangements. The majority of the unbalanced translocations previously detected by spectral karyotyping and/or array-CGH were detected amongst the inter-chromosomal rearrangements. A high number of intra-chromosomal rearrangements was also identified in both cell lines. For one of these two samples, RNA-sequencing generated ~ 500 000 reads of around 400 bp. Expression levels obtained by RNA-sequencing were compared to those measured using Affymetrix U133A GeneChip. Analysis is ongoing to search for chimaeric transcripts and mutations. Finally, we recently started whole genome sequencing with a high depth in order to detect all types of mutations in a NB patient.

Conclusions: Genome-wide massively parallel sequencing provides a more exhaustive and precise characterisation of rearrangements in tumor cells as compared to conventional strategies. It highlights the diversity of somatic rearrangements and allows to characterize structural variants to the base-pair level. It therefore represents a powerful approach to get insights into the mechanisms that underlie NB oncogenesis.

Perspectives in immunotherapy of neuroblastoma

Lode, Holger
University of Greifswald, Pediatric Hematology and Oncology, Greifswald, Germany

Immunotherapy of neuroblastoma has gained momentum as a result of efficacy reports in clinical trials. Three targeted approaches and expected added value to the treatment of this challenging disease will be discussed. First, the hallmark for immunotherapy in neuroblastoma combining passive immunotherapy using anti-gD2 antibodies with cytokines opened a new venue to further improve this approach with enhanced antibodies. Immuno-cytokine therapies combining anti-gD2 and a substantial set of prioritized monobonal antibody with the immunostimulatory activity of a cytokine in one molecule. Superior preclinical efficacy and activity in clinical trials promise a breakthrough for this approach and opens new opportunities also in combination with the following immunotherapeutic strategies. Second, cell based strategies are well under way to strengthen the effector arm of the immune system against neuroblastoma. Here the implementation of blood stem cell transplantation to provide for a new immune system and activated natural killer cells is a promising strategy. Such approaches can be refined by genetic modification of immune effector cells with chimeric receptors (CARs). CARs consist of an antibody-derived single chain Fv domain linked to the cytoplasmic signaling domain of the T cell receptor (TCR) chain and thereby retarget cellular activation pathways to tumor surface antigens. G2-specific CARs have been used to redirected T cells to neuroblastoma and have induced tumor regressions in patients with relapsing or refractory neuroblastoma. Future efforts aim at augmenting in vivo persistence and activity of G2-specific immune effector cells to provide sustained immune control of residual neuroblastoma cells.

Third, neuroblastoma is not MHC class I negative. Thus, active vaccination approaches to induce long lasting and persistent immunity against neuroblastoma associated antigens is the ultimate goal in a phase of immune reconstitution after blood stem cell transplantation. Here the use of DNA vaccination to create B7-1 and B7-2 costimulatory ligands have been demonstrated to be successful in vivo. GD2 mimotopes and T-cell vaccines (Tyrosine hydroxylase, MYCN) are promising preclinical approaches.

The final goal is to implement these strategies along a time line of immune reconstitution after blood stem cell transplantation and thereby create a most effective neuroblastoma immunotherapy protocol in the future.

The Neuroblastoma-TARGET project: Plans for sequencing, validation and frequency scans

Marti, John M; Atyeh, Edward; Asgharzadeh, Shahab; Seeger, Robert; Wei, Jin; Khosravi, Shadi
for the NBL-TARGET consortium, United States

The neuroblastoma TARGET (Therapeutically Applicable Research to Generate Effective Treatments) collaborative research initiative aims to discover novel therapeutic targets and genomic predictors of outcome in an unbiased systematic fashion. In year one of the project, we defined high resolution DNA copy number alterations and loss of heterozygosity using Illumina SNP arrays, and genome-scale expression signatures using Affymetrix HuEx arrays. In the second phase of the project, we are performing a comprehensive resequencing of the high-risk neuroblastoma genome. Two hundred samples will undergo solution phase exon capture followed by paired end resequencing (N=100 Illumina; N=100 AB Solid), and an additional 10 samples will undergo full genome resequencing using Illumina paired-end read technology. All samples will have paired-end DNA sequencing data. Finally, at least 110 of the samples will also have tumor RNA sequencing performed to detect expressed somatic variations. The first 100 exomes and 10 full genomes should be complete by July 2010, with the next 100 exomes to follow closely. RNAseq work is ongoing. Validation and frequency scans in up to 1500 cases (all risk groups) are being planned, as will integration with our genome wide association study to determine if any of the discovered germline variations may be predispositional alleles. Taken together, this project should define the mutational landscape of neuroblastoma and provide a rich resource for the international community of neuroblastoma investigators.

Finding variant needles in the neuroblastoma haystack: the Ghent approach

Vandesompele, Jo
behalf of all co-workers at CMGG, NXTGNT, NRC, Center for Medical Genetics, Ghent, Belgium

Massively parallel sequencing has heralded a new era in genomics in which sky is expected to be the limit. While throughput and cost per base almost exponentially grow and decrease, respectively, the data deluge and haze pose serious challenges. Especially in cancer genome resequencing projects, interpreting the sequence variants in order to separate the wheat from the chaff is a formidable task. Instead of performing whole genome resequencing or more focused exome sequencing, we chose to pursue alternative strategies to predict cancer genes that play a role in neuroblastoma.

Preliminary results will be presented for a PCR based targeted resequencing study on a cohort of 125 matched tumor/normal samples in which all human microRNA loci are amplified in an assembly to be sequenced. Innovative features of the study are the gene prioritization strategy based on SNP/scores and validated on resequencing data from other cancer types; the use of short reads and the low DNA input requirements through whole genome sample pre-amplification. In a second study, we evaluate RNA-seq on normalized cDNA libraries from neuroblastoma cell lines with at least one known mutation as a more powerful and focused alternative to DNA based exome sequencing to find sequence variants and structural aberrations such as fusion genes and splice isoforms. In a third and final study, we apply ChIP-seq on neuroblastoma cell lines to create a transcription factor binding map and to explore the possibility of finding sequence variants in the enriched genome loci.
With the recent demonstration by the Children's Oncology Group (COG) that ch14.18 + cytokine significantly improved outcome of patients with high risk neuroblastoma, an ongoing COG phase III trial to collect comprehensive toxicity profile for regulatory approval of ch14.18 is now underway. Immunochemotherapy with ch14.18 + cytokine is associated with significant toxicities, including pain, hypersensitivity reaction, acute vascular leak syndrome. There are several second generation targeted immunotherapeutic agents on our horizon to work in combination with the potentials for reduced toxicities and/or enhanced efficacy. HU14.18-I2L is a fusion protein of humanized anti-GD2 antibody (hu14.18) and IL-2. Twenty-seven pediatric patients with recurrent/refractory neuroblastoma and one with melanoma were treated with escalating doses of hu14.18-I2L. The maximal tolerated dose was determined to be 12 mg/m2/d, approximately 50% of ch14.18. Clinical toxicities were similar to those reported with hu14.18. Of 31 patients treated, 7 achieved complete or partial responses to hu14.18-I2L in this study; however, evidence of antitumor activity was noted in three patients (Neal et al. 2004). A phase II study of this immunocytokine is being planned. Hu14.18K322A is a humanized ch14.18 that shares identical C regions of IgG1-k as ch14.18 with the exception of a mutation to alanine at lysine 322 that limits its ability to fix complement and thereby reduces pain associated with a poor prognosis. While the ability of hu14.18K322A to activate complement was reduced, its ADCC capabilities were retained. Preclinical studies in rats confirmed that hu14.18K322 elicited significantly less allodynia than ch14.18. Sorokin et al. (2002) performed a phase I clinical trial of hu14.18K322 is ongoing at St. Jude Children's Hospital in Memphis, TN, USA. mAb1A7 is an anti-idiotypic antibody directed against a murine anti-GD2. This antibody is active in human neuroblastoma, and thereby mimic the original tumor antigen to which antibodies were developed and act as a surrogate tumor antigen. Active immunotherapy with anti-idiotypic antibodies should give rise to a gradual increase in cellular antitumor immunity. Thus, it may have the advantage of lower toxicity than passive immunotherapy with the relatively rapid infusion of high concentrations of mAbs. A pilot clinical trial of mAb 1A7 as a surrogate GD2 vaccine was conducted in patients with high risk neuroblastoma (Yu, Eskenazi et al. 2001). Thirty one patients with high risk neuroblastoma who achieved first or subsequent complete response (CR) or very good partial response (VGPR) were entered into this trial. The treatment was well tolerated with only transient local reactions, transient fever and chills in 4 patients and serum sickness in 1. There was no systemic toxicity, such as neuropathic pain which is often seen with infusion of anti-GD2 despite potent immunostimulatory effects. One patient died during first remission with no evidence of disease progression while 10 of 31 patients had remission. In the observed and anticipated low toxicity profile of mAb 1A7, and hu14.18K322A respectively, it may be possible to use ch14.18 with one of these products. Future clinical trials of mAb 1A7 vaccine or the mutant anti-GD2 mAb to document their therapeutic efficacy are warranted.

**WS26**

**Omega-3 fatty acids in cancer: The protectors of good and the killers of evil?**

Gleissman, Helena

Karolinska Institutet, Women's and Children’s Health, Stockholm, Sweden

Omega-3 fatty acids have been implicated in cancer prevention and treatment. Conventional chemotherapeutics are considered “double-edged swords”, as they kill the cancer cells but also strike the healthy cells causing severe morbidity and sometimes also mortality. Could omega-3 fatty acids in this setting work as a “sword and shield” instead, by being cytotoxic to cancer cells, but at the same time protect healthy cells from these deleterious effects? In addition, may our current diet with decreased omega-3/omega-6 ratio contribute to the increased cancer incidence, and thus, may an omega-3 enriched diet be used as a preventive measure against cancer? Here, our data concerning the toxicity of the omega-3 fatty acid docosahexaenoic acid in neuroblastoma, both in vitro and in vivo, will be summarized. Mechanisms behind the observed effects will be described as well. Till as immunomodulator the T1/2 of DHA. In addition, the potential use of omega-3 fatty acid in combination with conventional chemotherapy will be discussed.

**WS27**

**Optimizing drug development for neuroblastoma by close integration with adult oncology**

Reynolds, Patrick

Cancer Center, School of Medicine, Texas Tech University Health Sciences Center, Lubbock, TX, United States

Developing new drugs for neuroblastoma ultimately requires obtaining regulatory approval to enable routine and continual access to an active drug by the largest number of patients. While new agents targeting only neuroblastoma have been successfully developed, the current economic climate diminishes such opportunities for the foreseeable future. The substantial costs required for drug development and for insuring ongoing clinical supplies of a new agent will be surveyed. Developmental pathways will be reviewed for agents successfully incorporated into standard care for neuroblastoma (e.g. ch14.18 + mAb1A7), and for selected agents currently in clinical development (MBfG, BSO, fenretinide, safingol, ABT-751, hu14.18-I2L, ALK inhibitors). New drugs that have potential adult oncology indications likely will undergo more rapid development as the adult data informs pediatric clinical trials and funding is more accessible. Drugs that obtain regulatory approval in adult indications can be made available for treating neuroblastoma without conducting neuroblastoma drug registration studies, which are costly in time, money, and patients. Thus, future new agent development for neuroblastoma should, whenever possible, involve early collaborations with adult oncology investigators and should seek to maximally leverage recent legislation requiring pediatric drug development plans.
SS1
A public web-based analysis tool and database for high throughput and clinical data of neuroblastoma: introduction to practical use of R2
Koster, Jan; van Sluis, Peter; Ora, Ingrid; Caron, Huib; Molenaar, Jan; Versteeg, Roger
Dept. of Human Genetics, University of Amsterdam, Amsterdam, Netherlands
High throughput mRNA profiling is an efficient tool to identify expression levels for any gene in series of tumors and cell lines. Many tools have been developed for analysis of such data. However, most of them require specialist bioinformatic support or are time consuming or are not integrated in one user-friendly platform. We developed a tool for basic and advanced analysis of such data, called R2. This web-based tool enables swift and user-friendly analyses by any interested researcher. We have generated Affymetrix expression profiles of a series of 88 neuroblastoma tumors, as well as from a limited number of ganglioneuromas and ganglioneuroblastomas. The series is annotated for clinical and molecular parameters. R2 enables to analyse expression values for any gene, establish its prognostic value and find correlating expression patterns with other genes. It also permits global expression analysis and prognostic ordering for gene sets, which can be formed by KEGG pathways, functional groups, etc. Differentially expressed genes can efficiently be identified within annotated parameters, like age, stage, histology etc. Any resulting gene list can be analysed for pathway or gene ontology enrichment, or be visualized in heat maps. Finally, the R2 tool and database includes Affymetrix expression profiles of over 20,000 normal and tumor samples from other tissues, grouped per tumor or tissue type. This enables quick searches for expression profiles of specific genes over a wide range of tissues. The web-based R2 analysis tool and database will be made publicly available at the ANR.

SS2
Current and future strategies to improve outcomes in neuroblastoma: An update from the Children's Oncology Group Neuroblastoma Disease Committee
John M. Maris
United States
The long-term objectives of our committee rely on continued robust specimen collection and annotation to support the discovery and validation efforts required for a personalized approach to neuroblastoma diagnosis, prognostication, treatment and surveillance. Ongoing projects focused on discovery of oncogenic drivers, detection of rare residual tumor cells and identifying genomic signatures of tumor behavior will all be highlighted at ANR2010, and will be integrated into future clinical trials. For patients with high-risk disease, the recent demonstration that passive immunotherapy with ch14.18 combined cytokines (alternating GM-CSF and IL2) to improve antibody dependant cellular cytotoxicity dramatically improves survival rates when administered shortly after myeloablative therapy (Yu et al., NEJM in press) will provide the new baseline for which future studies will be compared. Ongoing clinical research is focused on further defining the toxicities associated with this regimen, and future studies will seek to further improve efficacy (e.g. hu14.18-IL2) and/or reduce toxicity. A parallel major goal is to improve the quality of induction/consolidation response to cytotoxic therapy. Major efforts include plans to test the efficacy of [111-]MIBG in frontline therapy in a randomized controlled trial, and to integrate molecularly targeted agents into the current chemotherapy backbone of induction therapy. Inhibitors of ALK, IGF1R and AURKA are lead candidates at this time, but it is clear that a major obstacle is a continued paucity of validated targets. Prospective identification of cases harboring mutated ALK receptors may provide for the opportunity for individualized therapy in the next generation of studies. Finally, the overall strategy for patients with non-high-risk disease will be to continue to utilize biomarkers allowing for reduction of cytotoxic therapy (Baker et al., NEJM in press). However, special emphasis on patient subsets with continued suboptimal outcomes, such as very young infants with INRG Stage MS disease, older children with Stage L2 disease and unfavorable genomic features, and the adolescents and young adults with any stage of disease, will require international cooperation and harmonization of approaches.
Email: Maris@email.chop.edu

SS3
Strategies to improve outcome and quality of life in patients with neuroblastoma: Activities of the SIOP European Neuroblastoma Group
Ladenstein, Ruth
Austria
No abstract available

SS4
Recent achievements and future strategies of GPOH to improve outcome for children with neuroblastoma
Simon, Thorsten
Germany
No abstract available
Fatty acids are precursors to a wide range of different lipid mediators that are suggested to play fundamental different roles in cancer development. Metabolic conversion of omega-6 fatty acids, represented by arachidonic acid, mostly gives rise to the pro-inflammatory eicosanoids, prostaglandins and leukotrienes, which are potent promoters of tumour growth. Omega-3 fatty acids, on the other hand, can give rise to anti-inflammatory and pro-resolving lipid mediators that have the capacity to inhibit tumour growth.

In humans, the omega-3 fatty acid, docosahexaenoic acid (DHA), is the most abundant fatty acid found in neural cells and the active lipid mediators of DHA shield normal neural cells against cellular insults through inhibition of oxidative stress and apoptotic processes. Conversely, neuroblastoma tumour cells are profoundly deficient in DHA but contain elevated levels of the omega-6 fatty acid arachidonic acid as well as enzymes involved in the conversion of arachidonic acid to prostaglandins and leukotrienes. Unlike normal neural cells, neuroblastoma cells are not able to convert DHA to protective lipid mediators. Instead, exogenous DHA is converted to lipid intermediates that are highly toxic resulting in apoptosis. Also, inhibition of prostaglandin or leukotriene synthesis induces mitochondrial-dependent apoptosis of neuroblastoma cells, whereas exogenous addition of these pro-inflammatory eicosanoids stimulates neuroblastoma cell proliferation. Neuroblastoma expresses both prostanoid and leukotriene receptors and binding of these eicosanoids to their cognate G-protein coupled receptors results in the activation of PI3K/Akt and Erk-mediated signal transduction. These findings suggest that both prostaglandins and leukotrienes are constituents of an autocrine survival loop in neuroblastoma.

In preclinical in vivo models, both DHA and agents that inhibit the production of prostaglandins, leukotrienes or their receptors have profound effects both on neuroblastoma development as well as growth and progression of established neuroblastoma tumours. Moreover, DHA and nonsteroidal anti-inflammatory drugs (NSAI�Ds) augment the toxic effects of several established cytostatic drugs in clinical use against neuroblastoma, suggesting these novel agents as potential new elements in clinical treatment protocols. Besides the direct inhibition of prostaglandin synthesis, NSAI�Ds have several side-effects that increase the anti-tumourigenic potential. In neuroblastoma NSAI�Ds significantly inhibit angiogenesis and directly act as an inhibitor of PI3K/Akt signalling resulting in reduced expression of Mycn. NSAI�Ds also enhance the chemosensitivity in neuroblastoma via downregulating HDm2 and augmenting p53 stability or differentially modulate p73 isoforms leading to enhanced apoptosis. In vivo, IL-6 induce the expression of cyclooxygenase-2 in neuroblastoma cells resulting in increased release of prostaglandin E2 and enhanced expression of IL-6 by bone marrow stromal cells making the bone marrow microenvironment favourable to the progression of metastatic neuroblastoma.

Taken together, these results from our group and others suggest that fatty acids and inhibition of their active lipid mediators have profound regulating effects on neuroblastoma initiation, progression and metastatic potential. Since, several compounds that inhibit these lipid mediators are available and tested for other purposes these drugs should be considered to be included in clinical testing as adjuvant to current therapeutic modalities.

Email: per.kogner@ki.se
There is a rich history of seeking to understand the genetic basis of neuroblastoma, beginning with the original description of aberrancies at the karyotypic level several decades ago. The Advances in Neuroblastoma Research Meeting has been the major venue to preview the seminal genetic discoveries in neuroblastoma, and these will be reviewed. Our laboratory has sought to build on this broad foundation to both understand how the host genome impacts susceptibility to develop neuroblastoma and how these events lead to the somatically acquired mutational events that lead to the diverse clinical phenotypes seen in the clinic. Here we will highlight some recent discoveries in neuroblastoma genetics from our laboratory, and discuss future plans designed to discover all of the major driver mutations in neuroblastoma.

**Heritable genetics.** We have discovered **ALK** as the major neuroblastoma predisposition gene. Activating mutations occur in the germline, but also somatically in 10-15% of primary tumors. Targeted inhibition strategies have proven effective in preclinical models, and a clinical trial is ongoing. **PHOX2B** loss of function mutations explain hereditary and sporadic neuroblastoma cases with associated Hirschsprung disease and/or congenital central hypoventilation syndrome. A third putative familial neuroblastoma locus has been tentatively mapped. In addition, by studying over 3,500 neuroblastoma cases compared to 10,000 controls, we have discovered multiple single nucleotide polymorphisms (SNPs) and copy number variations (CNVs) highly associated with neuroblastoma. The associations are phenotype specific, suggesting that neuroblastoma represents distinct diseases at the level of genetic initiation. Three proven associations result in somatic gain of function effects (**BARD1**, **NME7** and **LMO1**), and we now have evidence that these genes do indeed contribute to the malignant phenotype in high-risk neuroblastoma, thus providing possibilities for targeted therapeutics.

**Somatic genetics.** As part of the NCI-funded Therapeutically Applicable Research to Generate Effective Treatments (TARGET) project, we have generated high density SNP and Affymetrix HuEX array data on over 250 cases and used these data to further refine our genomic classification of tumors with an emphasis of refining risk classification. These data provide refined maps of copy number alterations in neuroblastoma, extend the prognostic impact of DNA and RNA signatures for predicting outcome, and have defined the frequency of mutation in over 100 regional candidate genes. The combined data further emphasize the central role of the myc family of proteins in neuroblastoma, and have identified several candidate therapeutic targets that are related to MYCN amplification and/or basal myc overexpression, such as **ALK**, **AURKA** and **CHK1**. We are currently performing a thorough resequencing of the neuroblastoma genome: 10 full genomes and 200 exomes, each with matched germline DNA, and tumor RNA sequencing in a subset. These data should provide a comprehensive catalogue of the majority (if not all) clinically relevant mutations. Ongoing epigenomic profiling will eventually be layered onto this dataset that will be freely available to all investigators. Taken together, the data generated from these projects should provide tractable therapeutic targets for rapid translation to the clinic. Moving past discovery efforts, to mechanistic understanding of DNA sequence variation, and defining the structure-function relationship of newly identified mutations, will be essential for these translational efforts.

Email: Maris@email.chop.edu
Copy number variations (CNVs) in neuroblastoma

Diskin, Sharon 1; Bosse, Kristopher 1; Mayes, Patrick 1; LaQuaglia, Michael 2; Attiyeh, Edward F. 3; Mosse, Yael P. 2; Laudenslager, Marc 2; Diamond, Maura 4; Norris, Geoffrey 4; Hou, Cuiping 5; Wang, Kai 5; Zhang, Haitor 6; Kim, Cecilia 5; London, Wendy 7; Devoto, Marcela 2; Li, Hongzhe 8; Hakonarson, Hakon 8; Maris, John M 1

1Children's Hospital of Philadelphia, Center for Childhood Cancer Research, Philadelphia, United States; 2Children's Hospital of Philadelphia, Center for Applied Genomics, Philadelphia, United States; 3Children's Hospital of Boston, Statistics, Boston, United States; 4Children's Hospital ofPhiladelphia, Genetics, Philadelphia, United States; 5University of Pennsylvania, Biostatistics, Philadelphia, United States; 6Children's Hospital of Philadelphia, Center for Childhood Cancer Research, Philadelphia, United States; 7Children's Hospital of Philadelphia, Center for Applied Genomics, Philadelphia, United States; 8University of Amsterdam, Human Genetics, Amsterdam, Netherlands;

Email: diskin@email.chop.edu

To date, we have genotyped over 3,500 NB cases and have reported two loci containing common SNPs (NEJM 2008, Nat Genet 2008) and a common CNV (Nature 2009) each highly associated with NB. We have discovered an additional CNV association within NME7 at 1q24.2 (see Maris, et al. ANR 2010 for additional SNP associations). This CNV is highly correlated with NME7 mRNA expression in LCLs (p < 0.0001). To investigate whether somatic alterations of NME7 also influence tumorigenesis, we analyzed tumor DNA copy number in 591 primary tumors and matched mRNA expression in a subset of 100 samples. We observed somatic gain of the NME7 locus in 24% of NBs (p = 3.3 x 10^-9). Tumor acquired somatic gain was also highly correlated with NME7 mRNA expression (p = 0.007), and Western blot confirmed a strict correspondence between mRNA and protein levels. Targeted knockdown of NME7 in NB cell lines resulted in decreased cell proliferation, restoration of contact inhibition, and decreased cell migration. Over-expression of NME7 in non malignant mouse neuroblasts. Inducible over-expression of LIN28B in neuroblastoma cell lines with low LIN28B mRNA expression levels was well accepted and induced a slight growth advantage. Over-expression of LIN28B was able to immortalize non malignant mouse neuroblasts. Finally, to study a functional role of LIN28B in vivo we performed correlation analysis using mRNA expression data (Affymetrix U133-plus2) and MIR expression data in 88 neuroblastoma samples using the R2 bio-informatic tool. Several miRNAs showed an inverse correlation with LIN28B mRNA expression. The 8 most significant inversely correlating miRNAs all belonged to the Let-7 cluster. This suggests that the LIN28B gene is functional and inhibits Let7 miRNAs processing in vivo.

Conclusions: Multiple members of the Let-7 miRNA family are repressed in human cancers. This was shown to result in malignant transformation through stabilisation of miRNAs coding for various oncogenes like MYC and RAS. The LIN28 and LIN28B RNA binding proteins inhibit processing of Let7 pre-miRNAs to mature miRNAs. This suggests that LIN28 and LIN28B could be involved in malignant transformation of cells but no genomic alterations of these genes have been identified.

Methods and results: We report a neuroblastoma tumor with high level DNA amplification of the LIN28B gene located on chromosome 6q21. In addition, the mRNA expression levels of LIN28B were strongly increased in a panel of 88 neuroblastoma tumors compared to normal tissues and many other human malignancies. Over-expression of LIN28B was highly correlated with a poor prognosis in neuroblastoma patients. We therefore investigated the functional role of LIN28B in neuroblastoma.

Lentiviral mediated silencing of LIN28B by various siRNA's resulted in strong neuronal differentiation in a panel of neuroblastoma cell lines, including several neuroblastoma TIC (Tumor Initiating Cells) lines. Inducible over-expression of LIN28B in neuroblastoma cell lines with low LIN28B mRNA expression levels was well accepted and induced a slight growth advantage. Over-expression of LIN28B was able to immortalize non malignant mouse neuroblasts. Finally, to study a functional role of LIN28B in vivo we performed correlation analysis using mRNA expression data (Affymetrix U133-plus2) and MIR expression data in 88 neuroblastoma samples using the R2 bio-informatic tool. Several miRNAs showed an inverse correlation with LIN28B mRNA expression. The 8 most significant inversely correlating miRNAs all belonged to the Let-7 cluster. This suggests that the LIN28B gene is functional and inhibits Let7 miRNAs processing in vivo.

Conclusion: We conclude that LIN28B is a potential tumor driving gene in neuroblastoma and we are currently unravelling the downstream signalling cascade.

Email: j.j.molenaar@amc.uva.nl
CAMTA1, a 1p36 tumor suppressor candidate, activates differentiation programmes in neuroblastoma cells

Henrich, Kai-Oliver1; Bauer, Tobias2; Ehmenn, Volker3; Deubzer, Hedwig4; Gogolin, Sina5; Fischer, Matthias6; Schwab, Manfred7; Westermann, Frank8

1DKFZ German Cancer Research Center, Tumor Genetics B030, Heidelberg, Germany; 2DKFZ German Cancer Research Center, Theoretical Bioinformatics B080, Heidelberg, Germany; 3University of Heidelberg, Pathology, Heidelberg, Germany; 4DKFZ Cancer Research Center, Clinical Cooperation Unit Pediatric Oncology, Heidelberg, Germany; 5University Children's Hospital, Pediatric Oncology, Cologne, Germany; 6DKFZ Cancer Research Center, Tumor Genetics B030, Heidelberg, Germany

**Background:** Deletion within distal 1p characterizes about 30% of neuroblastomas (NBs) and it is widely assumed that this region harbours genetic information mediating tumor suppression. The combination of recent fine mapping studies defined a 1p36 smallest region of consistent deletion pinpointing the CAMTA1 locus. Multivariate survival analysis identified low CAMTA1 expression as an independent predictor of poor survival and CAMTA1 is included in most of the recent prognostic NB expression classifier gene sets.

**Aim:** To analyze the potential effect of CAMTA1 on NB biology using inducible cell models.

**Methods:** Expression of CAMTA1 is low in NB cell lines compared to favourable tumors. We established stable NB cell models allowing Tet-inducible re-expression of CAMTA1. A specific antibody was generated to monitor CAMTA1 levels upon induction. The CAMTA1-induced phenotype was assessed using flow cytometry, colony formation, viability and soft agar assays. CAMTA1 regulation was assessed in neuroblastoma differentiation models. Transcriptome analysis upon CAMTA1 induction was done using whole genome microarrays.

**Results:** CAMTA1 induction in NB cells significantly decreased colony formation ability and growth rate. Growth inhibition was associated with induction of the cell cycle inhibitor p21 and an increase of the proportion of cells in G1/G0 phase. In neuroblastoma cells growing in soft agar, CAMTA1 induction inhibited the capacity of anchorage-independent growth. Further, CAMTA1 induction induced morphological changes and markers characteristic of neuronal differentiation. CAMTA1 expression was upregulated upon differentiation of NB cells induced by external stimuli. Time-resolved transcriptome analysis revealed 683 genes regulated upon CAMTA1 induction. Among CAMTA1 induced genes, GO terms related to neuronal function and differentiation were significantly enriched. Among CAMTA1 repressed genes, the majority of enriched GO terms related to cell cycle progression.

**Conclusion:** Together, our data imply that CAMTA1 is a 1p36 tumor suppressor candidate that inhibits features of malignant cells and is involved in neuronal differentiation.

**Email:** k.henrich@dkfz.de
Role of a novel inducible dependence receptor UNCSD in spontaneous regression of neuroblastomas: its functional cooperation with p53 and E2F1 for inducing programmed cell death

Zhu, Yuyan1; Li, Yuanyuan1; Haraguchi, Seiki2; Yu, Meng1; Ohira, Miki3; Nakagawa, Atsuko4; Isogai, Eriko5; Koseki, Haruhiko6; Nakamura, Yoko1; Arakawa, Hirofumi1; Nakagawa, Akira7

1Chiba Cancer Center Research Institute, Laboratory of Biochemistry and Innovative Cancer Therapeutics, Chiba, Japan; 2Chiba Cancer Center Research Institute, Laboratory of Embryonic & Genetic Engineering, Chiba, Japan; 3Chiba Cancer Center Research Institute, Laboratory of Cancer Genomics, Chiba, Japan; 4National Center for Child Health & Development, Department of Pathological Diagnosis, Tokyo, Japan; 5RIKEN Research Center for Allergy and Immunology, Laboratory of Developmental Genetics, Yokohama, Japan; 6National Cancer Center Research Institute, Division of Cancer Medicine and Biophysics, Tokyo, Japan

Background: The signals through NGF receptors (TrkA and p75NTR) may regulate induction of spontaneous regression in neuroblastomas (NBs). However, the molecular mechanism remains elusive. We have cloned a novel dependence receptor UNCSD, a member of netrin-1 receptors (DCD and UNC5 family), from the cDNA libraries generated from primary neuroblastoma tissues. Here we show that UNCSD is a novel inducible gene after NGF deletion and other stresses, and that it dramatically enhances the NGF-depletion-induced neuronal programmed cell death by cooperating with E2F1 and p53.

Methods: DNA and mRNA were extracted from 108 primary NBs. The mRNA expression and the protein/protein interactions were examined by a quantitative RT-PCR and immunoprecipitation experiments, respectively. Transcriptional activation was investigated by luciferase reporter assays. We generated Unc5D4 knockout mice and prepared MEF cells and sympathetic neurons.

Results: The significantly high expression of UncSD4, but not Unc5A-C, was observed in favorable NBs and associated with good prognosis (p=0.003). The ligand, netrin-1, was only weakly detected in stromal cells by immunohistochemistry. Like E2F1 and p53, the NGF withdrawal strongly upregulated UNCSD, but not Unc5A-C, in both favorable NB cells in primary culture and PC12 cells, suggesting that UNCSD is an inducible gene. In addition, UNCSD was a direct transcriptional target of p53. The NGF depletion as well as DNA damage induced cleavage of intracellular domain of UNCSD by caspases 2 and 3, and the cleaved fragment translocated into nucleus to form a transcriptional complex with E2F1, which in turn selectively transactivated pro-apoptotic genes including caspasases, Bid and E2F1 itself. This positive feedback loop dramatically enhanced apoptotic cell death through UNCSD. The analyses using MEF cells and sympathetic neurons obtained from the Unc5D4 knockout mice we generated supported the above observations.

Conclusion: Our results demonstrate that UNCSD contributes to NGF depletion-mediated programmed cell death in neuroblastoma via nuclear translocation of its intracellular fragment (UnICD) which acts as a co-activator of E2F1.

Email: lyuan@chiba-cc.jp

PL10

Metastatic neuroblastoma cancer stem cells display a mixed phenotype of tumor and niche origin required for survival

Hansford, Loen1; Monozova, Olena2; Lipman, Taliana3; Blakely, Kint1; Ohira, Miki5; Marrano, Paula4; Angelini, Paola2; Moffat, Jason1; Thiele, Carol4; Thorner, Paul1; Dick, John1; Nakagawara, Akira1; Irwin, Meredith3; Marra, Marco2; Kaplan, David1

1The Hospital for Sick Children, Department of Cell Biology, MaRS Centre, TDMT East Tower, Room 12-601, 101 College Street, Toronto, Canada; 2Canada’s Michael Smith Genome Sciences Centre, Bioinformatics, Vancouver, Canada; 3The Hospital for Sick Children, Department of Cell Biology, Toronto, Canada; 4University of Toronto, Department of Molecular Genetics, Toronto, Canada; 5Chiba Cancer Center Research Institute, Division of Biochemistry, Chiba, Japan; 6The Hospital for Sick Children, Paediatric Laboratory Medicine, Toronto, Canada; 7National Cancer Institute, National Institutes of Health, Pediatric Branch, Bethesda, United States; 8University Health Network, Division of Cell and Molecular Biology, Toronto, Canada

Neuroblastoma (NB) is a pediatric tumor of neural crest origin, and is the most common cancer of infancy. 50% of patients have metastases at diagnosis, of which 85% will die after multiple relapses from metastatic disease. We identified tumor-initiating cells (TICs) from bone marrow (BM) metastases of high-risk patients that are propagated as spheres in mouse models, and that have many properties of cancer stem cells, and form NB in mice with as few as 1 cell (see Van et al abstract). To understand patient relapse and disease progression, we compared NB-TICs from BM with those from tumor and brain metastases and SKPs, a normal pediatric stem cell counterpart, by cDNA microarray, flow cytometry, and whole genome shotgun sequencing transcriptome analysis. BM-derived NB-TICs expressed primitive neural crest and neuronal markers as well as hematopoietic markers from primitive, myeloid, and B-cell lineages and contained VDJ gene rearrangements, which are normally associated with B-cell leukemias. Hematopoietic genes were not expressed or expressed at very low levels in tumor-derived sphere lines and a line from an NB brain metastasis, however brain metastasis-derived TICs expressed CD133, while BM-derived NB-TICs did not. Furthermore, we found that shrRNA to CD74, which is upregulated in B-cell lymphoma and multiple myeloma and is a therapeutic target for those cancers, induced the rapid death of NB-TICs but not normal SKPs. Interestingly cells co-staining for the hematopoietic markers CD45 or CD74 and the neural neural progenitor marker nestin were found in BM smears of patients with relapsed NB in the BM.

We suggest that metastatic TICs from some tumors adopt resident niche-specific gene expression signatures, which may aid diagnosis and the development of novel treatments. We hypothesize that drugs used for leukemia may be efficacious therapeutics for metastatic NB, and are currently testing this hypothesis in mouse models.

Email: loen.hansford@sickkids.ca
CELL-THEMED SESSIONS

PL11
Cell-cell communication via ion fluxes in control of neuroblastoma cell cycle
Hyoshi, Hiromi1; Abdelhady, Shaimaa1; Sveinbjörnsson, Baldur1; Segerström, Lova2; Hansford, Loen1; Irwin, Meredith3; Kaplan, David1; Irwin, Meredith3; Kaplan, David3; Segerström, Lova2; Hansford, Loen1; Irwin, Meredith3; Kaplan, David3

Background: Ion channels control proliferation and self-renewal in stem cells and may act as tumor suppressors in cancer cells. We recently found an unexpected signaling pathway that controls proliferation via GABA gated ion channel activity and the DNA damage response (DDR) pathway (Nature 451, 2008). Our new data suggest yet another example of such a cell-cell signaling modality via the sodium pump, Na,K-ATPase.

Method/approach: We investigated primary human neuroblastomas, cultured neuroblastoma cell lines and TICs with immunostaining, electrophysiology, and qRT-PCR, and randomized nude mice with SH-SY5Y xenografts for targeted therapy or as controls.

Results: We found that low levels of ouabain (<10% pump inhibition) controlled Na,K-ATPase activity in neuroblastoma cells and mediated a fully reversible cell cycle block via the DDR pathway without causing DNA damage. A DDR pathway inhibitor reduced the cell cycle block, confirming a causal mechanistic link. Oral ouabain treatment of mice with xenografts showed that all tested non-MYcn amplified tumors expressed the critical Na,K-ATPase subunit for ouabain sensitivity, whereas MYcn amplified tumors did not.

Conclusion: Ouabain is synthesized by the adrenal glands, an environment where most neuroblastoma tumors reside. Therefore, we expect that ouabain sensitivity may have a physiological relevance for tumor growth. These data suggest novel options for targeted therapy.

Email: michael.andang@ki.se

PL12
PHOX2B is essential for peripheral sympathetic neuronal differentiation in the zebrafish
Luther II, William1; Stewart, Rodney1; Kanki, John1; Look, A. Thomas1; George, Rani E1

Background: Heterozygous mutations in PHOX2B have been identified in neuroblastoma, although the effects of these variants (gain- or loss-of-function) remain unclear. To determine the consequences of phox2b deficiency during embryogenesis, we studied the effects of phox2b knockdown on sympathetic nervous system development in the zebrafish model.

Method: One-cell embryos were injected with anti-sense morpholinos (MO) that block translation or mRNA splicing of zphox2b. Genes involved in sympathetic superior cervical ganglion (SCG) neurogenesis were monitored throughout development by RNA in-situ hybridization.

Results: Knockdown of zphox2b caused a significant decrease in th- and dbh-expressing neurons in the SCG that was rescued by overexpression of wild type (WT) human PHOX2B. Lack of apoptosis in the SCG of phox2b-deficient embryos was demonstrated by acridine orange staining. However, analysis of th- and phox2b expression in phox2b-deficient embryos revealed an increase in the numbers of phox2b-expressing cells in the SCG, with a concomitant decrease in the numbers of th-expressing cells compared to WT controls. Moreover, the SCG cells in phox2b-deficient embryos could not be induced to differentiate by retinoic acid (RA). In addition to increased phox2b expression itself, abrogation of zphox2b expression also led to increased expression of another pro-neurogenic marker zash1a, while the expression of markers indicative of more differentiated neurons zdhand, tfap2a, and gata3 were markedly decreased. To determine if phox2b and zash1a act redundantly with respect to differentiation, we injected embryos with zash1a MO and found only slight decrease in th-expression in the SCG. However, simultaneous knockdown of both zash1a and phox2b expression resulted in complete loss of th- and dbh-expression.

Conclusion: Our data show that loss of zphox2b function during development causes an increase in the number of neuroblasts at the expense of more differentiated cell types. We suggest that PHOX2B loss of function variants give rise to an undifferentiated phenotype that is more vulnerable to second hits that induce malignant transformation.

Email: rani.george@dfci.harvard.edu
PL13  
Infections linked to human cancers  
Zur Hausen, Harald  
Germany  
See page 54 for biography

PL14  
The Yin and Yang functions of the Myc oncoprotein in cancer development and as targets for therapy  
Arsenian Henriksson, Marie  
Karolinska Institutet, Department of Microbiology, Tumor and Cell Biology (MTC), Stockholm, Sweden  
See page 54 for biography

The MYC gene was originally identified in avian retroviruses as the oncogene responsible for inducing myelocytomatosis in birds. The cellular homologue, c-MYC, was found to be evolutionarily conserved. Later, MYCN and MYCL were found amplified in neuroblastoma and in small cell lung cancer, respectively. The MYC genes encode short-lived nuclear phosphoproteins with a half-life of 20–30 min that are subsequently ubiquitinated for proteasomal degradation. MYC is a basic Helix-Loop-Helix Leucine Zipper (bHLHZip) protein that heterodimerizes with the small bHLHZip protein Max resulting in dimers with DNA-binding ability at CACGTG and similar E-box sequences. The MYC proto-oncoproteins coordinate a number of normal physiological processes necessary for growth and expansion of somatic cells by controlling the expression of numerous target genes. Deregulation of MYC as a consequence of carcinogenic events enforces cells to undergo a transition to a hyperproliferative state. This increases the risk of additional oncogenic mutations that in turn can result in further tumor progression. However, MYC activation also provokes intrinsic tumor suppressor mechanisms including apoptosis, cellular senescence and DNA damage responses that act as barriers for tumor development, and therefore needs to be overcome during tumorigenesis. MYC thus possesses two seemingly contradictory “faces” here referred to as “Yin and Yang”. Observations that many tumor suppressor pathways remain intact but are latent in tumor cells opens the possibility that pharmacological inhibition of the Yin or activation of the Yang functions can prevail and offer new attractive approaches for treating diverse types of cancer including neuroblastoma.  
Email: marie.henriksson@ki.se
PL15

Identification of selective inhibitors of neuroblastoma stem cells - targeting the kinome

Grinshtein, Natalie1; Smith, Kristen1; Uehling, David2; Prakesh, Michael3; Isaac, Methvin1; Irwin, Meredith1; Datti, Alessandro4; Wrana, Jeff5; Al-awar, Rima3; Kaplan, David6

1Hospital for Sick Children, Cell Biology, Toronto, Canada; 2Hospital for Sick Children Toronto and The Scripps Research Institute, La Jolla, CA, Cell Biology, Toronto, Canada; 3Ontario Institute of Cancer Research, 4Samuel Lunenfeld Research Institute, SMART High Throughput Facility, Toronto, Canada; 5Samuel Lunenfeld Research Institute and University of Toronto, Department of Molecular Genetics, Toronto, Canada; 6Hospital for Sick Children and University of Toronto, Cell Biology and Department of Molecular Genetics, Toronto, Canada

Background: We previously isolated sphere-forming cells from neuroblastoma (NB) tumors and metastases with many of the properties of cancer stem cells, including the expression of stem cell markers, and the ability to self-renew in culture and form orthotopic metastatic tumors in immunodeficient animals with as few as a single cell (L. Hansford; S. Yan abstracts, ANR 2010).

Methods: To identify signalling pathways important for survival and self-renewal of NB tumor-initiating cells (TICs) as well as potential therapeutic targets we undertook a comprehensive high-throughput screening program using diverse chemical libraries including: (1) kinome library of 160 protein kinase inhibitors, including 33 used clinically, (2) 4400 bioactive compounds and neuroactive drugs of the Prestwick, Lopac, and Spectrum collections that we previously used to identify dequalinium-14, primaquine, and quinicrine as drugs that selectively kill TICs but not normal pediatric neural crest stem cells, and (3) 700,000 drug-like compounds of the Genomics Institute of the novartis research foundatin, which we are using to identify agents that promote NB TIC differentiation as detected by a MAP2 neuronal promoter-driven luciferase reporter.

Results: Here we report on our findings screening early passage NB TIC lines derived from bone marrow metastases from relapsed patients with the kinome library using a 96 hour growth/survival assay (Alamar Blue).

Conclusion: Our findings have identified candidate kinases that regulate primary NB TIC growth and survival, and suggest that PLK1 inhibitors may be an effective therapy for metastatic NB.

Email: dkaplan@sickkids.ca

PL16

A new Aurora kinase inhibitor (CCT241736) regulates Mycn protein expression and prevents neuroblastoma growth in vitro and in vivo

Vaughan, Lynsey1; Cullis, Elizabeth1; Barker, Karen1; Jamin, Yan1; Linardopoulos, Spiros1; Bavetsias, Vassilios1; Atrash, Butrus1; Blagg, Julian1; Pearson, Andrew1; Robinson, Simon1; Cheater, Louis1

1The Institute of Cancer Research, Paediatric oncology, Sutton, United Kingdom; 2The Institute of Cancer Research, Clinical magnetic resonance, Sutton, United Kingdom; 3The Institute of Cancer Research, Cancer Therapeutics, London, United Kingdom; 4The Institute of Cancer Research, Paediatric oncology, London, United Kingdom; 5The Institute of Cancer Research, Clinical magnetic resonance, London, United Kingdom

Background: Amplification of the MYCN oncogene occurs in 25% of neuroblastoma (NB) correlating with poor clinical outcome. We have examined indirect methods of destabilising Mycn by targeting key components of upstream signalling pathways responsible for maintenance of Mycn protein levels. Aurora kinases regulate cell cycle transit from G2 through to cytokinesis, are amplified in a variety of cancers. Recently, Otto et al also showed that Aurora stabilizes the Mycn protein by specifically interfering ubiquitination/degradation of Mycn in the proteasome. We have a significant chemistry effort targeted at developing refined Aurora A kinase inhibitors with enhanced ability to destabilize Mycn.

Method/Results: CCT241736 is a potent inhibitor of Aurora A with IC50 values in the nanomolar (nM) range. We show that CCT241736 inhibits phosphorylation of Histone H3 (Ser10) in vitro in a panel of isogenically derived wild-type (wt) N-myc and N-myc phosphomutant cell lines indicating Aurora Kinase inhibition. N-myc phosphomutants are resistant to destabilisation by broad spectrum PI3K blockade (Chesler, 2006). CCT241736 targeted wt N-myc expressing cells at nanomolar concentrations in SRB and MTS assays. In comparison, N-myc phosphomutant cells treated with CCT241736 show no decrease in the steady state levels of the N-myc protein on western blots and showed less decrease in cell viability. CCT241736 was significantly effective against tumour progression concomitant with destabilization of Mycn protein in a therapeutic intervention trial in the TH-MYCN model.

Conclusions: We conclude that targeting of Aurora A kinase destabilizes Mycn protein in vitro and in vivo and may have therapeutic efficacy in MYCN-amplified neuroblastoma. CCT241736 displayed promising in vivo and in vitro activity and refined CCT compounds targeted more specifically against Mycn are under development.

Email: lynsey.vaughan@icr.ac.uk
**PL17**

Mycn as a critical target of PI3K/mTOR inhibitors in neuroblastoma: paracrine effects on tumor vasculature  

Chen, Yvan1; Hackett, Chris1; Itsara, Melissa1; Grimmer, Matt2; Chesler, Louis3; Matthay, Katherine4; Weiss, William1  

1University of California, San Francisco, BMS- Neurology, San Francisco, United States; 2The Institute of Cancer Research, Pediatric Oncology, United Kingdom; 3University of California, San Francisco, Pediatric Hematology/Oncology, UCSF, San Francisco, United States; 4National Cancer Institute, Biomedical Informatics, Bethesda, United States; 5School of Medicine Cancer Center, Oncology, Lubbock, United States; 6University of Florida, Children's Oncology Group Statistics and Data Center, Gainesville, United States; 7Dana-Farber/Children's Hospital Boston Cancer Care, Children's Oncology Group Statistics and Data Center, Boston, United States; 8Pfizer Global Research and Development, Cancer Chemistry and Cancer Biology, La Jolla, United States  

Inhibitors of PI3K and of mTOR are now in clinical trials, with preclinical studies suggesting efficacy against tumor vasculature. We showed previously that this class of inhibitors also blocks Mycn protein, and that blockade of Mycn contributes to in vivo efficacy. Here, we extend this result, demonstrating that BEZ235 (Novartis), a dual inhibitor of PI3K and mTOR, led to decreased levels of Mycn protein and improved survival in mice transgenic for TH-MYCn. Murine tissues analyzed after BEZ235 treatment showed decreased proliferation and viability of both tumor cells, and tumor-associated endothelial and perivascular cells, with no untoward effects observed in normal retinal vasculature. To evaluate whether destabilization of Mycn contributes to the effects of BEZ235 on the tumor microenvironment, we co-cultured human endothelial and neuroblastoma cells, the latter transfected with vector, wt, or phospho-defective alleles of N-myc. Phospho-defective alleles of N-myc were unaffected by BEZ235 treatment, with cocultures showing increased levels of endothelial proliferation (angiogenesis) and increased levels of secreted Vascular Endothelial Growth Factor (VEGF) relative to tumor cells carrying vector or wild-type N-myc. Control co-culture experiments using TET21 cells (doxycycline regulated expression of Mycn), and using siRNA against MYCN confirmed a role for Mycn in driving paracrine VEGF signaling and promoting tumor angiogenesis. These results support: 1). Mycn as a critical target of PI3K/mTOR inhibitors, 2). A role for Mycn in sustaining tumor vasculature, in tumors driven by MYCN, and 3). PI3K/mTOR inhibition both in vitro and in vivo models to PF-02341066, an ATP-competitive, orally bioavailable small molecule inhibitor of ALK and MET, evaluate dose-dependent inhibition of phosphoprotein signaling, and begin to predict for resistance in the clinic.

**Results:** To date, we have sequenced and genotyped of 594 primary neuroblastomas and have identified non-synonymous sequence variations in 7.2% of samples (43/594), which grouped into four hotspots within the kinase domain. In the extracellular domain, we discovered and validated seven nonsynonymous sequence variations. We detected high-level amplification of ALK in 2.4% of tumors and show that ALK amplification and regional gain of the ALK locus are associated with increased ALK expression. We engineered human ALK cDNAs harboring the three most common germline mutations and the F1174L mutation, stably overexpressed these in retinal pigment epithelial cells, and show that these are gain-of-function mutations that induce differential constitutive kinase activation. We show that cytotoxicity to pharmacological ALK inhibition both in vitro and in vivo is dependent upon ALK genomic status, and correlates with abrogation of phospho-ALK and differential inhibition of downstream signaling pathways. We use homology modeling to predict a structural basis for differential activity against R1275Q and F1174L mutations.

**Conclusions:** Our data demonstrate that cytotoxicity to PF-02341066 is highly associated with ALK genomic status and evidence for constitutive activation, and provides the pre-clinical rationale for an ongoing phase 1/2 clinical trial in the Children's Oncology Group. Establishing the molecular mechanisms underlying the emergence of resistance, and understanding the structural basis of ALK inhibitory activity will be crucial for the development of ALK inhibition strategies.

**Email:** mosse@chop.edu

---

**PL18**

Exploitation of ALK as a therapeutic target in neuroblastoma  

Wood, Andrew1; Laudenslager, Marco1; Huglund, Elizabeth1; Courtwright, Joshua1; Plegaria, Jeffrey1; Carpenter, Eric1; Diskin, Sharon1; Attiyeh, Edward1; Cole, Kristina1; Toporovskaya, Yana1; Pawel, Bruce2; Zhao, Huaiqing1; Zhang, Junghui1; Reynolds, Patrik1; McGrady, Patrik1; London, Wendy1; McGue, Michael1; Marrone, Tam1; James, Christensen1; Maris, John1; Mosse, Yael1  

1Children's Hospital of Philadelphia, Oncology, Philadelphia, United States; 2Children's Hospital of Philadelphia, Pathology, Philadelphia, United States; 3University of California, San Francisco, Neurology, San Francisco, United States; 4National Cancer Institute, Biomedical Informatics, Bethesda, United States; 5School of Medicine Cancer Center, Oncology, Lubbock, United States; 6University of Florida, Children's Oncology Group Statistics and Data Center, Gainesville, United States; 7Dana-Farber/Children's Hospital Boston Cancer Care, Children's Oncology Group Statistics and Data Center, Boston, United States; 8Pfizer Global Research and Development, Cancer Chemistry and Cancer Biology, La Jolla, United States  

**Background:** The recent discovery of germline and somatic gain of function mutations in the receptor tyrosine kinase ALK provides a tractable therapeutic target for new drug development in neuroblastoma.

**Methods:** We report a comprehensive survey of ALK genomic status in all neuroblastoma phenotypic subsets. We determine the sensitivity of neuroblastoma in vitro and in vivo models to PF-02341066, an ATP- competitive, orally bioavailable small molecule inhibitor of ALK and MET, evaluate dose-dependent inhibition of phosphoprotein signaling, and begin to predict for resistance in the clinic.

**Results:** To date, we have sequenced and genotyped of 594 primary neuroblastomas and have identified non-synonymous sequence variations in 7.2% of samples (43/594), which grouped into four hotspots within the kinase domain. In the extracellular domain, we discovered and validated seven nonsynonymous sequence variations. We detected high-level amplification of ALK in 2.4% of tumors and show that ALK amplification and regional gain of the ALK locus are associated with increased ALK expression. We engineered human ALK cDNAs harboring the three most common germline mutations and the F1174L mutation, stably overexpressed these in retinal pigment epithelial cells, and show that these are gain-of-function mutations that induce differential constitutive kinase activation. We show that cytotoxicity to pharmacological ALK inhibition both in vitro and in vivo is dependent upon ALK genomic status, and correlates with abrogation of phospho-ALK and differential inhibition of downstream signaling pathways. We use homology modeling to predict a structural basis for differential activity against R1275Q and F1174L mutations.

**Conclusions:** Our data demonstrate that cytotoxicity to PF-02341066 is highly associated with ALK genomic status and evidence for constitutive activation, and provides the pre-clinical rationale for an ongoing phase 1/2 clinical trial in the Children's Oncology Group. Establishing the molecular mechanisms underlying the emergence of resistance, and understanding the structural basis of ALK inhibitory activity will be crucial for the development of ALK inhibition strategies.

**Email:** mosse@chop.edu
An RNAi screen of the protein kinome identifies CHK1 as a therapeutic target in neuroblastoma

Cole, Kristina; Huggins, Jonathan; Laquaglia, Michael; Huiderman, Chase; Aliyeh, Edward; Winter, Cynthia; Dickin, Sharon; Boase, Kristopher; Mayes, Patrick; Jagannathan, Jayanti; Norris, Geoffrey; Mosse, Yael; Manis, John
The Children's Hospital of Philadelphia and University of Pennsylvania School of Medicine, Division of Oncology / Department of Pediatrics, Philadelphia, PA, United States

Background: Despite intensification of therapy for neuroblastoma, survival is poor. Therefore, patients will benefit from treatment strategies that rationally exploit signaling pathways for which a tumor cell is selectively dependent. In an effort to identify novel therapeutic targets, we performed a comprehensive loss of function screen of the protein kinome in neuroblastoma cell models.

Method: Using a validated siRNA library targeting the human protein kinome, 529 individual kinase siRNAs were transfected into four neuroblastoma cell lines and substrate adherent growth was measured.

Results: Thirty kinase targets had broad activity in the RNAi screen, but the cell cycle checkpoint kinase CHK1 was the most potent. CHK1 mRNA expression was significantly higher in MYCN amplified (p < 0.0001) and high risk tumors (p < 0.05). Western blotting revealed that CHK1 is constitutively phosphorylated in 9 of 10 neuroblastoma cell lines and a panel of high risk primary tumors, but not in control cell lines or low risk primary tumors. As the next step in translation of our genetic screen, we tested two CHK1 tool compounds. Pharmacologic inhibition by SB21807 and TCS2312 showed cytotoxicity in 7 of 9 neuroblastoma cell lines with median IC50s of 564 nM (62-695 nM) and 548 nM (159-973 nM), respectively. In contrast, the control lines were resistant with high micromolar IC50s. There was a near perfect correlation of CHK1 phosphorylation and CHK1 inhibitor sensitivity. The mechanism of selective inhibition in neuroblastoma is unclear as we did not identify mutations in the coding exons of CHK1. However, cell cycle analysis suggests that CHK1 inhibition in neuroblastoma causes apoptosis during S-phase, consistent with its role in replication fork progression.

Conclusion: We have identified CHK1 as a potential therapeutic target in neuroblastoma and are currently extending our work to understand the mechanism of this tumor's apparent selective sensitivity to CHK1 inhibition. As CHK1 inhibitors are currently in phase I/II clinical trials as chemosensitizers, we are also focused on determining the in vivo efficacy of combination CHK1 inhibition with chemotherapy in neuroblastoma.

Email: colek@email.chop.edu
PL21

Genome-wide mapping of MYCN binding sites in neuroblastoma reveals e-box motif frequencies and associations with regions of DNA hypermethylation

Murphy, Derek1; Buckley, Patrick1; Bryan, Kenneth2; Das, Sudipto1; Alcock, Leah1; Foley, Niamh1; Prenter, Suzanne1; Bray, Isabella1; Watters, Karen1; Desmond, Higgins2; Stallings, Raymond L1.

1Royal College of Surgeons in Ireland, Department of Cancer Genetics & Children’s Research Centre, Our Lady’s Children’s Hospital, Dublin, Ireland; 2University College Dublin, Conway Institute, Dublin, Ireland

Background: Genomic amplification of MYCN, a member of the MYC family of oncogenic transcription factors, is a powerful prognostic indicator of poor clinical outcome in neuroblastoma (NB). In this study we have characterised MYCN genome-wide promoter occupancy in various NB cell lines and correlate these patterns with regions of DNA hypermethylation.

Methods: MYCN chromatin immunoprecipitates from SK-N-AS, Kelly and SHEP-21 (containing a repressible MYCN transgene) were applied to microarrays (NimbleGen) representing all annotated promoters in the genome. Only sites identified by two independent MYCN antibodies that recognize different epitopes were used in our analyses. In order to evaluate MYCN binding with respect to other genomic features, we determined the methylation status of all annotated CpG islands and promoter sequences using methylated DNA immunoprecipitation (MeDIP).

Results: Assessment of E-box usage within consistently positive MYCN binding sites revealed a predominance for the CATGTG motif (p = 0.0016), with significant enrichment of additional motifs CATTGT, CACTGT, CACACTG in the MYCN amplified state only. Gene ontology analysis revealed enrichment for the binding of MYCN at promoter regions of numerous molecular functional groups including DNA helicases and mRNA transcriptional regulation in cell lines over-expressing MYCN. A highly significant positive correlation between MYCN binding and DNA hypermethylation was identified upon integration of MYCN ChIP-chip and MeDIP data. This association was also detected in regions of hemizygous loss, indicating that the observed association occurs on the same homologue.

Conclusion: These findings suggest that MYCN binding occurs more commonly at CATGTG as opposed to the classical CACGTG E-box motif, and that disease associated overexpression of MYCN leads to aberrant binding to additional weaker affinity E-box motifs in neuroblastoma. The co-localization of MYCN binding and DNA hypermethylation further supports the dual role of MYCN, namely that of a classical transcription factor affecting the activity of individual genes, and that of a mediator of global chromatin structure.

Email: derekmurphy3@rcsi.ie

PL22

Accurate prediction of neuroblastoma outcome based on miRNA expression profiles

Schlaud, Stefan1; Schulte, Johannes1; Schowe, Benjamin1; Mestdagh, Pieter1; Kaderali, Lars1; Kalaghati, Prabhav1; Vermeulen, Joelle1; Brockmeyer, Ben1; Pajtel, Kristian1; Thor, Theresa1; Spelmann, Frank2; Morik, Katharina2; Eggert, Angelika1; Vandesompele, Jo3; Schramm, Alexander1

1University Children’s Hospital Essen, Pediatric Oncology and Hematology, Essen, Germany; 2University of Dortmund, Department of Informatics, Dortmund, Germany; 3Ghent University Hospital, Center for Medical Genetics Ghent (CMGG), Ghent, Belgium; 4University of Heidelberg, Bioquant, Heidelberg, Germany

Background: Identification of new biomarkers and therapeutic targets is mandatory to improve risk stratification and survival rates of neuroblastoma (NB). MicroRNA (miRNA) expression is deregulated in most cancers, including NB. The purpose of this study was to evaluate miRNAs as NB biomarkers and identify miRNAs involved in NB tumor biology and prognosis.

Method/approach: miRNA expression was analyzed in 69 NB patients using stem-loop RT-qPCR. Patient outcome was predicted based on miRNA expression patterns using support vector machines (SVM). Survival times were analyzed with Cox regression-based models (CASPAR).

Results: Of the 430 miRNAs analyzed, 307 were readily detectable. Prediction of event-free survival (EFS) with SVM and CASPAR were highly accurate, and reached 86.7% for SVM on a training set. Five-year EFS was 19% for patients predicted by CASPAR to have a poor outcome versus 78% for patients predicted to have long-term survival. Validation in an independent test set yielded accuracies of 94.7%(SVM) and 5y-EFS probabilities (CASPAR) of 25% for predicted poor outcome versus 100% for predicted long-term survival. Kaplan-Meier analysis revealed that both classifiers effectively separated patients with adverse clinical course (p<0.001). MYCN-amplification was highly correlated with deregulated miRNA expression, including miRNAs of the miR-17-92 cluster, the miR-181 family and miR-34a. Interestingly, 37 miRNAs correlated with expression of the TrkA neurotrophin receptor (p<0.05). Overexpression of TrkA in vitro regulated 6 of 11 mirnAs further analyzed, suggesting a functional relationship. Among the miRNAs most significantly correlated with TrkA expression in vivo was miR-542-5p. This miRNA was also induced upon TrkA overexpression in vitro, was inversely correlated with MYCN amplification in NB tumors and was a marker of EFS in the corresponding NB patients (p<0.001).

Conclusion: NB patient outcome prediction using miRNA expression is feasible and effective. Specific miRNAs such as miR-542-5p are likely to be important in NB tumor biology, and may qualify as potential therapeutic targets.

Email: johannes.schulte@uni-due.de
PL23

Individual survival time prediction from gene-expression and/or global genomic data of neuroblastoma patients using CASPAR
Oberthuer, Andre1; Kalaghagi, Prabhav2; Kahliert, Yvonne1; Hero, Barbara1; Berthold, Frank1; Brors, Benedikt1; Ellis, Roland1; Fischer, Matthias1; Kadenali, Lars1
1University of Cologne Children’s Hospital, Pediatric Oncology and Hematology, Cologne, Germany; 2University of Heidelberg, Viroquant Research Group Modeling (BQ26), Bioquant, Heidelberg, Germany

Background: Both array-CGH (aCGH) and gene-expression (GE) data have been used to predict outcome of neuroblastoma patients. However, integration of information from both platforms is not established. Here, we report on an extension of our CASPAR algorithm that allows using data from either platform alone or in combination to predict individual survival time (as a continuous variable) of neuroblastoma patients.

Methods/approach: GE and aCGH data were generated from 128 neuroblastoma patients. Then, CASPAR was applied to GE data alone, aCGH data alone and to combined GE+aCGH data (Comb) using a leave-one-out crossvalidation. In addition, a simple genomic predictor based on intrachromosomal variation (VAR) was built. Subsequently, CASPAR predicted OS times for the total cohort and for patients with 11q-deletion (n=37), 17q-gain (n=71) and MYCN-amplified disease (n=27). Prediction accuracy was assessed by Kaplan-Meier analyses, and ROC curve analyses.

Results: For the total cohort, CASPAR separated patients with distinct outcome with high accuracy from all data sets (GE: 5y-OS 0.81 (patients with predicted long survival) vs. 0.28 (predicted short survival); aCGH 0.77 vs. 0.46; Comb 0.82 vs. 0.23; VAR 0.78 vs. 0.37; all p<0.0001).

Predictions from GE data alone and combined GE+aCGH data achieved highest accuracies as determined by area under the ROC curve (AUC) calculation (GE: 0.86±0.07; Comb: 0.87±0.03; aCGH alone 0.73±0.06). Remarkably, predictions based on VAR were similar to predictions on full aCGH data (AUC 0.75±0.06), indicating that global genomic information can be condensed without losing predictive capability. Similar results were observed for subgroups with del11q and gain17q, while outcome of MYCN-amplified patients could not be predicted accurately with neither data set.

Conclusion: CASPAR is the first algorithm able to predict patients’ individual OS time from either GE or aCGH data or a combination of both. In case of neuroblastoma, expression information alone appears to reflect tumor behavior more accurately than global genomic information, and combined information from aCGH and GE data does not significantly improve prediction accuracy.

Email: andre.oberthuer@uk-koeln.de

PL24

Identification of multiple protein disrupting mutations in stage 4 neuroblastoma using next generation sequencing transcriptome analysis
Badgett, Thomas; Guo, Xiang; Wei, Jun S; Song, Young K; Johansson, Peter; Wen, Xinyu; Chen, Qingrong; Tolman, Catherine; Yeh, Susan; Khan, Javed
National Cancer Institute, NIH, Pediatric Oncology Branch, Gaithersburg, United States

Background: Neuroblastoma is a small round blue cell tumor of childhood. Fifty percent of patients present with high risk disease and despite aggressive multimodal therapy approximately 60% percent of these patients die from their disease. Currently, only a handful of molecular alterations are known to influence prognosis, but no clear mechanism of pathogenesis has been demonstrated.

Method/approach: We sequenced the transcriptomes of 20 stage 4 tumors, including ten MYCN amplified and ten MYCN non-amplified samples, using massively parallel sequencing technology. In our analysis pipeline, 50 nucleotide filtered reads are aligned to the reference human genome (hg 18). Reads that align were analyzed for: 1) base coverage, 2) transcript expression levels, 3) calling SNVs and 4) determination of damaging SNVs by Sorting Intolerant From Tolerant (SIFT) analysis.

Results: Initial analysis of the first six samples, yielded an average of 86.6 million uniquely mapped reads per sample. On average we detected the expression of 6,000 genes to a depth of 10x. The RNA seq expression profile correlated well with expression array data from the same sample (r=0.62), while the sequencing data identified an additional 3,000 genes, not detected by microarray. Using the SAMtool, an average of 1,255 nonsynonymous SNVs were detected per sample. Of these nonsynonymous SNVs, 69-160 per sample were predicted by the SIFT algorithm to be damaging. Interestingly, 10 different genes had damaging nonsynonymous SNVs in at least 20% of the samples.

Conclusion: Next generation sequencing of transcriptomes is a powerful and more sensitive method than microarrays for expression profiling and allows for the identification of novel transcripts including non-coding RNAs. Here we report the most extensive profiling of the neuroblastoma transcriptome to date. We identified several hundred protein disrupting SNVs, and of these 10 were commonly altered. Ongoing analysis is underway to validate our results. The identification of recurrent genetic alterations will assist in developing a better understanding of the mechanisms of pathogenesis of neuroblastoma and lead to new therapeutic targets.

Email: badgetttt@mail.nih.gov
PL25
Modeling neuropsychiatric disorders in the mouse
Capecchi, Mario
United States
See page 71 for biography

PL26
Genetic and developmental therapeutic studies in a transgenic mouse model for high-risk neuroblastoma
Weiss, Bill
University of California, United States
See page 71 for biography

Neuroblastoma is the third most common tumor of childhood. Amplification of MYCN is the best-characterized genetic marker for neuroblastoma, and generally marks high-risk disease. We targeted expression of MYCN to the peripheral neural crest of transgenic mice to generate a mouse model for this disease. We and others have characterized this model, and have demonstrated significant genetic and biological parallels with high-risk neuroblastoma. The talk will focus on our use of this model to dissect basic biology and genetics in neuroblastoma and as a platform for developmental therapeutics.

Email: waweiss@gmail.com
Abstract Book 101

PL27

Widespread dysregulation of miRNAs by MYCN amplification and chromosomal imbalances in neuroblastoma: Association of miRNA expression with survival

O'Sullivan, Maureen; Stallings, Raymond L; Vandesompele, Jo; Speleman, Frank; London, Wendy B; McGrady, Foley, Niamh H; Murphy, Derek M; Alcock, Leah; Mestdagh, Pieter; Higgins, Desmond G; De Preter, Katleen; O'Meara, Anne; Druy, Alexander; Tsaur, Grigory; Popov, Alexander; Verzhbitskaya, Tatyana; Shorikov, Egor; Savulev, Leonid; Fekina, Larisa; Bray, Isabella; Bryan, Kenneth; Prenter, Suzanne; Buckley, Patrick G; Conway Institute of Biomolecular and Biomedical Research, Dublin, Ireland; University of Florida, Children's Oncology Group (COG) Statistics and Data Center, Gainesville, United States; University College Dublin, Conway Institute of Biomolecular and Biomedical Research, Dublin, Ireland; Royal College of Surgeons in Ireland, Cancer Genetics, Dublin, Ireland; 5Our Lady's Children's Hospital, Departments of Oncology and Pathology, Dublin, Ireland.

Background: miRNAs regulate gene expression at a post-transcriptional level and their dysregulation can play major roles in the pathogenesis of different forms of cancer, including neuroblastoma. The purpose of this study was to identify patterns of differential miRNA expression predictive of outcome in neuroblastoma.

Methods: We analyzed a set of neuroblastoma (n = 145) that is broadly representative of the genetic subtypes of this disease for miRNA expression (430 loci by stem-loop RT qPCR) and for DNA copy number alterations (array CGH). The tumors were stratified and then randomly split into a training set (n = 96) and a validation set (n = 49) for data analysis.

Results: Thirty-seven miRNAs were significantly differentially expressed in MYCN amplified relative to MYCN single copy tumors, indicating a potential role for MYCN in either the direct or indirect dysregulation of these loci. We also identified a significant correlation between miRNA expression levels and DNA copy number, indicating a role for large-scale genomic imbalances in the dysregulation of miRNAs. To directly assess if miRNA expression was predictive of clinical outcome, we used the Random Forest classifier to identify miRNAs most significantly associated with poor overall survival (OS). A 15 miRNA signature predictive of OS with 72.7% sensitivity and 86.5% specificity in the validation set was calculated for every marker.

Conclusion: There is widespread dysregulation of miRNA expression in neuroblastoma tumors caused by over-expression of MYCN and by large-scale chromosomal imbalances. We show a miRNA signature predictive of clinical outcome, and capable of subdividing 11q- cases into two distinct clinical groups. This work highlights the potential for miRNA mediated diagnostics and therapeutics.

Email: lbray@rcsi.ie

PL28

Evaluation of PHOX2B, tyrosine hydroxylase (TH), GD2 and ELAVL4 expression for minimal residual disease (MRD) detection in neuroblastoma patients

Druy, Alexander; Tsaur, Grigory; Popov, Alexander; Verzhbitskaya, Tatyana; Shorikov, Egor; Savulev, Leonid; Fekina, Larisa; Bray, Isabella; Bryan, Kenneth; Prenter, Suzanne; Buckley, Patrick G; Conway Institute of Biomolecular and Biomedical Research, Dublin, Ireland; University of Florida, Children's Oncology Group (COG) Statistics and Data Center, Gainesville, United States; University College Dublin, Conway Institute of Biomolecular and Biomedical Research, Dublin, Ireland; 5Our Lady's Children's Hospital, Departments of Oncology and Pathology, Dublin, Ireland.

Background: miRNAs regulate gene expression at a post-transcriptional level and their dysregulation can play major roles in the pathogenesis of different forms of cancer, including neuroblastoma. The purpose of this study was to identify patterns of differential miRNA expression predictive of outcome in neuroblastoma.

Methods: We analyzed a set of neuroblastoma (n = 145) that is broadly representative of the genetic subtypes of this disease for miRNA expression (430 loci by stem-loop RT qPCR) and for DNA copy number alterations (array CGH). The tumors were stratified and then randomly split into a training set (n = 96) and a validation set (n = 49) for data analysis.

Results: Thirty-seven miRNAs were significantly differentially expressed in MYCN amplified relative to MYCN single copy tumors, indicating a potential role for MYCN in either the direct or indirect dysregulation of these loci. We also identified a significant correlation between miRNA expression levels and DNA copy number, indicating a role for large-scale genomic imbalances in the dysregulation of miRNAs. To directly assess if miRNA expression was predictive of clinical outcome, we used the Random Forest classifier to identify miRNAs most significantly associated with poor overall survival (OS). A 15 miRNA signature predictive of OS with 72.7% sensitivity and 86.5% specificity in the validation set was calculated for every marker.

Conclusion: There is widespread dysregulation of miRNA expression in neuroblastoma tumors caused by over-expression of MYCN and by large-scale chromosomal imbalances. We show a miRNA signature predictive of clinical outcome, and capable of subdividing 11q- cases into two distinct clinical groups. This work highlights the potential for miRNA mediated diagnostics and therapeutics.

Email: lbray@rcsi.ie

Results:

<table>
<thead>
<tr>
<th>Marker</th>
<th>Diagnostic sensitivity (DS)</th>
<th>Specificity (Sp)</th>
<th>Positive predictive value (PPV)</th>
<th>Negative predictive value (NPV)</th>
<th>Overall correct prediction (OCP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHOX2B+</td>
<td>0.915</td>
<td>1.000</td>
<td>1.000</td>
<td>0.902</td>
<td>0.952</td>
</tr>
<tr>
<td>TH+</td>
<td>0.872</td>
<td>0.947</td>
<td>0.953</td>
<td>0.857</td>
<td>0.917</td>
</tr>
<tr>
<td>ELAVL4+</td>
<td>0.447</td>
<td>1.000</td>
<td>0.100</td>
<td>0.594</td>
<td>0.702</td>
</tr>
<tr>
<td>GD2+</td>
<td>0.383</td>
<td>0.974</td>
<td>0.947</td>
<td>0.561</td>
<td>0.655</td>
</tr>
<tr>
<td>PHOX2B or TH+</td>
<td>0.957</td>
<td>0.946</td>
<td>0.957</td>
<td>0.946</td>
<td>0.952</td>
</tr>
</tbody>
</table>

Despite relatively high Sp of GD2 and ELAVL4 expression evaluation, these markers had low DS. Due to relatively low OCP GD2 and ELAVL4 were excluded from further analysis. In contrast, TH and PHOX2B showed both high DS and Sp. As OCP for both TH and PHOX2B was high, we estimated applicability of MRD monitoring approach where samples were defined positive in case of either PHOX2B or TH expression higher than TL. In comparison with PHOX2B, TH addition led to higher DS, but lower Sp, while OCP remained stable.

Conclusions: In our series PHOX2B and TH were the most sensitive MRD markers in NB pts. TH addition did not bring significant benefits in comparison with PHOX2B only. GD2 and ELAVL4 assessment did not show any relevance for MRD monitoring.

Email: tsaur@mail.ru
PL29

QRT-PCR for TH and Phox2B mRNA in peripheral blood and bone marrow from children with high risk neuroblastoma predicts overall survival; a SIOPEN molecular monitoring group study

Viprey, Virginie; Corrias, Maria; Tchirkov, Andrei; Swerts, Kaat; Krijnen, Wouter; Vichi, Alexis; Dallorso, Sandro; Gregory, Walter; Lukash, Roberto; Brock, Penelope; Malette-Canuet, Dominique; Laureys, Genevieve; Malis, Josef; Papadakos, Vasillis; Bican, Pavel; Ladenstein, Ruth; Burchill, Susan

1Leeds Institute of Molecular Medicine, Children’s Cancer Research Group, Leeds, United Kingdom; 2Gaslini Institute, Paediatric Oncology, Genoa, Italy; 3Centre Jean Perrin, Radiotherapy, Clermont-Ferrand, France; 4University Hospital Gent, Haematology, Gent, Belgium; 52nd Medical Faculty Charles University, Paediatric Oncology, Prague, Czech Republic; 6University of Leeds, Clinical Trials Research Unit, Leeds, United Kingdom; 7Istituto Nazionale Tumori di Milano, Paediatric Oncology, Milan, Italy; 8Great Ormond Street Hospital, Paediatric Oncology, London, United Kingdom; 9Institut Gustave Roussy, Paediatric Oncology, Villejuif, France; 10University Hospital Gent, Paediatric Oncology, Gent, Belgium; 11Agia Sofia Children’s Hospital, Paediatric Haematology and Oncology, Athens, Greece; 12Roosevelt University Hospital, Paediatric Oncology, Banska Bystrica, Slovakia; 13CCRI/St. Anna Children’s Hospital, Children’s Cancer Research Group, Vienna, Austria

Aim: To determine the clinical significance of detecting Phox2B and/or tyrosine hydroxylase (TH) mRNA by QRT-PCR in bone marrow (BM) and peripheral blood (PB) from children entered into the HR-NBL-1/SIOPEN trial.

Methods: BM and PB samples were collected and processed according to SOPs (Viprey et al, 2007, EJC, 43, 341-350; Viprey et al, 2008, J. Pathol, 216, 245-252). QRT-PCR was performed on BM samples from 230 children at diagnosis (Dx) and 175 pre-myeloablative therapy (preMAT) for TH mRNA, and from 199 children at Dx and 159 preMAT for Phox2B mRNA. TH mRNA was also measured in 224 and 156 PB samples at Dx and preMAT respectively. Phox2B was measured in 212 and 144 PB samples at Dx and preMAT respectively.

Results: The frequency of detecting TH and Phox2B mRNA in BM at Dx was 93% (214/230) and 86% (197/230) respectively, and preMAT 82% (144/175) and 58% (92/159). TH and Phox2B were highly correlated at Dx (r=0.92, p<0.001) and preMAT (r=0.77, p<0.001).

The BM-Dx values for TH and Phox2B predicted strongly for survival and relapse, showing independent predictive power in this high risk group of children. These factors predominated in multivariate Cox model analyses, with an additional β2 of 13.4 (p=0.0003) for analysis using TH and allowing for preMAT (HR=3.14 [95% cI 2.02-4.89] and 3.28 [95% cI 1.99-5.39] respectively for TH and preMAT) and sample type (BM or PB). Hazard ratios (Hrs) for survival and Phox2B mrna levels showed a clear threshold effect in predicting survival. The threshold log value differed depending on time point (Dx or preMAT) and sample type (BM or PB). Hazard ratios (HRs) for survival were 3.14 (95% CI 2.02-4.89) and 3.28 (95% CI 1.99-5.39) respectively for TH and Phox2B in BM at Dx using the threshold cut-off. Similar effects were observed for values in the PB at Dx (HR=2.99 [95% CI 1.61-5.50] for TH and HR=3.30 [95% CI 1.96-5.57] for Phox2B) and for values in the BM preMAT (HR=2.39 [95% CI 1.44-3.96] for TH and HR=2.89 [95% CI 1.65-5.09] for Phox2B).

Conclusion: Phox2B and TH mRNA detected by QRT-PCR in BM and PB from children with high risk neuroblastoma at diagnosis and preMAT predicts overall survival.

Email: s.a.burchill@leeds.ac.uk

PL30

Analyses of mIBG scoring as a prognostic indicator in patients with stage 4 neuroblastoma. A Children’s Oncology Group (A2973) report

Yanik, Gregory; Parisi, Margaret; Shulkin, Barry; Naranjo, Ariene; Kreissman, Susan; London, Wendy; Villablanca, Judy; McGrady, Patrick; Matthay, Kathleen

1University of Michigan, Pediatrics, Hematology-Oncology, Ann Arbor, MI, United States; 2Seattle Children’s Hospital, Radiology, Seattle, WA, United States; 3St. Jude Children’s Research Hospital, Radiology, Nuclear Medicine, Memphis, TN, United States; 4Children’s Oncology Group, Biostatistics, Gainesville, FL, United States; 5Duke University Medical Center, Pediatrics, Hematology-Oncology, Durham, NC, United States; 6Children’s Oncology Group, Biostatistics, Boston, MA, United States; 7Children’s Hospital of Los Angeles, Pediatrics, Hematology-Oncology, Los Angeles, CA, United States; 8University of California, San Francisco, Pediatrics, Hematology-Oncology, San Francisco, CA, United States

Background: Over the past 2 decades, radiolabeled metaiodobenzylguanidine (mIBG) has proven to be a highly sensitive marker for the detection of neuroblastoma (NBL). Recently, a semiquantitative mIBG scoring method (Curie score) has been developed. The aim of this study was to correlate mIBG scores with outcome in a group of uniformly treated patients (pts).

Methods: Newly diagnosed pts with stage 4 NBL enrolled on COG A3973 were examined. MIBG scans were evaluated at the time of diagnosis (n=280), post-induction (n=274), post-transplant (n=203), and upon completion of biotherapy (n=99). Pts with non-mIBG avid disease at diagnosis (Dx) were excluded (n=29). For each time point, mIBG scans were evaluated at 10 anatomic sites. Scans were read by 2 observers, using a semiquantitative scoring method. Individual sites were scored 0-3, based upon extent of disease at each site. Absolute scores (cumulative score at each time point) and relative scores (absolute to initial score; ratios) were correlated with event free (EFS) and overall survival (logrank test).

Results: The median Curie score at Dx was 12 (range 1-30). There was no correlation between Curie score at Dx and EFS. In contrast, pts with a Curie score > 5 following induction therapy had a significantly worse EFS when compared to those with a score < 5 (3-yr EFS: 8.3±4.6% vs 41.5±3.5%, p < 0.0001). The presence of MYcn amplification was strongly associated with a 3-yr EFS <15%. Curie scores (0 vs >0) post-induction had a greater impact in pts with MYcn amplified tumors (3 yr EFS: 45.2±7.0% vs 15.0±8.0%, p=0.014) than those with MYcn non-amplified tumors (3 yr EFS: 45.4±5.8% vs 35.9±6.8%, p=0.088). Pts with Curie scores > 0 post-transplant had a lower EFS than those with a Curie score of 0 (3-yr EFS: 28.6±6.5% vs 45.2±4.6%, p=0.023). Relative scores (>0.5, >0.25) were highly significant, but did not change results using absolute scores at any time point.

Conclusion: Pts with Curie scores >5 after induction have a 3 yr EFS <10% and should be considered for alternative therapy regimen.

Email: gyanik@umich.edu
Characterization of neuroblastoma imaging studies using F-18-DOPA PET/CT

Tzen, Kai-Yuan1; Lu, Meng Yao2; Chang, Hsiu-Hao3; Lin, Kai-Hsin1; Jou, Shih-Ting2; Yang, Yung-Li2; Lin, Dong-Tso3; Hsu, Wen-Ming2
1National Taiwan University Hospital, Nuclear Medicine, Taipei, Taiwan; 2National Taiwan University Children Hospital, Pediatrics, Taipei, Taiwan; 3National Taiwan University Hospital, Surgery, Taipei, Taiwan

Objectives: Tumors of ganglion cell origin including ganglioneuroma, neuroblastoma and ganglioneuroblastoma are common tumors in children. Iodinated MIBg and FDG PET-CT are the choice for functional imaging studies. In the recent year, F-18 DoPA has emerged as a new diagnostic tool for neuroendocrine tumors. We try to apply and characterize the functional status of the neuroblastoma in limited number of cases of our institution.

Methods: After 100 mg of carbidopa was given orally for 60 mins, the patients was injected with 200 MBq (5.4 mci) of F-18-DOPA and wait for 90 mins for imaging. Whole body imaging was performed using PET/CT. The patients also received the standard I123-MIBg, FDG PET/CT imaging studies.

Results: Nineteen patients with neuroblastoma were enrolled in this study. Their ages ranged from 0.5-12.8 years old. All patients had F-18-DOPA PET scan. Three patients were primary diagnosis/staging of disease and 16 cases were restaging of disease. Three primary diagnosis patients showed positive uptake of F-18-DOPA in primary and metastasis lesions. In restaging patients, five patients without uptake of F-18-DOPA showed negative standard imaging studies. Eleven patients with uptake of F-18-DOPA showed only 6 positive I123-MIBg and 7 positive FDG PET/CT. In organ-region-specific analysis, there were different uptake pattern in 3 imaging studies.

Conclusion: No study on the possible role of F-18-DOPA in neuroblastoma has been published yet. In our study, we found a major drawback of FDG PET/CT was lack of visualization of lesions in the liver and cranium because of high physiologic activity. Another disadvantage of FDG PET/CT was not disease-specific. F-18-DOPA is a better substrate for the cell membrane norepinephrine transporter than MIBg and a more specific substrate for neuroblastoma cells than FDG. F-18-DOPA might provide more additional information than FDG PET/CT in this area. F-18-DOPA positivity indicates the ability of tumor cells to accumulate and the ability to decarboxylate F-18-DOPA by AADC in a well differentiated tumor or tumor component. This might indicate better prognosis. The clinical significance needs further follow up.

Email: lmy1079@gmail.com

Clinical and biological features predictive of survival after relapse of neuroblastoma: A study from the International Neuroblastoma (NB) Risk Group (INRG) Database

Castel, Victoria1; Matthay, Kate K2; Monclair, Tom3; Pearson, Andrew D2; Cohn, Susan L4; London, Wendy B5; Castel, Victoria1; Matthay, Kate K2; Monclair, Tom3; Pearson, Andrew D2; Cohn, Susan L4; London, Wendy B5
1H.U La Fe, Pediatric Oncology Unit, Avda de Campanar 21, Valencia, Spain; 2University of California, San Francisco Children’s Hospital, San Francisco, United States; 3Rikshospitalet University Hospital, Division of Surgery, Oslo, Norway; 4Institute of Cancer Research and Royal Marsden Hospital, Institute of Cancer Research and Royal Marsden Hospital, Surrey, United Kingdom; 5University of Chicago, Pritzker School of Medicine, Chicago, IL, United States; 6Dana Farber Harvard Cancer Care/Children’s Hospital Boston, Dana-Farber Harvard Cancer Care/Children’s Hospital Boston, Boston, MA, United States

Background: In NB, most patients (pts) who relapse eventually die. Prognostic factors are used to stratify treatment at diagnosis, but typically not at the time of relapse. Our goals were to determine a) which factors were predictive of time to death post-relapse; b) if time from diagnosis until relapse/progression has a predictive role.

Methods: Retrospective analysis included INRG pts with first event of relapse, progressive disease, or secondary malignancy (excluding pts whose first event was death). Time from diagnosis until event (“time-to-first-event”) was calculated and analyzed as <1 year (yr) vs ≥1 yr. 5-yr estimates of overall survival (OS ± standard error), time from first event until death or last contact, are presented (lifeetable methods). Time-to-first-event was tested in a multivariable Cox model (adjusting for nonproportional hazards) with clinical and biologic factors; hazard ratios (HR) for increased risk of death post-relapse were calculated.

Results: From 8,800 INRG pts, 2,286 experienced a non-death first event. Median time to relapse was 13.2 months (mo) (range: 1 day to 11.4 yrs). The 5-yr OS after first event was 20%±1%. Time-to-first-event (HR=1.8), age >18 mo (HR=2.3), INSS stage 4 (HR=3.4), MYCN amplified (HR=2.8), diploidy (HR=1.6), high MKI (HR=2.0), undifferentiated grade (HR=1.6), and 1p aberration (HR=1.7) were significantly predictive of death after relapse (p<0.0001), but not 11q aberration. Compared to pts whose first event occurred <6 mo from diagnosis, pts who relapsed 6–<18 mo from diagnosis had increased risk of death, while relapses ≥18 mo from diagnosis had decreased risk of death. Shorter time-to-first-event was not independently predictive of death after adjustment for undifferentiated grade, high MKI, MYCN amplification, or diploidy. We found the same results, when we analyzed relapses in stage 4– than at 18m.

Conclusions: Time to first relapse is a significant predictor of time to death after relapse; the risk of death is higher for pts who relapse within 6–<18 mo, but lower for pts who relapse ≥18 mo from diagnosis. Stratification of relapsed NB pts according to the timing of first relapse, age, stage, MYCN, and MKI, and diploidy is important in retrieval study designs.

Email: castel_vic@gva.es
**PL33**

Topotecan-vincristine-doxorubicin in metastatic neuroblastoma failing to respond to rapid COJEC. Preliminary results of a SIOPEN Group Study

**Authors:**
- Amoroso, Lorena
- Makin, Gui
- Ladenstein, Ruth
- Laureys, Genevieve
- Lukach, Roberto
- Castel, Victoria
- Brock, Peppy
- Thomas, Caroline
- Valente-Couanet, Dominique
- Garaventa, Alberto

1. Istituto Giannina Gaslini, Paediatric Oncology, Genoa, Italy; 2. University of Manchester, Paediatric Haematology, Manchester, United Kingdom; 3. Children’s Cancer Research Institute, Applied Clinical Research and Statistics, Vienna, Austria; 4. University Hospital, Paediatric Haematology Oncology, Gent, Belgium; 5. Istituto Nazionale Tumori, Paediatric Haematology Oncology, Milan, Italy; 6. Hospital Universitario La Fe, Paediatric Oncology, Valencia, Spain; 7. Great Ormond Street Hospital, Paediatric Oncology, London, United Kingdom; 8. Centre Hospitalier Universitaire, Paediatric Haematology Oncology, Nantes, France; 9. Institut Gustave Roussy, Paediatric Oncology, Villejuif, France.

**Background:** This study has evaluated activity and toxicity of the Topotecan-Vincristine-Doxorubicin (TVD) combination administered to patients (pts) with stage 4 neuroblastoma failing to achieve remission after induction therapy (rapid COJEC) according to the HR-NBL-1 SIOPEN protocol.

**Methods:** Pts above 1 year of age with stage 4 neuroblastoma, who failed to achieve metastatic remission with rapid COJEC were eligible. Topotecan was administered at 1.5 mg/m2/day for 5 days, followed by 48-hour infusion of vincristine, 2 mg/m2, and doxorubicin, 45 mg/m2. Tumor response was assessed after 2 TVD courses, according to the INSS criteria. Pts achieving CR or VGPR (metastatic CR) underwent myeloablative therapy (MAT) made of BU-MEL or CEM, followed by PBSC rescue. Pts who achieved PR received 2 further TVD courses and then were re-assessed. In case of CR or VGPR, treatment was continued according to HR-NBL-1 (BU-MEL) standard arm. Pts who failed to achieve PR after 2-4 TVD courses, or developed PD were withdrawn from the study according to Hr-nBL-1 SIOPEN protocol. Twenty-six /66 patients are presently alive. Toxicity was mostly hematopoietic. 49 pts experienced grade 4 neutropenia, 44 grade 4 thrombocytopenia and 11 grade 4 anemia.

**Results:** Sixty-six pts who did not achieve CR or VGPR after rapid COJEC were enrolled in the study. After 2 TVD courses, responses of 51 assessable pts included CR in 3, VGPR in 11, PR in 17, MR in 6, NR in 12. PD in 2 (overall response rate 60 %). Twenty-three pts who achieved CR or VGPR or PR (metastatic CR) received MAT (random BU-MEL vs CEM) according to protocol. Twenty-six/66 patients are presently alive. Toxicity was mostly hematopoietic. 49 pts experienced grade 4 neutropenia, 44 grade 4 thrombocytopenia and 11 grade 4 anemia after the first course. 43 pts developed grade 4 neutropenia, 35 grade 4 thrombocytopenia and 12 grade 4 anemia after the second course. Systemic antibiotic therapy required hospitalization for 19 pts after the first course, and for 15 pts after the second. Results were recorded in the SIOPEN-R-NET database.

**Conclusion:** TVD combination was active and tolerable in pts with metastatic neuroblastoma after treatment with rapid COJEC.

Email: albertogaraventa@ospedale-gaslini.ge.it

---

**PL34**

Suppression of human anti-mouse antibody response by rituximab plus cyclophosphamide permits continuation of anti-GD2 immunotherapy

**Authors:**
- Modak, Shalakee
- Kushner, Brian H.
- Kramer, Kim
- Cheung, Irene Y.
- Cheung, Nai-Kong V.

Memorial Sloan-Kettering Cancer Center, Pediatrics, New York, United States

**Background:** Anti-GD2 monoclonal antibodies (MoAb) are effective against high risk NB. Human anti-mouse antibodies (HAMA) arising after mouse or chimeric MoAb treatment can interfere with MoAb binding to GD2 and neutralize the benefit of further immunotherapy. We hypothesized that destruction of B and T cells by the combination of rituximab and cyclophosphamide may suppress preformed HAMA and allow MoAb therapy to continue.

**Method/approach:** Ultra-high risk NB patients receiving anti-GD2 immunotherapy with mouse anti-GD2 MoAb 3F8 plus GMCSF (NCT00072358) who developed HAMA (>1000U/ml) were treated with intravenous (IV) rituximab (375mg/m2/dose on days 1 and 15) plus IV cyclophosphamide (750mg/m2 on day 16) (R-C). Patients resumed immunotherapy when HAMA levels subsided.

**Results:** 41 patients with elevated HAMA titers received R-C. Patients had previously received a median of 4 (range 1-10) cycles of 3F8/GMCSF with median HAMA titer of 2521 (range 1034-21385) U/ml. 6 patients developed allergic reactions to rituximab: 4 completed R-C; 1 received a single dose of rituximab while 1 could not complete the first dose and was ineligible for response. Pneumonia or unexplained infections were not observed in any patient. HAMA titers were abrogated in 30/40 (75%) evaluable patients at a median of 60 (range 9-245) days but remained >1000U/ml in 10(25%) patients at a median of 127 (range 13-511) days after R-C. 28/30 R-C-responsive patients went on to receive further 3F8/GMCSF. 17/26 evaluable patients (65%) continued to be HAMA-negative after rechallenge receiving a median of 3 (range 1-9) further 3F8/GMCSF cycles. 9/26 (35%) redeveloped HAMA that persisted for a median of 141 (range 17-395) days after 3F8 rechallenge.

**Conclusion:** R-C therapy was safe and well tolerated in heavily pretreated patients with NB. It suppressed preformed HAMA response and permitted continuation of MoAb therapy in most patients. This is the first report of an effective strategy to suppress ongoing host immune response against IgG proteins. It may have general application in suppressing or delaying MoAb responses, thereby rendering MoAb immunotherapy more effective.

Email: modaksh@mskcc.org
PL35

Long term outcome: the price of treatment for surviving high-risk neuroblastoma

Herl, Barbara1; Thorsten, Simon1; Dilico, Dagmar2; Krensm, Bernard1; Grigull, Lorenz2; Scheel-Walter, Hans-Gerhard3; Berthold, Frank1

1University of Cologne, Pediatric Oncology and Hematology, Cologne, Germany; 2University of Bonn, Pediatric Oncology and Hematology, Bonn, Germany; 3University of Essen, Pediatric Oncology and Hematology, Essen, Germany; 4Hannover Medical School, Pediatric Oncology and Hematology, Hannover, Germany; 5University of Tuebingen, Pediatric Oncology and Hematology, Tuebingen, Germany

Background: Megatherapy has been proven to be superior in randomized trials and is currently the backbone of most treatment regimen for high risk neuroblastoma. However, long term outcome and the risk of late effects have not been addressed in these patients so far.

Methods: We analysed our previously published cohort of 295 randomized high risk neuroblastoma patients (stage 4 or MYCN amplified) with special focus on late recurrences and therapy related late effects. After induction chemotherapy, patients were randomized to megatherapy with stem cell rescue or to maintenance therapy (4 cycles of oral cyclophosphamide).

Results: The cohort is currently followed for up to 12 years (median observation time: 8.4 years). The long-term results confirmed the better outcome after megatherapy in the “as-randomized”, “as-treated” and “treated-as-randomized” analysis. Megatherapy (n=143) was early complicated by pneumonia in ten, other severe infectious complications in eight, veno-occlusive disease in eight and renal failure in three patients. Five patients died due to megatherapy-related complications. Only one out of 119 patients treated with maintenance therapy developed pneumonia. No patient died to maintenance therapy related complications. Relapses occurred later in patients treated with megatherapy (74/143 pts., 8-85 months, median 21 months) than in patients with maintenance therapy (83/119 pts., 7-60 months, median 16 months, p=0.001). Patients died up to nine years after diagnosis (megatherapy: median 30 months; maintenance therapy: 20 months, p<0.001). One patient of each arm developed secondary leukaemia. Major late effects were analysed in 109 patients surviving five years or longer (megatherapy n=88, maintenance n=41), and were more often found in patients of the megatherapy arm: hearing loss 72% vs. 51% (p=0.04), tubular damage 18% vs. 12% (n.s.), hypothyroidism 19% vs. 2% (p=0.02), focal nodular hyperplasia of the liver 10% vs. 0% (p=0.04), impaired growth 10% vs. 0% (p=0.04).

Conclusion: Megatherapy proved effective with respect to long term outcome, but is complicated by acute toxicity and by an increased risk of long term effects.

Email: barbara.hero@uk-koeln.de

PL36

Long-term toxicity in survivors of ENSG5 trial for children with high-risk neuroblastoma

Moreno, Lucas1; Vaidya, Sucheta1; Pinkerton, Rosa2; Lewis, Ian J3; Imeson, John1; Ellershaw, Caroline1; Machin, David1; Pearson, Andrew DJ1

1The Royal Marsden Hospital. Institute of Cancer Research, Paediatrics, Sutton, United Kingdom; 2Royal Children’s and Mater Children’s Hospitals, Paediatrics, Brisbane, Australia; 3St.James’ University Hospital, Paediatrics, Leeds, United Kingdom; 4University of Leicester, CCLG Data Centre, Leicester, United Kingdom

Background: Due to the use of intensive therapies survivors of high-risk neuroblastoma potentially face many complications. Our aim is to provide a long-term follow-up of metastatic neuroblastoma survivors included in the ENSG5 protocol from 1990 to 1999.

Methods: Patients were randomised to receive the same induction drug doses but in one arm the dose intensity was 1.8 times greater (OPEC/ OJEC vs. CQJEC), surgical removal of primary tumour and high-dose melphalan with stem cell rescue. 262 children were randomized, 62 survived more than 5 years and 57 of them were analyzed. Information from ENSG5 yearly updated database was gathered and questionnaires were sent to participating centres (73.7% responses).

Results: Median follow-up was 12.87 (6.88-16.49) years. Overall, 44 children (77.2%) developed at least one complication and these were severe in 11 cases (19.5%). Twenty-eight children (49.1%) developed hearing loss. This was severe (Brock grade 3 and 4) in 5 (8.8%), 9 patients (15.8%) developed decreased GFR, but no cases of renal failure or tubulopathy were documented. Endocrine complications (28.1% of children) included mainly hypogonadism, delayed growth and delayed puberty. Neurocognitive issues (behavioral problems, speech or learning difficulties) were reported in 12 cases (21%). Three children developed second malignancies 5 years after diagnosis: one localized osteosarcoma, one carcinoma of the parotid gland and one anaplastic ependymoma. No haematologic malignancies/myelodysplasia were documented. There were no deaths in remission during follow-up. There were no differences between the two induction treatment arms.

Discussion: This study presents a homogeneous cohort of high-risk neuroblastoma survivors from a multi-institutional randomized trial with a low profile of long-term toxicity compared to previous studies. However, 3 cases of secondary malignant solid neoplasms have developed so far. Long-term toxicity was not increased in patients receiving more intensive CQJEC chemotherapy compared to standard arm. It is likely that with current more intensive treatment regimens using radiotherapy for local control, the burden on survivors could increase.

Email: lucas.moreno@icr.ac.uk
Parallel session 1 – Targeting kinases OR1–OR07

OR1
The KidsCancerKinome: Validation of Aurora kinases as potential drug targets in neuroblastoma and other pediatric tumors

Westerhout, Ellen1; Kool, Marcel1; Molenair, Jan1; Strobbe, Peter2; den Boer, Monique2; Segers, Stephanie2; Clifford, Steven3; Delattre, Olivier4; Benetkiewicz, Magdalena1; Lanvers, Claudia1; Pieters, Roel1; Pietsch, Torsten1; Holst, Marcel1; Renshan, Jannet1; Shipley, Janet5; Serra, Massimo6; Scotlandi, Katie7; Geegerger, Birgit8; Vassal, Gilles9; Degrand, Olivier10; Verschuer, Arnaud10; Versleg, Roger10; Caron, Hubert10

1 Academic Medical Center, Human Genetics, Amsterdam, Netherlands; 2Erasmus MC-Sophia Children’s Hospital, Pediatric Oncology, Rotterdam, Netherlands; 3University of Newcastle, Northern Institute for Cancer Research, Newcastle, United Kingdom; 4The Curie Institute, The Department of Pediatric Oncology, Paris, France; 5University Hospital Münster, Pediatric Haematology and Oncology, Münster, Germany; 6The Erasmus MC-Sophia Children’s Hospital, Pediatric Oncology, Rotterdam, Netherlands; 7The University of Bonn Medical School, Department of Neuropathology, Bonn, Germany; 8Institute of Cancer Research, Molecular Carcinogenesis, Sutton, United Kingdom; 9Instituti Ortopedici Rizzoli, Laboratory of Oncologic Research, Rizzoli, Italy; 10The Institut Gustave-Roussy, Department of Pediatric Oncology, Villejuif, France; 11France Europe Innovation, Administrative and financial management, Paris, France; 12Academic Medical Center, Pediatric Oncology, Amsterdam, Netherlands

KidsCancerKinome (KCK) is a translational research effort connecting 9 European labs with 2 European SME’s aimed at the systematic investigation of the human protein kinase family to validate novel drug targets and develop targeted therapies. Many novel kinase inhibitors are under development for adult oncology and KCK will test their in vitro activity against the tumor-driving kinases identified in this program. Successful small molecule inhibitors will be taken further to in vivo validation in established xenograft models of the six childhood tumor types. The experimental approach encompasses: 1. target presence analyses (mRNA and protein expression of the human kinase) 2. molecular validation of kinase tumor dependency (RNAi) 3. kinase mutation analysis 4. in vitro drug efficacy testing 5. in vivo proof-of-principle of drug efficacy

We have validated Aurora kinase A and B as potential drug targets in six highly malignant pediatric tumor types (i.e. Ewing sarcoma, osteosarcoma, rhabdomyosarcoma, neuroblastoma, medulloblastoma and ALL). The stepwise procedure started with the extensive analyses of expression of human kinases using Affymetrix mRNA profiles of over 500 tumors and cell lines. Clustering analyses on the combined data of all tumor types revealed a cluster containing many G2M kinases that showed significantly higher expression patterns than in the reference tissues. Prominently present in the G2M cluster were Aurora kinase A and B, which expression could be correlated to poor prognosis in the individual tumor types in further analyses. Subsequently, lentiviral shRNA-mediated knockdown of AURKA and AURKB protein expression has been performed in one tumor line per species, each tumor type to evaluate the kinases for their potential as drug targets. Inhibition of the Aurora kinases resulted in significant phenotypes in several pediatric tumor cell lines ranging from growth inhibition to extensive cell death. The knockdown of AURKA or AURKB often leads to induction of apoptosis, although preceded by a different type of cell arrest. These findings were promising for further evaluation of Aurora kinase inhibitors in the core panel of sensitive pediatric tumor cell lines.

Email: e.m.westerhout@amc.uva.nl

OR2
Inhibition of Aurora-A as an approach to control N-Myc levels in neuroblastoma

Brockmann, Markus1; Chester, Louise2; Ellers, Martin3

1 University of Würzburg, Biozentrum, Physiologische Chemie II, Würzburg, Germany; 2 Institute of Cancer Research, McElwain Laboratories, Paediatric Oncology, Neuroblastoma Drug Development, London, United Kingdom

The oncogene MYCN belongs to the MYC family of transcription factors with the basic region/helix-loop-helix/leucine zipper domain (bHLHZip). Amplification of MYCN in neuroblastoma is one of the strongest predictors of aggressive disease, resistance to therapy and poor prognosis. To explain the aggressive phenotype of MYCN-amplified neuroblastoma and in order to identify potential molecular targets for the therapy of these tumors, we used a RNA-interference screen to identify a small group of genes that are required for the growth of MYCN-amplified neuroblastoma cells, but largely dispensable in cells without MYCN-amplification. One of the identified genes encodes Aurora-A. We have previously shown that Aurora-A has a critical function in stabilizing the N-Myc protein (Otto et al., 2009 Cancer Cell). We have now found that several small molecule inhibitors of the Aurora-A kinase reduce N-Myc protein levels in neuroblastoma cells, demonstrating that catalytically active Aurora-A is required to maintain N-Myc protein levels. Our results show that Aurora-A uses two distinct mechanisms to stabilize N-Myc: First, Aurora-A interacts in a kinase-independent manner with both N-Myc and the ubiquitin ligase SCFFbxw7 and promotes the synthesis of non-K48-linked ubiquitin chains that do not support degradation. We found that Aurora-A recruits the ubiquitin-conjugating enzyme UbcH5 that can conjugate to K11, K63 in addition to K48 ubiquitin chains, providing a mechanistic basis for these observations. Second, Aurora-A inhibitors decrease phosphorylation of GSK-3β at Ser 9, demonstrating that Aurora-A directly or indirectly controls phosphorylation at this site. As a result, inhibition of Aurora-A activates GSK3, promoting N-Myc degradation. We are currently testing whether Aurora-A is a direct Gsk3 kinase and whether inhibition of Aurora-A and of Akt, a key kinase for this residue, synergize in regulating Gsk3β Ser9 phosphorylation and N-Myc levels.

Taken together, our data indicate that small molecule inhibitors of Aurora-A may be a tool to inhibit MYCN activity for neuroblastoma tumor therapy.

Email: Markus.Brockmann@uni-wuerzburg.de

OR3
Molecular analysis and therapeutic targeting of the PI3K/Akt/mTOR pathway in paediatric neuroblastomas

Wood, Paul1; Ashley, David2; Collinane, Carleen3; Kinross, Kathryn4; Poortinga, Gretchen5; Ardley, Kerry5; McArthur, Grant6

1 Peter MacCallum Cancer Centre, Molecular Oncology, and Royal Children’s Hospital, Children’s Cancer Centre, Melbourne, Australia; 2 Royal Children’s Hospital, Children’s Cancer Centre, and Royal Children’s Hospital, The Murdoch Children’s Research Institute, Melbourne, Australia; 3 Peter MacCallum Cancer Centre, Translational Research Laboratory, Melbourne, Australia; 4 Peter MacCallum Cancer Centre, Molecular Oncology, Melbourne, Australia; 5 Peter MacCallum Cancer Centre, Molecular Oncology, and St. Vincent’s Hospital, Department of Medicine, Melbourne, Australia

Background: The PI3K/AKT/mTOR cell-signalling pathway plays a key role in major cellular functions including cell growth, survival and angiogenesis. Dereactivation of this pathway is observed in MYCN amplified neuroblastoma (NBL), making it an attractive target for inhibitors of this pathway. Novel imaging modalities such as small animal ultrasound (US) and fluoro-deoxy glucose-positron emission tomography (FDG-PET) are emerging as useful tools for evaluating interventions in murine models.

Method/approach: Homozygous TH-MYCN transgenic mice underwent serial abdominal US from four weeks of age until neuroblastomas greater than 75mm3 were detected. Tumour volumes were calculated and baseline FDG-PET scans were performed followed by a 7-day intervention with PF04691502, a combined PI3K/mTORC1 inhibitor. Repeat US and FDG-PET were performed at 48 hours. Tumour:background (T:B) ratios for the radio-labelled FDG tracer were used as a measure of avidity. Tumours were harvested for western blot (WB) and immunohistochemistry (IHC) analysis of key proteins in the PI3K/AKT/mTOR as well as markers of senescence, apoptosis and angiogenesis.

Results: A significant decrease in the FDG uptake was observed following treatment with PF04691502 (T:B ratios 4.6±1.8 reduced to 1.7±0.3) when compared with vehicle (T:B ratios 3.5±0.7 compared to 5.0±0.8 post treatment, p<0.001). Treatment with PF04691502 also improved survival at 7 days (100%) compared with vehicle (33.3%), p<0.01. WB analysis demonstrated decreased levels of MYCN. WB and IHC showed reduced tumour vascularity as evidenced by a significant decrease in CD31 staining and the induction of apoptosis.

Conclusion: Both small animal FDG-PET and US have been validated as robust tools for use in the TH-MYCN transgenic mouse model. PF04691502 significantly decreased uptake of FDG, suggesting inhibition of metabolism and/or tumour viability. Treatment with PF04691502 induced apoptosis, reduced expression of MYCN and also reduced tumour vascularity. These data indicate the PI3K/AKT/mTOR pathway is a promising therapeutic strategy in MYCN amplified neuroblastoma, targeting multiple cellular mechanisms.

Email: paul.wood@petermac.org
OR4
PI3K inhibitors prime neuroblastoma cells for chemotherapy in vitro and in vivo by the balancing of pro-apoptotic Bcl-2 proteins and increased mitochondrial apoptosis
Bender, Ariane1; Opel, Daniellea; Naumann, Ivonnea; Kappler, Rolandb; Friedland, Loric; Debatin, Klaus-Michaeld; Fulda, Simone;
1Ulm University, Ulm, Germany; 2Klinikum rechts der Isar, Technical University Munich, Pediatric Surgery, Munich, Germany; 3Genentech, South San Francisco, United States

Aberrant activation of the PI3K/Akt/mTOR cascade is a characteristic feature of many cancers and has been associated with poor prognosis. We recently identified Akt activation as a novel predictor of poor outcome in neuroblastoma. Therefore, we investigated whether inhibition of PI3K/Akt signaling presents a novel approach for chemosensitization of neuroblastoma. Here, we provide first evidence that the PI3K inhibitor PI103 synergistically induces apoptosis in combination with various anticancer drugs including Doxorubicin, Etoposide, Topotecan, cisplatin, Vincristine and Taxol. Mechanistic studies reveal that PI103 cooperates with Doxorubicin to downregulate Mcl-1, to upregulate Noxa and Bim levels and to inhibit Bim phosphorylation. This shifted ratio of pro- and anti-apoptotic Bcl-2 proteins results in increased Bax conformational change, loss of mitochondrial membrane potential, cytochrome c release, caspase activation and apoptosis upon combined treatment with PI103 and Doxorubicin. Knockdown of Mcl-1 enhances Doxorubicin-induced apoptosis, while silencing of Noxa, Bax/Bak or p33 reduces Doxorubicin-mediated cell death. The central role of the mitochondrial pathway for chemosensitization is underscored by Bcl-2 overexpression, which inhibits Bax activation, mitochondrial perturbations, clearance of caspases and apoptosis. Interestingly, the caspase inhibitor zVAD.fmk blocks caspase activation and apoptosis without interfering with Bax activation and mitochondrial outer membrane permeabilization. This places mitochondrial events upstream of caspase activation. PI103 and Doxorubicin cooperate to induce apoptosis in patients’ derived primary neuroblastoma cells, supporting the clinical relevance of the results. Most importantly, combined treatment with PI103 and Doxorubicin is superior to either agent alone to suppress tumor growth in an in vivo model of neuroblastoma. By demonstrating that PI3K inhibitors such as PI103 prime neuroblastoma cells for chemosensitization-induced apoptosis in vitro and in vivo, these findings have important clinical implications for the development of targeted therapies to increase chemosensitivity of neuroblastoma.

Email: simone.fulda@uniklinik-ulm.de

OR5
PLK1 is a novel target for high-risk neuroblastoma therapy
Ackermann, Sandra1; Feket1; Fink1; Vreugdenhil, Erik H. J.3; van de Water, Bob3; Versteeg, Rogier2; Fitzsimons, Carlos P.1; Vreugdenhil, Erno1
1Academic Medical Center, Department of Human Genetics, Amsterdam, Netherlands; 2Academic Medical Center, Department of Human Genetics, Amsterdam, Netherlands; 3LACDR, Leiden University, Division of Toxicology, Leiden, Netherlands

Background: High-risk neuroblastoma remains a therapeutic challenge for pediatric oncologists. The Polo-like kinase 1 (PLK1) is a key regulator of eukaryotic cell division and serves as a negative prognostic marker in many human cancers. This enzyme is a target of the novel small-molecule inhibitor BI 2536, which has already shown clinical efficacy in adult malignancies. In this study, we investigated the effect of BI 2536 on neuroblastoma cells in vitro and in vivo to explore PLK1 as a potential target in high-risk neuroblastoma therapy.

Methods: Oligonucleotide-microarray profiles of 476 neuroblastoma specimens were analyzed and mRNA levels of PLK1 were correlated with prognostic markers and outcome. To explore the effect of PLK1 inhibition on growth properties of neuroblastoma cells, seven cell lines were treated with various concentrations of BI 2536 and changes in cell proliferation and cell cycle distribution were determined. Furthermore, nude mice with IMR-32 neuroblastoma xenografts were treated with various doses of BI 2536.

Results: Up-regulation PLK1 transcript levels is associated with markers of unfavorable prognosis like disseminated stage 4, age >18 months and MYCN amplification (p<0.001 each). Moreover, high PLK1 expression is significantly correlated with unfavorable gene expression-based classification and adverse patient outcome (p<0.001 each). Analysis of PLK1 protein levels revealed high expression levels of PLK1 in all seven neuroblastoma cell lines and in samples from patients with poor outcome. On treatment with nanomolar doses of BI 2536, all seven neuroblastoma cell lines showed reduced proliferation, cell cycle arrest and cell death with LC50 values ranging from 2.2 nM to 31.2 nM. BI 2536 treatment of nude mice bearing IMR-32 neuroblastoma xenografts resulted in significant reduction of tumor growth (p<0.001).

Conclusions: High expression of PLK1 is significantly associated with high-risk neuroblastoma and unfavorable patient outcome. Inhibiting its function with BI 2536 has strong anti-tumor activity on high-risk neuroblastoma cells and has the potential to become a novel therapeutic approach for neuroblastoma.

Email: sandra.nowacki@uk-koeln.de

OR6
MicroRNA-184 inhibits neuroblastoma cell proliferation and promotes apoptosis by targeting the AKT2 3'UTR
Foley, Niamh H1; Bray, Isabella; Murphy, Derek M; Ryan, Jacqueline; Tivnan, Amanda; Buckley, Patrick; Ray, Stalings
Department of Cancer Genetics, Royal College of Surgeons in Ireland & Children’s Research Centre, Our Ladys Children’s Hospital, Department of Cancer Genetics, Dublin, Ireland

Background: Prior studies indicate that miR-184 is significantly under-expressed in MYCN amplified neuroblastoma (NB) tumors and that ectopic up-regulation of miR-184 results in the induction of a caspase mediated apoptotic pathway in NB cell lines (Cancer Res. 2007;67:976). Here, we elucidate the target responsible for the molecular mechanism of this pro-apoptotic effect.

Method/Results: MI-184 is computationally predicted to target the 3’UTR of AKT2. Given that AKT2 is a member of the PI3K pathway, one of the most potent pro-survival pathways in cancer, the interaction between miR-184 and AKT2 was further explored. Analysis of primary NB tumors indicated a significant inverse correlation between miR-184 and AKT2 expression, while transfection of miR-184 into Kelly or SK-N-AS cell lines resulted in a highly significant decrease in both AKT2 mRNA and protein levels. Conversely, transfection of the miR-184 antagonist into these cell lines led to increased AKT2 protein and a statistically significant increase in the rate of cell proliferation. siRNA mediated knock-down of AKT2 resulted in decreased cell proliferation through the induction of significant apoptosis, while ectopic up-regulation of AKT2 using an expression plasmid led to increased cell proliferation, mimicking the effect of miR-184 knockdown. Co-transfection of miR-184 with an AKT2 expression plasmid lacking the miR-184 target site rescued cell proliferation, indicating that miR-184 exerts pro-apoptotic effects primarily through the targeting of AKT2. Finally, the inverse correlation between MYCN and miR-184 levels was experimentally confirmed using the SHEP-TET cell line containing a repressible MYCN transgene. Suppression of MYCN resulted in an 8 fold increase in miR-184 levels. Using chromatin immunoprecipitation based methods we also demonstrate that MYCN binds weakly to an up-stream region of miR-184 containing e-box motifs previously demonstrated to bind MYCN.

Conclusion: MYCN induces a tumorigenic effect in part through down regulating miR-184, leading to increased AKT2 protein. MI-184, as an inhibitor of AKT2, could be of potential benefit in microRNA mediated therapeutics.

Email: niamhfoley@rcsi.ie

OR7
Exploring a new therapy for neuroblastoma: silencing of doublecortin-like kinase using RNA-interference
Verissimo, Carla S.1; Molenaa, Jan J.; Meerman, John1; Puigvert, Jordi C.; Lamers, Fieke; Rotman, Maarten1; van Kuik-Romeijn, Petra1; Danen, Erik H.J.2; van der Water, Bob1; Versteeg, Rogier2; Fitzsimons, Carlos P.1; Vreugdenhil, Erno1
1LACDR, Leiden University, Division of Medical Pharmacology, Leiden, Netherlands; 2Academic Medical Center, Department of Human Genetics, Amsterdam, Netherlands

Background: Doublecortin-like kinase (DCLK) is a member of the PI3K pathway, one of the most potent pro-survival pathways in cancer. This enzyme is a target of the novel small-molecule inhibitor of DCLK, BI 2536. We also found that DCLK knockdown in vitro and in vivo resulted in decreased cell proliferation and differentiation of neuroprogenitor cells. Gene expression profiling revealed a high expression of these transcripts in neuroblastoma patients. Furthermore, these transcripts are endogenously expressed specifically in neuroblasts but are not found in other cell types. Suppression of DCLK by short interfering RNA (siRNA) disrupted the mitotic spindles in neuroblastoma cells and gene expression profiling revealed numerous differentially expressed genes indicating apoptosis. Apoptotic cell death of neuroblastoma cells by DCLK knockdown was further confirmed by several assays. Interestingly, mitochondria were the most affected cell component after DCLK knockdown. We also found in neuroblastomas a significant correlation between DCLK expression and genes related with mitochondria activity. Furthermore, we showed a successful delivery of siRNA targeting DCLK to neuroblastoma cells by using specific peptide-siRNA conjugates. In conclusion, silencing of the DCLK gene by siRNA interference is a novel potential therapeutic approach for neuroblastoma with the promise of combining high specificity with fewer side effects. Peptide-siRNA conjugates might be the tool needed for specific neuroblastoma delivery.

Email: c.verissimo@lacdr.leidenuniv.nl
**OR8**

**HIF-2α maintains an undifferentiated state in neural crest-like human neuroblastoma tumor-initiating cells**

**Pietras, Alexander1; Hansford, Loen M.2; Johnson, A. Sofie3; Bridges, Edith4; Stjärend, Jonas1; Gisselsson, David5; Rehn, Matslåd; Beckman, Daniel3, 5; Siv, Alexander6; Noguera, Rosa6; Navarro, Samuel6; Cammenga, Jörg7; Fredlund, Erik2; Kaplan, David R.1; Påhlman, Sven1**

1Lund University, Center for Molecular Pathology, Malmö, Sweden; 2Lund University, Molecular Medicine and Gene Therapy, Lund, Sweden; 3Lund University, Clinical Genetics, Lund, Sweden; 4Lund University, Center for Molecular Pathology, Malmö, Sweden; 5University of Valencia, Pathology, Valencia, Spain

**Background:** Cancers are phenotypically heterogeneous and hypoxia, being one cause of heterogeneity, dedifferentiates neuroblastoma cells towards a neural crest-like phenotype. Low stages of tumor cell differentiation are frequently coupled to advanced disease and high hypoxia-inducible factor 2α (HIF-2α) protein levels predict poor outcome in neuroblastoma.

**Method/approach:** We aimed to phenotypically characterize neuroblastoma tumor-initiating/stem cells (TICs) isolated from the bone marrow of patients with high-risk neuroblastoma with a focus on HIF expression and regulation and stem cell/necrosis marker genes as well as markers of SNS differentiation.

**Results:** Here, we identify HIF-2α as a marker of normoxic neural crest-like neuroblastoma TICs isolated from bone marrow of patients with high-risk neuroblastoma. Knockdown of HIF-2α reduced VEGF expression and induction of sympathetic neuronal differentiation markers while expression of neural crest-associated genes diminished. Xenograft tumors of HIF-2α-silenced cells were widely necrotic, poorly vascularized and resembled the bulk of tumors in clinical neuroblastomas by expressing sympathetic neuronal markers including tyrosine hydroxylase, while control tumors were immature, welldifferentiated and stroma-rich. HIF-2α-silenced xenograft tumors were high in HIF-1α, a feature that unlike HIF-2α was not associated with adverse clinical outcome and correlated negatively with advanced clinical stage and thus tumor spread in human neuroblastoma.

**Conclusion:** We conclude that HIF-2α is required for maintaining an aggressive undifferentiated phenotype of neuroblastoma TICs. As low expression of differentiation markers predict poor outcome in neuroblastoma and angiogenesis is crucial for macroscopic tumor growth, HIF-2α is an attractive target for neuroblastoma therapy.

**Email:** alexander.pietras@med.lu.se

---

**OR9**

**Identification of signaling pathways and drug candidates using primary neuroblastoma cancer stem cells by phosphoproteomics and transcriptome sequencing**

**Vojvodic, Milijana1; Morozova, Olena2; Blakely, Kim3; Grinstein, Natalie3; Hansford, Loen1; Smith, Kristen1; Tong, Jiefei1; Taylor, Paul1; Irwin, Meredith2; Moffat, Jason4; Moran, Mike5; Mann, Marco3; Kaplan, David2**

1University of Toronto and Hospital for Sick Children, Toronto, ON, Canada; 2University of Illinois at Chicago, Anatomy and Cell Biology, Chicago, Illinois, United States; 3Children's Hospital of Philadelphia, NAP Core, Philadelphia, United States

**Background:** Metastatic NB in vivo with 1 cell (L. Hansford; S. Y. an abstracts, ANR).

A major cause of fatality in neuroblastoma (NB) is relapse in the bone marrow (BM). Tumor-initiating (TICs) isolated from the BM of relapsed patients have many properties of cancer stem cells and form metastatic NB in vivo with 1 cell (L. Hansford; S. Yan abstracts, ANR). These cells express both neural-specific and hematopoietic genes that we hypothesize are required for survival in the BM niche. To identify the signaling pathways required for the survival of TICs, as well as drugs that will be cytotoxic on a patient-specific basis, we performed two types of complementary analyses: (1) phosphoproteomics by mass spectroscopy (MS) to identify kinases of constitutively active signaling pathways, and (2) next-generation RNA sequencing to reveal genes that are highly expressed in TICs compared to normal pediatric neural crest stem cells and a panel of cancer tissues. The presence of TIC-enriched transcripts at the protein level was confirmed using MS. Three constitutively activated signaling pathways were identified, FGF2 and insulin receptor, beta1-integrin, and B-cell receptor (BCR) and their activated effectors including Lyn, LCK, SYK, and FAK. Treatment of TICs with Src and Syk inhibitors, including dasatinib, bosutinib, and R406, which are used clinically for hematopoietic malignancies, or shRNA knockdown of Src family members was rapidly cytotoxic. Transcriptome profiling also identified genes of the BRCA1 DNA damage response, CHK checkpoint control, and G2/M DNA damage checkpoint regulation as highly upregulated. One gene of the BRCA1 pathway, Aurora B kinase, which has not been considered as a drug target in NB, was analyzed further. Aurora B knockdown or treatment with AZD1152, a selective Aurora B inhibitor in phase I clinical trials for AML, was cytotoxic to TICs. This work is the first high-resolution analysis of the transcriptome, proteome, and phosphoproteome of NB TICs and identifies candidate TIC-enriched proteins and transcripts for development as therapeutic targets. Furthermore, we suggest that targeting hematopoietic survival pathways, which have thus far not been predicted to play a role in NB, may provide new drug therapies.

**Email:** dkaplan@sickkids.ca

---

**OR10**

**Induced stable neuroblastoma cancer stem cells**

**Ikegaki, Naohiko1; Regan, Paul2; Fox, Autumn3; Jacobs, Joshua4; Rappaport, Enric1; Tang, Xao1**

1University of Illinois at Chicago, Anatomy and Cell Biology, Chicago, Illinois, United States; 2Children’s Hospital of Philadelphia, NAP Core, Philadelphia, United States

**Background/Aim:** Cancer stem cells (CSC) are known for their phenotypic drift toward non-CSC. An established tumor would therefore contain a mixture of CSC and non-CSC. To understand the biology of NB CSC and to identify effective therapeutic agents against them, one needs to establish and characterize stable NB stem cells induced by small molecules (i.e., induced CSC or iCSC).

**Methods:** Sphere forming culture conditions without growth factors were used with epigenetic modifier treatments to establish stable iCSC from NB cell lines SKNAS and SKNBEx2(C). TaqMan real-time PCR, gene expression profiling, and Western blot assay examined the expression of genes and proteins (stemness factors, stem cell markers). MTS assay examined the effect of chemotherapeutics on iCSC.

**Results:** Short-term treatments of NB cells with epigenetic modifiers significantly enhanced the expression of stemness factors (SOX2, OCT4, KLF4, LIN28, NANOG) and stem cell markers (ABC2G2, CD133, CD44, CXCR4) in the iCSC as compared to monolayer and sphere cultures that were not treated with epigenetic modifiers, resulting in expression levels equivalent to those in NT2 teratocarcinoma cells. The established iCSCs also retained high-level expression of MYC or MYCN. Even with the short treatment of epigenetic modifiers, the stemness phenotype of the iCSC has been stable over 150 days. The iCSC conferred clonal expansion, a fundamental characteristic of CSC, and clonal iCSC populations retained the characteristics of the bulk iCSC. Gene expression profile analysis of SKNAS-iCSC revealed that they expressed elevated levels of genes involved in NOTCH/Delta, Wnt/Fzd pathways and genes expressed highly in other CSC types (BIN1, ENPP2, PDPN, THY1). Genes for some isoforms of the stem cell marker ALDH were also up-regulated in the iCSC. MYC-destabilizing agents were >100-fold more effective against SKNAS-iCSC over the monolayer counterpart in MTS growth assay. Tumor seeding ability of SKNAS iCSC in vivo is currently being evaluated.

**Conclusion:** This study opens doors to a better understanding of the true nature of NB CSC as well as CSC in general.

**Email:** ikegaki@uic.edu

---
Inhibition of global DNA methylation induces differentiation of human neuroblastoma tumor-initiating cells  

**Background:** In neuroblastoma, low stage of differentiation correlates with aggressive disease and poor outcome. Human bone marrow-derived neuroblastoma tumor-initiating cells (TICs) are characterized by an immortal phenotype, a normal neural crest-like phenotype, expression of typical markers such as nestin and ID2. We have recently shown that HIF-2α maintains an undifferentiated state of neuroblastoma TICs and knockdown of this protein results in induced differentiation towards a sympathetic neuronal lineage. Here, we have studied the effects of epigenetic modification and growth factor treatment on neuroblastoma TIC lineage specification and differentiation.

**Method/approach:** Neuroblastoma TICs were treated with DNA methylation inhibitor S-Aza-2’-deoxycytidine (DAC) in combination with growth factors involved in normal sympathetic neuronal and glial development, respectively. The effects were analyzed by quantitative RT-PCR and immunofluorescence.

**Results:** Induction of global DNA methylation by S-Aza-2’-deoxycytidine induces differentiation of neuroblastoma TICs, giving rise to a mixed population of cells with both neuronal lineage features with induced expression of SNS markers, such as pII-tubulin and neurofilaments, as well as Schwannian/glial lineage features with expression of typical glial markers GFAP and S100β. Additional growth factor treatment induces lineage-restricted differentiation, with nerve growth factor (NGF) and neurotrophin-3 (NT-3) driving TIC differentiation into a sympathetic neuronal lineage whereas ciliary neurotrophic factor (CNTF) and glial cell derived neurotrophic factor (GDNF) drive differentiation into a distinct glial lineage. Further, response to DNA methylation inhibition and growth factor treatment is lineage-stage specific as differentiation is induced only in cells devoid of SNS marker expression, as tested in classical neuroblastoma cell lines.

**Conclusion:** Neuroblastoma TICs are immature and neural crest-like and resemble normal neural crest cells in that they have the capacity to differentiate into distinct ganglionic and glial/Schwann cell populations.

Email: sofie.johnsson@med.lu.se

---

**OR12**

Exploiting the embryonic environment to reprogram cancer stem cells in neuroblastoma

**Method/approach:** Inhibition of global DNA methylation by S-Aza-2’-deoxycytidine (DAC) in combination with growth factors involved in normal sympathetic neuronal and glial development, respectively. The effects were analyzed by quantitative RT-PCR and immunofluorescence.

**Results:** Induction of global DNA methylation by S-Aza-2’-deoxycytidine induces differentiation of neuroblastoma TICs, giving rise to a mixed population of cells with both neuronal lineage features with induced expression of SNS markers, such as pII-tubulin and neurofilaments, as well as Schwannian/glial lineage features with expression of typical glial markers GFAP and S100β. Additional growth factor treatment induces lineage-restricted differentiation, with nerve growth factor (NGF) and neurotrophin-3 (NT-3) driving TIC differentiation into a sympathetic neuronal lineage whereas ciliary neurotrophic factor (CNTF) and glial cell derived neurotrophic factor (GDNF) drive differentiation into a distinct glial lineage. Further, response to DNA methylation inhibition and growth factor treatment is lineage-stage specific as differentiation is induced only in cells devoid of SNS marker expression, as tested in classical neuroblastoma cell lines.

**Conclusion:** Neuroblastoma TICs are immature and neural crest-like and resemble normal neural crest cells in that they have the capacity to differentiate into distinct ganglionic and glial/Schwann cell populations.

Email: sofie.johnsson@med.lu.se
OR17
Galectin-1 modulates immune response towards a state of tolerance in neuroblastoma
Soldati, Rocio1; Berger, Elsa1; Zenclussen, Ana C2; Jorch, Gerhard1; Salatino, Mariana2; Rabinovich, Gabriel A1; Feist, Stefan2
1Otto-von-Guericke University, Department of Pediatrics, Magdeburg, Germany; 2Otto-von-Guericke University, Experimental Oncology, Magdeburg, Germany; 3Instituto de Biología y Medicina Experimental, Laboratorio de Immunopatología, Buenos Aires, Argentina
Background: Galectin-1, a highly conserved glycan-binding protein, endows dendritic cells (DC) with a regulatory phenotype which contributes to sustain a tolerogenic microenvironment at sites of tumor growth. Galectin-1 is upregulated in neuroblastoma (NB) patients and its expression is associated with poor outcome. We investigated the role of galectin-1 as a modulator of the anti-NB immune response in syngeneic NB mouse model.
Method/approach: Galectin-1 expression and secretion by murine (NXS-2) and human (LAN-1, Kelly, SK-N-AS) NB cells was evaluated by westernblot and immunohistochemistry. The effect of NB supernatant on I-A/K expression on bone marrow derived DC (BMDC) as an indicator for DC maturation was analyzed by flow cytometry. Next, we stably transfected NXS-2 cells with galectin-1 antisense DNA (LAG-1) to suppress galectin-1 expression (LAG-1-NXS-2). LAG-1-NXS-2 was injected into A/J mice s.c. and its growth was compared to that of NXS-2 (galectin+). Cytoxic Cr51 assays were performed to evaluate NB cell lysis.
Results: Galectin-1 is overexpressed in all tested human and murine NB cell lines at 36 months in contrast to the very weak expression in A/J mouse organs. We found galectin-1 in NB cell supernatant, which suppressed DC maturation as indicated by decreased I-A/K expression in the CD11c population (32%) in comparison to DC matured in medium (64%). LAG-1-NXS-2 s.c. injection prevented the tumorgrowth in contrast to s.c. NXS-2. We could not find metastases in the livers of the LAG-1- NXS-2 group. In contrast, on average of 6 metastases were detected in the livers of the NXS-2 group. Splenocytes from mice receiving s.c. injections of LAG-1-NXS-2 showed 20-45% higher NXS-2 target cells lysis (82%; E:T 100:1) compared to splenocytes from mice injected with NXS-2 (37%; E:T 100:1).
Conclusion: Galectin-1 is secreted by NB cells which may inhibit DC maturation thus leading to increased tumor growth and dissemination. This effect may explain the state of tolerance observed in NB patients. Galectin-1 is therefore an interesting target to develop novel anti-NB immunotherapeutic approaches.
Email: stefan.fest@med.ovgu.de

OR18
Leukomalades activates anti-tumor functions of NK cells and overcomes immune suppression by IL-6 and TGFβ1
Xu, Yibing; Sun, Jiangping; Wu, Hong-Wei; Sheard, Michael; Tran, Hung; Wan, Zeiheng; Liu, Cathy; Seeger, Robert
Childrens Hospital Los Angeles, Hem-Onc, Los Angeles, United States
Background: Tumor progression occurs from residual disease in 40% of high-risk neuroblastoma patients. Effective natural killer (NK) cell-based immunotherapy may improve outcomes for these patients. The melanoma antigen (MAGE-A3) and cell membrane phagocytoses, which includes IL-6 and TGFβ1, can suppress NK functions and promote tumor growth. We determined the ability of lenalidomide, an immunomodulatory agent, to activate NK cell anti-tumor functions (direct cytoxicity, ADCC, secretion of cytokines) and to overcome NK suppression by IL-6 and TGFβ1.
Methods: Normal NK cells from donors were activated with IL-2 ± leukomalades with CD16 stimulation (anti-CD16 mAb or anti-CD2 mAb ch14.18 with neuroblastoma cells) for 24-72 hrs. Direct cytotoxicity and ADCC with ch14.18 were then determined by co-culturing NK cells with calcin-AM labeled neuroblastoma cells for 6 hrs and then quantifying loss of calcein from target cells. Cytokine release was quantified with a BD Cytometric Bead Array (CBA) assay or with individual ELISAs.
Results: Activation of NK cells for direct cytotoxicity and for ADCC with ch14.18 against multi-drug sensitive and resistant neuroblastoma cell lines was increased by adding leukomiadine to IL-2. Leukomiadine increased NK secretion of IL-2, GM-CSF, IFNγ, TNFa, MIP1α, and MIG, release of granzyme A and B, and synthesis of perforin but decreased secretion of IL-6, IL-10 and TGFβ1. IL-6 + sIL-6R and TGFβ1 suppressed IL-2 + CD16 activation of NK ADCC and secretion of IFNγ, but leukomiadine reversed this suppression. Leukomiadine increased IL-6 and TGFβ1 mediated phosphorylation of STAT3 and SMAD2/3 respectively in NB cells. Leukomiadine significantly improved the frequency of long-lived NK cells which possess high granzyme A and B levels.
Conclusion: Leukomiadine enhances anti-tumor cytotoxicity and cytokine secretion by NK cells and overcomes immune suppression by IL-6 and TGFβ1. These data support clinical testing of leukomiadine with anti-tumor cell mAbs in patients with recurrent, high-risk neuroblastoma.
Email: ykuyu@chiha.usc.edu
**OR19**

**Treatment of high risk neuroblastoma with autologous T lymphocytes transfected to recognize GD2**

Liu, Enli; Liu, Hao; Gee, Adrian; Yvon, Eric; Rooney, Cliona; Heslop, Helen; Bremer, Matthew

*R* Texas Children’s Hospital/Baylor College of Medicine, Pediatric Oncology, Center for Cell and Gene Therapy, Houston, United States; *B* Baylor College of Medicine, Center for Cell and Gene Therapy, Houston, United States; *C* Texas Children’s Hospital/Baylor College of Medicine, Pediatric Oncology, Houston, United States

**Background:** Adoptive transfer of tumor directed T cells may offer an alternative to standard approaches for patients with advanced stage neuroblastoma who cannot be expanded in vivo, actively migrate through tissue planes, and use direct and indirect cytotoxic mechanisms to kill tumor cells. Two major limitations have been the ability of tumors to downregulate MHC expression and decrease susceptibility to antigen-specific T cell killing, and the lack of co-stimulatory molecules leading to incomplete T cell activation and poor survival. We attempted to overcome these limitations by generating an MHC-independent chimeric antigen receptor (CAR) targeting GD2, a tumor antigen expressed on almost all neuroblastoma cells. We transduced activated T cells (ATC), and Epstein Barr virus-specific cytotoxic T lymphocytes (EBV-CTL) with a distinguishable GD2-CAR, infused them into patients with high-risk neuroblastoma, and evaluated the safety, persistence and clinical response after infusion.

**Method/approach:** This was a Phase I, dose-escalating, safety trial administered to GD2-ATC and EBV-CTL in high-risk neuroblastoma patients.

**Results:** Nineteen patients received autologous ATC and EBV-CTL transduced with GD2-CARs. No dose limiting toxicities were identified. Twelve of 18 patients had detectable GD2 T cells/CTLs within the peripheral blood 6 weeks post-infusion. Of 4 followed >1 year continue to have detectable GD2 T cells/CTL populations. Clinically, 8 subjects had no evidence of disease at the time of infusion. In 3-24 months and 1 is alive with disease (AWD) 29 months after infusion. Of 11 with relapsed/resistant disease: 7 had bulky disease, 3 had solitary bone lesions, and 1 had bone marrow disease. 2 patients with bulky disease had evidence of tumor necrosis, and a 3rd is AWD 25 months post-infusion. The subject with bone marrow disease cleared within 6 weeks. Lastly, 2 complete responses were seen in those with bone lesions: >4 years and >12 months post-infusion.

**Conclusion:** Treatment of high-risk neuroblastoma with adoptively transferred T cells expressing GD2 CARs appears safe and can be associated with both long term persistence and anti-tumor activity.

**Email:** culouso@txccc.org

**OR20**

**A novel lentiviral-transduced dendritic cell vaccine targeting the survivin antigen is effective against neuroblastoma**

Liu, Daofeng; Song, Liping; Wei, Jie; Metelitsa, Leonid

*University College London, Institute of Child Health, London, United Kingdom; 2University College London, Cancer Institute, London, United Kingdom; 3University College London College, Cancer Institute, London, United Kingdom

**Background:** The inhibitor of apoptosis protein survivin emerges as a promising target for neuroblastoma. We have previously reported the recent generated survivin minigene DNA vaccine was able to induce a protective CD8 T cell-mediated immune response using attenuated Salmonella typhimurium (aSL) as carrier in a NB model. However, the potential of survivin minigen S-high were generated in HEK293T cells by CaCl2 precipitation expression. (2) We codon-optimised the entire CAR sequences for improved expression.

**Results:** To manage possible toxicity, we have incorporated an Casp9 suicide gene within the CAR vector. Co-expression of Casp9 induced by an inert small molecule inhibitor allows >95% killing of transduced T cell after a single exposure.

Lastly, 2 complete responses were seen in those with bone lesions: >4 years and >12 months post-infusion.

**Conclusion:** Our highly optimised retroviral cassette incorporating additional safety features for the treatment of neuroblastoma with a CAR, alongside lymphodepleting condition is improving responses in a follow up clinical study.

**Email:** j.anderson@ich.ucl.ac.uk

**OR21**

**Immunotherapy for neuroblastoma by GD2 specific chimeric antigen receptor**

Anderson, John; Thomas, Simon; Himoudi, Nourredine; Pule, Martin

*1University College London London, Institute of Child Health, London, United Kingdom; 2University College London College, Cancer Institute, London, United Kingdom

**Background:** Chimeric antigen receptors (CARs) are single molecules comprising the antigen-binding moiety of a monoclonal antibody with activation motifs from endogenous T cell receptors and/or costimulatory receptors. T-cells transduced with CARs are able to recognize and kill target cells expressing the cognate antigen. This technology allows the generation of large numbers of T-cells specific to any cancer antigen without requiring T-cell selection/expansion. We have previously tested a GD2-specific CAR transduced autologous T-cells in a clinical trial in refractory neuroblastoma (Pule et al, Nat Med, 2008) with promising clinical responses.

**Methods:** We have made a series of refinements to the CAR and the adoptive transfer methodology used in the initial clinical study, with a view to a follow-on study.

**Results:** We have performed the following modifications to the anti-GD2 CAR:

1. We have humanised the original murine antibody (muk666) component of the CAR and added co-stimulatory domains. When expressed in T-cells, the humanised CAR mediates specific lysis of GD2-bearing neuroblastoma cells to comparable levels as the murine derivative, and undergoes two fold greater antigen specific proliferation as well as greater specific l2 and IFNγ secretion.

2. We codon-optimised the entire CAR sequences for improved expression.

3. To manage possible toxicity, we have incorporated an Casp9 suicide gene within the CAR vector. Co-expression of Casp9 induced by an inert small molecule inhibitor allows >95% killing of transduced T-cell after a single exposure.

4. We improved the vector cassette to include a Genome Scaffold Attachment Sequence to create more homogenous bright expression, improving expression and suicide gene activity.

5. We have generated syngeneic models ofElukase and GD2 expressing tumours in which bioluminescence imaging successfully demonstrates safety, efficacy and kinetics of tumour kill within an immunoreplete host.

**Conclusion:** Our highly optimised retroviral cassette incorporating additional safety features for the treatment of neuroblastoma with a CAR, alongside lymphodepleting condition is improving responses in a follow up clinical study.

**Email:** j.anderson@ich.ucl.ac.uk

**OR22**

**NKT cells co-localize with tumor-associated macrophages in neuroblastoma in an innate response to tumor-induced hypoxia**

Liu, Dao; Song, Ming; Wei, Jie; Metelitsa, Leonid

Texas Children's Cancer Center, Baylor College of Medicine, Department of Pediatrics, Houston, United States

**Background:** The potential importance of CD1d-restricted Va24-invariant Natural Killer T cells (NKT) for antitumor immunity and potential therapeutic effects has been demonstrated in multiple models of cancer as well as in cancer patients. However, the mechanism by which in NKTs mediate antitumor responses against solid tumors, mostly through CD1d-negative, has remained enigmatic. We recently reported that instead of attacking tumor cells directly, NKTs target CD1d-positive tumor-associated macrophages (TAMs) that play essential roles in tumor progression (JCI 2009).

The observed co-localization of NKTs with TAMs in primary human neuroblastoma (NB) we hypothesized that TAMs actively chemoattract NKTs. To test this hypothesis, we used a multiplex quantitative RT-PCR to analyze changes in the expression of CC and CXC chemokine genes in primary human monocytes upon co-culture with human NB cells in normoxic (20% O2) and hypoxic (1% O2) conditions. We found that hypoxia alone selectively up-regulated CCL20 gene expression that was confirmed at the protein level by ELISA. Of interest, co-culture with NB cells even in normoxia resulted in a cell contact-dependent up-regulation of CCL20 in monocytes and the effect was further amplified up to 70 fold in hypoxia (N=8, P<0.001, t-test). All primary human INKTs expressed high levels of CCR6, the only receptor for CCL20 and the expression was not affected by hypoxia for at least 24 h. In functional experiments we found that co-culture of NB cells with monocytes was significantly more chemoactive for NKT cells than either NB cells or monocytes alone and this effect of the co-culture was further enhanced in hypoxia. Anti-CCL20 neutralizing mAb strongly inhibited NKT-cell in vitro migration towards tumor-conditioned hypoxic monocytes. Furthermore, in vivo neutralization of human CCL20 in NO/SCID mice prevented co-localization of adaptively transferred human INKT cells with TAMs within human neuroblastoma xenografts. TAMs via CCL20 production attract INKTs inside tumor tissues that reveals a novel mechanism of an innate response to hypoxia and should be exploited for cancer immunotherapy.

**Email:** lsmeteli@txccc.org
Parallel session 4 –
p53 and molecular targets OR24–OR32

OR23
Bone marrow response evaluation with a quantitative device identifies prognostic groups in patients over 18 months
Ambros, Inge M.; Pölscher, Ulrike; Ziegler, Andrea; Modritz, Ditha; Gadner, Helmut; Ladenstein, Ruth; Ambros, Peter; Sikorski, Denae; Shohet, Jason
1CCRI, Children’s Cancer Research Institute, St. Anna Kinderkrebsforschung, Tumour Biology, Vienna, Austria; 2CCRI, Children’s Cancer Research Institute, St. Anna Kinderkrebsforschung, SIIRP, Vienna, Austria; 3St. Anna Kinderspital, CCRI, Children’s Cancer Research Institute, St. Anna Kinderkrebsforschung, SIRP, Vienna, Austria; 4St. Anna Kinderspital, Paediatric Oncology, Vienna, Austria

Background: Bone marrow (BM) based response criteria are still lacking or controversial in stage 4 neuroblastomas. We hypothesized that the dynamics of BM clearing mirrors the response to cytotoxic treatment and is thus able to identify subgroups of stage 4 patients with unfavourable prognosis.

Methods: BM samples from 81 stage 4 patients registered in two neuroblastoma Trials were tested with a fully automatic fluorescence based device which combines GD2 based immunocytology and subsequent molecular-cytogenetic analyses of identical cells (automatic immunofluorescence plus FISH, AIPF). Inclusion criteria of the study were: BM specimens at diagnosis and given time points during treatment and genomic information on the primary tumour. After exclusion of 37 patients (tumor cell free BMs at diagnosis, lack of data or material), 44 patients (age 0 to 239 months, 219 BM specimens, median observation time 8.2 years) remained for whom a complete data set was available.

Results: BM clearing after 2 to 4 cycles of chemotherapy was achieved by 28 patients (63.6%) and was significantly associated with overall survival (OS) in patients above 18 months of age at diagnosis (p<0.0001, Logrank test) but not in the younger age group. Stage 4 patients below 18 months of age had a good prognosis irrespective of BM clearing and tumour genetics. None of the genetic markers, like MYCN amplification (MNA), 1p and 11q loss and 17q gain showed a correlation with OS in this patient cohort. For MNA, a tendency with BM clearing was observed (p=0.059, Fisher’s Exact Test).

Conclusion: The determination of BM clearance reaches the so far highest prognostic impact in stage 4 neuroblastoma patients over 18 months of age making accurate BM monitoring an important tool for risk assessment in this patient group.

Email: peter.ambros@ccri.at

Parallel session 4 –
p53 and molecular targets OR24–OR32

OR24
Cooperative induction of apoptosis through p53 signaling and mTOR inhibition in neuroblastoma
Barbieri, Eveline; Chen, Zaowen; Kim, Eugene; Patterson, Danielle; Sokorski, Denae; Shohet, Jason
1Texas Children’s Cancer Center and Baylor College of Medicine, Houston TX, United States; 2Texas Children’s Hospital and Baylor College of Medicine, Houston TX, United States

Aim: We investigated the role of Sestrin1 and Sestrin2 in p53-mediated apoptosis in neuroblastoma and the interaction of mTOR and p53 pathways after their simultaneous blockade using the mTOR inhibitor, Temsirolimus and the MDM2 inhibitor, Nutlin 3a.

Methods: We used microarray expression profiling and quantitative real-time PCR to define the transcriptional response to MDM2 inhibition in primary neuroblastoma lines. We studied growth and apoptosis of neuroblastoma lines in the setting of concurrent therapy with Temsirolimus and Nutlin 3a. Flow cytometric analysis was used to assess the status of phospho-S6 ribosomal protein (mTOR) signaling pathway in patients above 18 months of age at diagnosis. We studied growth and apoptosis of primary neuroblastoma lines in the setting of concurrent therapy with Temsirolimus and the MDM2 inhibitor, Nutlin 3a.

Results: We show that the global transcriptional response to Nutlin is p53-dependent and that Sestrin1 and Sestrin2 are significantly upregulated in response to Nutlin in neuroblastoma cells. With MTT and Tunnel assays we demonstrate a p53-dependent synergistic effect of combined Nutlin 3a and Temsirolimus treatment on cell growth and apoptosis. Flow cytometric analysis of the phospho-S6 ribosomal protein demonstrates a profound dephosphorylation of S6 in vitro when low dose Nutlin 3a is combined with Temsirolimus. Additional in vivo studies suggest that mTOR inhibition reduces tumor burden and phospho-S6 of neuroblastoma xenografts in nude mice. Conclusions. We conclude that MDM2 inhibition and p53 driven Sestrin1 and Sestrin2 activation may enhance the apoptotic response to mTOR inhibition. Further in vitro and in vivo studies will support the novel therapeutic strategy of combined MDM2 and mTOR inhibition for relapsed and de novo neuroblastoma.

Email: ekbarbiz@txccc.org
OR25
Repressed p53 stress responses in normal perinatal cells provides a susceptibility to N-Myc oncosogenesis as an initiating event in embryonal malignancy

Smrzka, Oskar1; Beissbarth, Tim2; Sturm, Dominik3; Pfister, Stefan4; Ding, Han-Fei7; Marshall, Glenn8; Neil1; Chen, Bernard1; van Bekkum, Margo1; Ellis, Tammy3; Norris, Murray4; Haber, Michelle5; Kim, Eugene6; Shohet, Jason3; Wainwright, Brandon9; Deng, Han-Fei10; Marshall, Glenn11

Children’s Cancer Institute Australia for Medical Research, Molecular Carcinogenesis, Sydney, Australia; 2Department of Biochemistry and Cancer Biology, University of Toledo College of Medicine, Toledo, Ohio, United States; 3Institute for Molecular Bioscience, University of Queensland, Queensland, Australia; 4Children’s Cancer Institute Australia for Medical Research, Molecular Diagnostics, Sydney, Australia; 5Children’s Cancer Institute Australia for Medical Research, Experimental Therapeutics, Sydney, Australia; 6Texas Children’s Hospital, Children’s Cancer Center, Houston, Texas, United States; 7Medical College of Georgia, Molecular Oncology Program, Augusta, Georgia, United States; 8Children’s Cancer Institute Australia for Medical Research 2. Sydney Children’s Hospital, Molecular Carcinogenesis, Sydney, Australia

Embyonal cancers which are not required for organogenesis must be deleted by metalloproteases. Our finding is that unique specific embryonal rests will form postnatally and may later become cancerous. We have previously shown that N-Myc expression caused neuroblast or granule neuone precursor (GNP) cells rest as tumor-initiating events in murine models of neuroblastoma (TH-MYCN transgenic) and medulloblastoma (Pchtr1+/- hemizygous knockout). However, prolonged Myc expression in normal cells should trigger protective apoptosis or senescence barriers via stress response signals involving p53 and p19ARF. Here we show that exogenous N-Myc expression instead caused resistance to trophic factor withdrawal-induced death in perinatal precursor cells for neuroblastoma (neuroblasts, medulloblastoma-potentiated precursors or both). CDK4, a transcriptional target of the MYC proteins, is overexpressed or amplified. Our work has potential clinical implications.

Email: c.poehler@dkfz.de

OR27
Addiction of MYCN amplified neuroblastomas to B-MYB underscores a reciprocal regulatory loop

Gualdrini, Francesco; Sala, Arturo1; Gualdrini, Francesco; Sala, Arturo2

UCL Institute of Child Health, Molecular Haematology and Cancer Biology, London, United Kingdom

Background: Amplification of MYCN is the most important genetic aberration in neuroblastoma. B-MYB is a transcription factor of the MYB family associated with advanced neuroblastoma stages and whose over-expression confers drug resistance to neuroblastoma cells. In this study, we investigated the relationship between B-MYB and MYCN in neuroblastoma.

Method/approach: Expression of MYCN and B-MYB in neuroblastoma patients was assessed in silico (Oncomine, Genesapiens, Oncogenomics). For transcription studies, we carried out transient transfection and luciferase assays, ChIP and gel shift analyses. Infection by lentiviral vectors carrying B-MYB and MYCN shRNAs were performed for functional studies.

Results: The expression of B-MYB is significantly associated to that of MYCN in neuroblastoma samples and strongly predicts poor patients survival. B-MYB and MYCN are bound to the promoter of each other in living neuroblastoma cells. B-MYB is required for the expression of the MYCN amplicon and the proliferation of MYCN amplified, but not MYCN non-amplified, neuroblastoma cells.

Conclusion: In this study we identify B-MYB as key MYCN downstream effector, required for expression of the MYCN amplicon and whose inhibition causes synthetic lethality in MYCN amplified cells. In the light of our study, we hypothesize that in neuroblastoma, following MYCN amplification, hundreds of copies of the gene in DM bodies or HSRs chromosomes causes accumulation of MYCN oncoprotein and activation of B-MYB expression. This, in turn, will initiate an aberrant regulatory cycle where B-MYB will enhance the expression of MYCN and vice versa. This theory should explain the apparent paradox of a ubiquitous factor -B-MYB- driving the expression of a tissue specific factor –MYCN- and clarify why MYCN is not activated in non-neuronal tumours where B-MYB is overexpressed or amplified. Our work has potential clinical implications. Given the absolute requirement for B-MYB expression, tumours with MYCN amplification should be exquisitely sensitive to B-MYB targeting, indicating that the search for small molecule inhibitors of B-MYB is warranted.

Email: a.sala@ich.ucl.ac.uk
OR28

Combined massively parallel sequencing and synthetic lethal screening identifies multiple druggable targets in neuroblastoma

Chen, Qing-Rong1; Azorsa, David2; Song, Young1; Wei, Jun3; Badgett, Tami1; Guo, Xiang1; Johansson, Peter1; Wen, Xinyu1; Yeh, Susan1; House, Catherine1; Maris, John1; Khan, Javed2

1National Cancer Institute, Pediatric Oncology Branch, Gaithersburg, United States; 2Translational Genomics Research Institute, Pharmaceutical Genomic Division, Scottsdale, United States; 3University of Pennsylvania, Children's Hospital of Philadelphia, Philadelphia, United States

Background: Despite aggressive multimodal treatments the overall survival of patients with high risk neuroblastoma remains poor. Rational methods are needed to identify novel targets and select therapeutic strategies for patients with neuroblastoma. RNAi-based screening has become a powerful tool to identify drug targets for cancer therapy and recent advances in next generation sequencing make it possible to identify mutations in all of the protein coding genes in a massively parallel manner.

Methods: We took a combined approach of parallel sequencing and siRNA based synthetic lethal screening to identify mutated genes whose inhibition leads to growth suppression or synergizes with topotecan, a topoisomerase I inhibitor currently used to treat high-risk neuroblastoma. We screened siRNAs designed against ~7000 druggable targets of the human genome in 4 neuroblastoma cell lines representing both MYCN amplified and non-amplified tumors. In parallel we sequenced the transcriptome and whole exome of these cell lines.

Results: Many important biological processes, including cell cycle, phosphorylation, and protein modification, were significantly enriched in 173 growth suppressive siRNAs common to all 4 cell lines. By integrating the siRNA functional data with sequencing analyses at depth, we identified ~40 candidate genes that are essential for cancer cell proliferation and also have non-synonymous damaging coding mutations in the 4 different neuroblastoma cell lines. Further validations are underway to establish if the same mutations are present in neuroblastoma tumor samples.

Conclusions: Taken together, our integrative approach of siRNA screening and massively parallel sequencing identifies multiple druggable targets in neuroblastoma.

Email: cheng@niah.nih.gov

OR29

Tumor regression and curability of preclinical neuroblastoma models by the novel targeted camptothecin EZN-2208

Pastorino, Fabio1; Loi, Monica2; Sapra, Pujit3; Becherini, Pamela3; Cilli, Michael4; Ihan, Robin5; Ribatti, Domenico3; Greinberger, Lee M5; Horak, Ivan D3; Ponzoni, Mirco2

1G. Gaslini Children's/H/Italian NB Foundation, Laboratory of Oncology, Genoa, Italy; 2G. Gaslini Children's H, Laboratory of Oncology, Genoa, Italy; 3Enzon Pharmaceuticals, Enzon Pharmaceuticals, Piscataway, NJ, United States; 4National Cancer Institute, Animal Research Facility, Genoa, Italy; 5University of Bari, Department of Human Anatomy, Bari, Italy

Background/Aims: Treatment of neuroblastoma (NB) is successful in less than half of patients with high-risk disease. Here, the anti-tumor activity of a water soluble pegylated SN38 drug conjugate (EZN-2208), was evaluated in preclinical models of human NB.

Methods: The in vitro cytotoxicity of EZN-2208 was tested by counting trypan blue dye- and annexin-V-positive cells, while its therapeutic efficacy was evaluated, in terms of survival, anti-tumor and anti-angiogenic activities, in subcutaneous, pseudometastatic and orthotopic NB animal models.

Results: In vitro, EZN-2208 was about 100-fold more cytotoxic than CPT-11 in a panel of NB cell lines, by inducing apoptosis/necrosis and p53 expression and by reducing HIF-1α/ HIF-2α expression. Compared to the SN38 equivalents of CPT-11, EZN-2208 led in vivo to a significant tumor regression in subcutaneous luciferase-transfected xenografts and to 100% of disease-free mice in the pseudometastatic model. In the orthotopic model, EZN-2208-treated mice showed a dramatic arrest and regression of primary tumor growth. Long term survival was seen in 100% of EZN-2208-treated animals with tumors almost disappeared, as assessed by staining histological sections of tumors with antibodies recognizing NB cells and cell proliferation. At MTD, while CPT-partially prolonged mice survival, all immunocompetent and immunodeficient, EZN-2208-treated NB-bearing mice were cured. Compared to CPT-11, EZN-2208 significantly reduced the number of radiating vessels invading the tumor implanted onto the chorioallantoic membranes. Mechanistic experiments showed statistically significant enhanced TUNEL and Histone H2ax staining and decreased VEGF, CD11, MMP-2 and MMP-9 expression in tumors removed from EZN-2208-treated mice. In orthotopic NB model resistant to Doxorubicin (D), Cisplatin, Vincristine (V), Fenretinide and Topotecan (T), EZN-2208 induced 100% curability. EZN-2208 blocked tumor relapse after TVD-combined treatment.

Conclusion: EZN-2208 could be considered the most promising novel anti-NB agent, to be administered in different clinical settings.

Supported by Italian NB Foundation and Enzon Pharmaceuticals

Email: fabiopastorino@ospedale-gaslini.ge.it

OR30

Selective targeting of neuroblastoma tumor initiating cells by a telomerase inhibitor IMETELSTAT

Lipman, Talatana1; Fujitani, Mayumi2; Hansford, Loen3; Clarke, Ian4; Harley, Calvin5; Tressler, Robert6; Malkin, David2; Walker, Erin7; Dirks, Peter2; Sylvain, Baruchel8; Kaplan, David9; Tabori, Uri10

1Hospital For Sick Children, The Arthur and Sonia Labatt Brain Tumor Research Centre, Toronto, Canada; 2Geron Corporation, Research, Menlo Park CA, USA, United States; 3Hospital For Sick Children, Cell Biology, Toronto, Canada; 4The Arthur and Sonia Labatt Brain Tumor Research Centre, Cell biology, Toronto, Canada; 5Hospital For Sick Children, Hematology Oncology, Toronto, Canada

The ability of neuroblastoma (NB) to recur after maximal therapy can be related to limited replicative potential of tumor initiating cells (TIC) and telomerase activation. The role of telomerase activation and its inhibition role in neuroblastoma TIC is still unknown.

Aims: To demonstrate that telomerase-dependent telomere maintenance is critical to the survival of the self-renewing neuroblastoma TICs , targeting telomerase activity may selectively block tumorigenicity.

Methods: Telomere maintenance was assessed for normal stem cells (SKPs, NSC, MSC) and TICs NB12, NB88R2 and NB122R isolated from NB bone marrows. NSC and NB TICs were treated with Imetelstat 5 μM (Geront). Telomerase activity was assessed by Telomeric Repeat Amplification Protocol (TRAP) assays. Telomere length was assessed by TRF assay and stem cell renewal was assessed by sphere forming assays.

Results: NB TIC lines exhibited very short telomeres and high telomerase activity. Strikingly, normal stem cells revealed undetectable telomerase activity and very long telomers. Inhibition of telomerase by Imetelstat of 3 different neuroblastoma TICs lines resulted in a dramatic loss of replicative potential after 3-5 weeks of treatment, accompanied by rapid telomere attrition and loss of the sphere forming ability. In contrast, normal neural stem cell lines were insensitive to telomerase inhibition even after 12 weeks of therapy.

Animals xenografted with NB12 TIC which were pre-treated with Imetelstat failed to form tumors (p<0.009). TICs harvested from these animals were subjected to sphere forming assays.

TICs NB12 were Xenografted in NOD/SCID mice then treated with IP Imetelstat (30mg/kg twice a week) tumor volumes and survival were monitored. TIC harvested from these animals were subjected to sphere forming assay.

Conclusion: Telomerase inhibition causes tumor growth delay and irreversible loss of TIC self-renewal capacity. Targeting TIC with telomerase inhibitors may represent a new therapeutic approach in NB.

Email: sylvain.baruchel@sickkids.ca
OR31
ABCC transporters influence multiple aspects of neuroblastoma biology, as well as clinical outcome, independent of cytotoxic drug efflux
Haber, Michelle1; Henderson, Michelle1; Porro, Antonio1; Munoz, Marcia1; Iraci, Nunzio2; Xue, Cheng3; Murray, Jayne1; Flemming, Claudia1; Smith, Janice1; Fletcher, Jamie1; Gherardi, Samuele2; Kwek, Alan1; Russell, Giovanni2; Norris, Murray1; Amanda1; London, Wendy3; Buxton, Allen4; Ashton, Lesley1; Sartorelli, Alan5; Cohn, Susan6; Schwab, Manfred7; Marshall, Glenn1; Perini, Giovanna1; Norris, Murray1
1Children’s Cancer Institute Australia, Randwick, NSW, Sydney, Australia; 2University of Bologna, Department of Biology, Bologna, Italy; 3Children’s Hospital Boston, Dana-Farber Harvard Cancer Care, Boston, United States; 4Children’s Oncology Group Statistics and Data Center, Gainesville, Florida, Gainesville, United States; 5Yale University School of Medicine, Connecticut, USA, New Haven, United States; 6The University of Chicago, Department of Pediatrics, Chicago, United States; 7German Cancer Research Center (DKFZ), Division of Tumor Genetics, Heidelberg, Germany

Background/Aims: We have previously shown that high levels of the multidrug transporters ABCC1/MRP1 (J Clin Oncol 24:1546, 2006) and ABCC4/MRP4 (Mol Cancer Ther 4:547, 2005), are strongly predictive of poor outcome in neuroblastoma. Although the prognostic significance of ABCC1 may be explained in terms of cytotoxic drug resistance, none of the drugs used to treat children in these studies were ABCC4 substrates. This suggests that multidrug transporters can contribute to the malignant phenotype, independent of cytotoxic drug efflux, as we have recently outlined (Nat Rev Cancer, 10:147, 2010).

Methods: A MYCN-driven transgenic mouse neuroblastoma model was crossed with an Abcc1-deficient mouse strain or alternatively, treated with an ABCC1 inhibitor. ABCC genes were suppressed using siRNA or overexpressed by stable transfection. Quantitative PCR was used to examine the clinical significance of ABCC family gene expression in a large prospectively accrued cohort (n=209) of primary neuroblastomas. Survival curves were compared with a logrank test.

Results: Pharmacological inhibition or genetic depletion of ABCC1 significantly inhibited neuroblastoma development in MYCN transgenic mice, while knockdown of ABCC1 or ABCC4 resulted in reduced proliferation and migration, and enhanced morphological differentiation of cultured cells. Analysis of a large neuroblastoma cohort confirmed the predictive power of ABCC4 expression and revealed that low ABCC3 expression was highly predictive of reduced survival (p<0.001). These results were confirmed by analysis of a large publicly available neuroblastoma gene expression database. No other ABCC transporter genes were predictive of clinical outcome. Expression levels of ABCC1, ABCC3 and ABCC4 were independently prognostic for outcome and their combined expression pattern defined a subgroup of patients with particularly poor survival. Over-expression of ABCC3 reduced neuroblastoma cell migration and proliferation.

Conclusions: Transporters of the ABCC subfamily can influence important biological characteristics of neuroblastoma independently of their role in drug efflux and represent attractive targets for therapeutic intervention.

Email: mhaber@ccia.unsw.edu.au

OR32
EZH2 mediates epigenetic silencing of candidate neuroblastoma tumor suppressor gene Casz1
Wang, Chunxi1; Woo, Chan-Wook2; Liu, Zhuhui3; Wei, Jun4; Song, Young2; Wang, Lileng5; Marquez, Victor6; Khan, Javed7; Ge, Kai8; Thiele, Carol9
1National Cancer Institute, Pediatric Oncology Branch, Bethesda, Maryland, United States; 2Seoul University School of Medicine, Pediatrics Branch, Seoul, Republic of Korea; 3NIH, National Institutes of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland, United States; 4National Cancer Institute, Lab of Chemical Biology, Bethesda, Maryland, United States

Genes from the Polycomb group (PCG) are epigenetically silenced in cancer, and EZH2, the catalytic subunit of the PRC2 complex, is deregulated in a variety of cancers. EZH2 mediates PRC2-mediated H3K27me3 methylation, which targets genes for transcriptional repression.

Background: EZH2 is amplified in prostate cancer and breast cancer and is the only PCG member expressed at higher levels in glioblastoma and Ewing’s sarcoma. We previously showed that high expression of EZH2 and EED associated with poor prognosis in NB tumors (58 patients, median value, EZH2: P=5.87 x 10^-4, EED: P=2.6 x 10^-3). Chromatin Immunoprecipitation (ChiP) assays show increased H3K27me3 at the Transcriptional Start Site of Casz1, which decreased after Depsi treatment. ChiP analysis also reveals binding of PRC2 complex subunits (EZH2, EED and SUZ12) to the Casz1 transcription start site in an area co-incident with increased H3K27me3 binding. PRC2 complex binding also decreased after Depsi treatment. Targeting of EZH2 using siRNA to EZH2 led to increased Casz1 expression (2 fold) in NB cells. Also Casz1mRNA is 3-fold higher in EZH2-/- mouse embryonic fibroblasts (MEFs) compared to EZH2+/+ MEFs. Treatment of NB cells with a small molecule inhibitor of EZH2, DZNep, caused a 2-fold increase in CASZ1 expression. Our data shows CASZ1 expression is epigenetically silenced by PRC2 complex and the decrease of PRC2 complex expression and binding leads to increased CASZ1 expression. This is consistent with a model in which aberrant EZH2 mediated epigenetic gene silencing of Casz1 contributes to NB tumorigenesis.

Email: c47a@nih.gov
OR33

Discovery of epistatic genetic interactions associated with high-risk neuroblastoma

Capasso, Marco\(^\d\); Boese, Kristopher\(^\d\); Diek, Sharoin\(^\d\); Moise, Yael\(^\d\); Isolacci, Achille\(^\d\); Devoto, Marcellin\(^\d\); Maris, John\(^\d\)\footnote{CEINGE Biotecnologie Avanzate, Genova, Via Comunale Margherita, 482, 80145, Napoli, Italy; Children's Hospital of Philadelphia, Center for Childhood Cancer Research, 3501 Civic Center Blvd, CTRB 3060, Philadelphia, United States; Università degli Studi di Napoli "Federico II", Genetics, Napoli, Italy; Children's Hospital of Philadelphia, Genetics, Philadelphia, United States}

Background: We have recently demonstrated that polymorphisms in the BRCA1 interacting gene BARD1 are associated with a high-risk neuroblastoma (NBL) using a genome-wide association approach (Capasso, Nat Genet 2009). The mechanism by which BARD1 impacts susceptibility to NBL remains undefined. Here, using a two-locus analytic method, we sought to identify genes that might increase susceptibility to NBL development by their interaction with BARD1.

Methods: We performed an interaction analysis based on a genome-wide SNP array dataset comprising 1433 cases and 3221 controls. Six databases (BIND, BIOGRID, MINT, HPRD, STRING, IntAct) were queried to identify the proteins known or predicted to interact with BARD1. A regression analysis method implemented in PLINK was utilized to test for pairwise interactions between BARD1 SNPs and SNPs of candidate interacting genes.

Results: A total of 109 proteins were identified that were known or predicted to interact with BARD1. Twenty-two of these were identified in three or more databases surveyed, and were the focus of subsequent interaction analyses. The most significant interactions were found between the intrinsic SNPs rs195851 and rs1880709 of the PTN gene (pleiotropin) and rs17487792 of BARD1 (OR=1.45, P=9.0x10\(^{-5}\); OR=1.39, P=7.1x10\(^{-8}\)). The PTN SNPs showed no effect in the single SNP analysis (OR=0.97, P=0.49). The top three SNPs that showed highest association with NBL (OR=1.36, P=7x10\(^{-5}\)) were the most common genotypes at the two loci combined was 9.69. SNP rs17487792 is in LD (r\(^2\)=0.96) with rs2070096, and rs919581) relative to the most common genotype at the two loci.

Conclusions: PTN and BARD1 variants may act in an epistatic fashion to promote NBL tumorigenesis. Future work will focus on identifying the mechanisms for this interaction, and discovering if somatically acquired alterations in these genes may also contribute to a high-risk NBL phenotype.

Acknowledgements: This work was in part supported from OPEN

Email: capasso@ceinge.unina.it

OR34

Accumulation of segmental alterations determines progression in neuroblastoma

Schleiermacher, Gudrun\(^\d\); Janoueix-Lerosey, Isabelle\(^\d\); Ribeiro, Agnes\(^\d\); Klijianenko, Jerzy\(^\d\); Couturier, Jerome\(^\d\); Pieron, Gaelle\(^\d\); Mosseri, Veronique\(^\d\); Vallet, Alexandre\(^\d\); Ager, Nathalie\(^\d\); Plantaz, Dominique\(^\d\); Rubie, Hervé\(^\d\); Valsecchi-Couanet, Dominique\(^\d\); Toulousse, France; Institut Curie, Service de Biostatistiques, Paris, France; Institut Curie, Unité de Génétique Somatique et Cytogénétique, Paris, France; Institut Curie, Département d'Anatomopathologie, Paris, France; Institut Curie, Laboratoire d'OncoGénomique Moléculaire, Lyon, France; Institut Curie, Département d'Oncologie Pédiatrique, Paris, France; Institut Curie, Laboratoire d'Oncologie Pédiatrique, Lyon, France; Institut Curie, Département d'Oncologie Pédiatrique, Paris, France

Background: Neuroblastoma is characterized by two distinct types of genetic profiles, consisting of either numerical or segmental chromosome alterations. The latter are associated with a higher risk of relapse, even when occurring together with numerical alterations. We explored the role of segmental alterations in tumor progression and the possibility of evolution from indolent to aggressive genomic types.

Methods: A retrospective cohort study was conducted on ALL patients with a known ALL-GE. After matching, 30 relapsed patients were compared with 30 non-relapsed patients. The patients were further divided into subgroups with numerical or segmental alterations. Univariate and multivariate logistic regression analysis methods were applied to identify factors associated with relapse.

Results: The univariate analysis revealed that the presence of segmental alterations at diagnosis was significantly associated with a higher risk of relapse (OR=3.5). When controlling for currently used risk factors: patients at high molecular risk and with surgery alone. A higher number of chromosome breakpoints was also usually observed at relapse in cases with segmental alterations at diagnosis. Such an evolution was not linked to secondary effects of cytotoxic treatments since it was observed even in cases treated with surgery alone. A higher number of breakpoints was correlated with higher age at diagnosis, higher stage of disease, and a higher risk of relapse and a poorer outcome.

Conclusion: These data emphasize the importance of segmental alterations, suggesting that tumor progression is directly linked to the accumulation of segmental alterations in neuroblastoma. This possibility of genomic evolution should be taken into account in treatment strategies of low- and intermediate-risk neuroblastoma and should warrant biological reevaluation at the time of relapse. *(GS and IJL contributed equally to this work.)*

Email: gudrun.schleiermacher@curie.net

OR35

Improved outcome prediction of children with neuroblastoma using a miRNA signature

Mestligh, Peter\(^\d\); De Preter, Katleen\(^\d\); Vermeulen, Jordi\(^\d\); Naranjo, Arlene\(^\d\); Bray, Isabelle\(^\d\); Castel, Victoria\(^\d\); Chen, Califu\(^\d\); Eggert, Angelika\(^\d\); Hogarty, Michael D\(^\d\); London, Wendy B\(^\d\); Noguera, Rosa\(^\d\); Schramm, Alexander\(^\d\); Schulte, Johannes\(^\d\); Stalling, Raymond V\(^\d\); Versteeg, Laureys\(^\d\); Van Cauwenberghe, Jo\(^\d\); Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium; University of Florida, Children's Oncology Group, Gainesville, United States; Royal College of Surgeons in Ireland, Department of Cancer Genetics, Dublin, Ireland; Children's Hospital of Philadelphia, Oncopaediatric Unit, Valley, Spain; Applied Biosystems, Foster City, United States; Université de Bourgogne, Children’s Hospital Essen, Department of Pediatric Oncology and Haematology, Essen, Germany; The Children's Hospital of Philadelphia, Division of Oncology, Philadelphia, United States; Children's Hospital Boston/Dana-Farber Harvard Cancer Center, Children's Oncology Group, Boston, United States; University of Valencia, Department of Pathology, Medical School, Valencia, Spain; Academic Medical Center, Department of Human Genetics, Amsterdam, Netherlands; Ghent University Hospital, Department of Paediatric Hematology and Oncology, Ghent, Belgium

Background: More accurate assessment of prognosis is important to further improve the choice of risk-related therapy in neuroblastoma (NB) patients.

Method/approach: 430 human mature microRNAs (miRNAs) were profiled on two patient subgroups with maximally divergent clinical courses. Univariate logistic regression analysis was used to select miRNAs that correlated with NB patient survival. Subsequently, a 25-miRNA gene signature was built using 54 training samples, tested on 197 test samples, and validated on an independent set of 278 tumors.

Results: The 25-miRNA signature significantly discriminates the test patients with respect to progression-free survival (PFS) and overall survival (OS) (p<0.0001). Multivariate analysis indicates that the miRNA signature is a significant independent predictor of PFS and OS after controlling for currently used risk factors: patients at high molecular risk have a 5-fold higher risk for relapse/progression and a 6-fold higher risk to die from disease compared to patients at low molecular risk. Patients with increased risk for both a shorter PFS and OS can also be identified in the cohort of high-risk patients based on currently used risk factors, showing the potential of this signature for improved clinical management in this subgroup. These results were confirmed in an external validation set, in which the signature is also independently statistically significant for PFS and OS. A separate logistic model for PFS and OS for the 25-miRNA signature and a previously published 59-miRNA signature was performed on a subgroup of 236 samples and shows that the 25-miRNA signature is an independent significant predictor for PFS, as is the 59-miRNA signature for OS. Currently, we are evaluating the possibility of combining the power of both miRNA and miRNA signatures in order to establish an integrated and even more improved prognostic classification.

Conclusion: Based on miRNA expression data for an unprecedentedly large number of more than 500 NB patients, we established and validated a robust miRNA classifier, able to identify a cohort of high risk NB patients at greater risk for adverse outcome. *(shared 1st aut)*

Email: Katleen.DePreter@UGent.be
OR36
Gene expression-based classification improves risk estimation of neuroblastoma patients
Oberthuer, Andre; Hero, Barbara; Berthold, Frank; Jaraeva, Dilafraz; Faldum, Andreas; Kahler, Yvonne; Asgharzadeh, Shahab; Seeger, Robert; Scaruffi, Paola; Torloni, Gian Paolo; Janoueix-Lerosey, Isabelle; Delatte, Olivier; Schleiermacher, Gudrun; Vandewoude, Jo; Vermeulien, Joel; Spelteman, Frank; Noguera, Rosa; Riqueras, Marta; Béard, Jean; Vallet, Alexander; Avigdor, Smadar; Yaniv, Isaac; Westermann, Frank; Westermann, Holger; Grundy, Richard; Gans, Schardt, Katharina; Schwab, Manfred; Els, Roland; Warnat, Patricia; Kaderali, Lars; Simon, Thorsten; DeCarolis, Boris; Theissen, Jessica; Westerman, Frank; Boris, Brenz, Fischer, Matthias
1University Children's Hospital, Pediatric Oncology, Leipzig, Germany; 2German Cancer Research Center, Department of Theoretical Bioinformatics (B080), Heidelberg, Germany; 3University Clinic Mainz, Institute for Medical Biometry, Epidemiology and Informatics, Mainz, Germany; 4Children's Hospital Los Angeles, Children's Center for Cancer and Blood Diseases, Los Angeles, United States; 5Children's Hospital Los Angeles, Children's Center for Cancer and Blood Diseases, Los Angeles, California, United States; 6National Institute for Cancer Research, Translational Pediatric Oncology, Genova, Italy; 7Institut Curie, INSERM Unit 830, Paris, France; 8Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium; 9University of Valencia, Department of Pathology, Valencia, Spain; 10Institut Gustave Roussy, Department of Tumor Genetics, Villejuif, France; 11Schneider Children's Medical Center, Pediatric Hematology Oncology, Petah Tikva, Israel; 12University of Leipzig, Department of Pediatric Oncology and Hematology, Leipzig, Germany; 13University of Leicester, Children's Cancer Leukaemia Group, Leicester, United Kingdom; 14University of Newcastle upon Tyne, Institute of Human Genetics, Newcastle, United Kingdom; 15German Cancer Research Center, Department of Tumor Genetics (B030), Heidelberg, Germany; 16University Children's Hospital, Pediatric Oncology, Cologne, Germany

Background: The usage of neuroblastoma clinical phenotypes and corresponding therapeutic regimens make accurate risk estimation at the time of diagnosis an essential prerequisite for treatment decisions. This study aimed at evaluating the potential clinical impact of a pre-defined gene-expression-based classifier in neuroblastoma patients. Methods/approach: Gene-expression profiles of 440 internationally collected neuroblastoma specimens (Germany, n = 325; other countries, n = 115) were investigated by microarray analysis. Of the German samples, 125 were examined prospectively as part of the trial NB2004. Patients were classified as either favorable or unfavorable by a 144-gene PAM classifier established previously on a separate set of 77 patients. PAM classification results were compared with those of current prognostic markers and risk estimation strategies. Results: Patients with divergent outcome were reliably distinguished by the PAM classifier (favorable, n = 249, and unfavorable, n = 191; 5-year EFS 0.84±0.03 vs. 0.35±0.04, 5-year OS 0.98±0.01 vs. 0.65±0.05, respectively; both p < 0.001). Different clinical courses were robustly discriminated in both the German and international cohorts, as well as in the prospectively analyzed samples (p < 0.001 for both EFS and OS each). In subgroups with clinical low, intermediate and high risk, the PAM predictor significantly separated patients with divergent outcome (low risk, 5-year OS 1.0 vs. 0.75±0.10, p < 0.001; high risk, 0.84±0.03 vs. 0.38±0.04, 5-year OS 0.98±0.01 vs. 0.65±0.05, respectively; both p < 0.001). Clinical features were considered in observed patients currently considered as non-high risk (n = 188): 5-year OS EFS 0.87±0.02 vs. 0.44±0.07, 5-year OS 1.0 vs. 0.83±0.06; both p < 0.001. Conclusion: Gene expression-based classification using the 144-gene PAM predictor can contribute to improved therapy stratification of neuroblastoma patients. Email: matthias.fischer@uk-koeln.de

OR37
Genomic portrait of tumor progression using next-generation sequencing
We, Jun; Johannson, Peter; Guo, Xiang; Badgett, Tom; Song, Young; Wen, Xinyi; House, Catherine; Yeh, Susan; Khan, Jawed
NCI/NIH, National Human Genome Research Institute, Bethesda, United States
Background: Genetic alterations are thought to enable cancers to proliferate and survive more effectively, or to resist cytotoxic therapies. However, the molecular basis of tumorigenesis and progression is not fully understood. Methods/approach: In order to understand the genetic aberrations underlying tumor progression in refractory neuroblastoma, we performed the next-generation sequencing on the whole transcriptomes and exomes for three selected high-risk stage 4 neuroblastoma patient at diagnosis, after cytotoxic therapies, and at death. In parallel, we performed whole genome sequencing on patient’s constitutional genomic DNA at time of diagnosis to establish the genetic background. Results: Whole transcriptome sequencing of three samples yielded total >500 million mappable reads representing >14,000 genes (>20,000 transcripts). The average coverage for each transcript is >14X, while >50% genes have an average per-base-coverage of >10. Single nucleotide variant (SNV) analysis of transcriptome sequencing data identified approximately 10,000 exonic SNVs of which ~4000 are in the coding regions and about half of them are nonsynonymous. Using SIFT analysis, we predicted approximately 400 SNVs to be damaging. Of them, >150 SNVs are shared among two or more samples. In addition, 188 SNVs were present at diagnosis and in the second sample after cytotoxic therapies. These deleterious mutations that arise during therapies are currently being validated by whole exome sequencing, and investigated for pathway disruption that may lead to refractory disease. Conclusion: In summary, we present the most comprehensive genomics analysis of a neuroblastoma genome to date in a single patient during the course of the disease. Our data show that multiple passenger and driver mutations are found in the tumor biopsies indicating the presence of significant genomic instability of these tumors. This approach will allow us to develop tumor progression models and potential targeted therapies against mutated gene products in neuroblastoma. Email: weij@mail.nih.gov

OR38
A multi-locus technique for risk evaluation of patients with neuroblastoma
Ambros, Inge M.; Brunner, Bettina; Bedwell, Claire; Beiske, Klaus; Béard, Jean; Bow, Nick; Combret, Valerie; Courtieu, Jerome; Deffennar, Raffaela; Gross, Nicole; Jeison, Marta; Lune, John; Marques, Barbara; Martinsson, Tommy; Mazocco, Katia; Noguera, Rosa; Schleiermacher, Gudrun; Spelteman, Frank; Stalling, Rayn; Torloni, Gian Paolo; Tweedle, Deborah; Vaid, Aly; Van Roy, Nadine; Vilamoon, Eva; Ziegler, Andrea; Schreier, Günther; Agner, Gerhard; Drobois, Mario; Lardenius, Ruth; Amann, Gabriele; Schouten, Jan; Pöhler, Gabriele
1Children’s Cancer Research Institute, Tumour Biology, Vienna, Austria; 2University of Newcastle upon Tyne, Institute of Human Genetics, Newcastle upon Tyne, United Kingdom; 3Oulu University Hospital, Department of Pathology, Oulu, Finland; 4Children’s Cancer Research Institute, Tumour Biology, Vienna, Austria; 5Institut Gustave Roussy, Medical Pathology and Biology Department, Villejuif, France; 6Centre Léon Bérard, Laboratoire de Recherche Translationalle, Lyon, France; 7Institut Curie, Service de Génétique Oncologique, Paris, France; 8National Institute for Cancer Research, Translational Paediatric Oncology, Genova, Italy; 9University Hospital, Pediatric Oncology Research, Department of Pediatrics, Lusanne, Switzerland; 10Children’s Cancer Research Institute of Israel, Pediatric Hematology Oncology, Petach Tikvah, Israel; 11Newcastle University, The Medical School, Northern Institute for Cancer Research, Newcastle upon Tyne, United Kingdom; 12Istituto Nazionale di Ricerca e Trattamento dell’Ematologia Oncologica, Rome, Italy; 13University of Gothenburg, Institute of Biomedicine, Department of Clinical Genetics, Gothenburg, Sweden; 14Medical School of Valencia, Department of Pathology, Valencia, Spain; 15Institut Curie, Centre de Recherche, Paris, France; 16University of Ghent, Center for Medical Genetics, Ghent, Belgium; 17Royal College of Surgeons in Ireland, Department of Cancer Genetics, Dublin, Ireland; 18Newcastle University, Northern Institute for Cancer Research, Department of Cellular Pathology, Newcastle upon Tyne, United Kingdom; 19Charles University, Department of Paediatric Haematology and Oncology, Prague, Czech Republic; 20Ghent University Hospital, Centre for Medical Genetics, Ghent, Belgium; 21University of Technology, Safety & Security Department, Vienna, Austria; 22St. Anna Kinderspital, Paediatric Oncology, Vienna, Austria; 23Medical University of Vienna, Department of Pathology, Vienna, Austria; 24MRCC Holland, MRC Holland, Amsterdam, Netherlands; 25Children’s Cancer Research Institute, SIRIR, Vienna, Austria
Background: Precise and comprehensive analysis of tumour genetics is essential for the most accurate risk evaluation and treatment of neuroblastoma. The multi-locus approaches fulfill the present-day requirements. Here, we present the establishment of the multiplex ligation-dependent probe amplification technique (MLPA) for neuroblastoma. Methods: A neuroblastoma specific MLPA kit was designed by the SIOPEN (SIOP Europe Neuroblastoma) Biology Committee in cooperation with MRC Holland. The kit used in this study contained target sequences for 106 genetic loci corresponding to 19 chromosomal arms and reference loci. The validation was performed by fluorescence in situ hybridization (FISH, n = 125), BAC array (aCGH, n = 39) and by SNP array (n = 10). Dilution experiments for determination of minimal tumour cell percentage were performed as well as testing of reproducibility which was checked by inter-laboratory testing involving nine laboratories. Results: Inter-technique validation showed a high concordance rate (99.5%) as well as the inter-laboratory MLPA testing (kappa 0.95, p < 0.01) with seven discrepant out of 1490 results (0.5%). Validation of MLPA results by SNP and aCGH showed a single discordance out of 190 consensus results (0.5%). The test results led to the formulation of interpretation standards and to a revision of the kit. The minimal amount of tumour cell content was fixed at 60% to detect segmental aberration, for detection of amplification, it can be lower. Conclusions: The recently designed neuroblastoma specific MLPA kit not only covers the chromosomal regions demanded by the International Neuroblastoma Risk Group (INRG) for therapy stratification but also includes all hitherto described genetic loci of possible prognostic interest for future studies. Moreover, the technique turned out to be cost effective, reliable and robust with a high inter-laboratory and inter-technique concordance. Email: ambros@cri.at
OR39
Detecting the cutting edges. Highly sensitive and absolute specific detection of MYCN amplified neuroblastoma cells by amplicon-fusion-site (AFS) PCR
Weber, Axel1; Taube, Sylvia; Starke, Sven; Bergmann, Eckhard; Christiansen, Nina Merete; Christiansen, Holger
Children’s Hospital / University of Leipzig, Department of Pediatric Hematology, Oncology and Hemostaseology, Leipzig, Germany

Background: To detect tumor cells as sensitively and specifically as possible is one major goal of cancer diagnostics. It is indispensable to develop new, feasible tools to reliably classify tumor stage at time of initial diagnosis and to monitor the response to therapy as well as recurrence of disease as early as possible.

Method/approach: We mapped the amplified genomic regions (ampGR) around the proto-oncogene MYCN from about 40 primary human neuroblastomas and 3 neuroblastoma cell lines, using a high resolution Tiling Array (HR-TA). Based on the HR-TA data we were able to precisely describe the telomeric and centromeric borders of the ampGR and to deduce virtual fusion sites of the connected ampGRs (amplicon-fusion-sites (AFS)). These AFS served as blueprints for a first AFS-PCR primer design. The specific AFS-PCR fragments were then sequenced. Based on the exact AFS sequences we were able to establish high sensitive, quantitative real time PCR assays (final AFS-PCR).

Results: All ampGR and thus, all AFS identified were absolute tumor cell specific and unique for each patient. AFS-PCR was highly sensitive and uncovered one tumor cell out of $10^6 - 10^7$ control cells. We successfully proved the “in-vivo” practicability of AFS-PCR by detecting and quantifying the specific AFS-DNA of MYCN amplified neuroblastomas in peripheral blood (PB) and bone marrow (BM) samples of the corresponding patients.

Conclusion: AFS-PCR promises an important contribution to an exact definition of the tumor stage of MYCN amplified neuroblastoma at time of initial diagnosis, monitoring the response to therapy by quantifying smallest amounts of minimal residual disease (MRD) or recurrent disease over time. Furthermore, AFS-PCR is not limited to a specific tumor type but is rather transferable to every entity of malignancy, provided that the individual tumor cells harbour ampGR. Once established, AFS-PCR represents a powerful but nevertheless feasible, personalized diagnostic tool for a large number of cancer patients including children with MYCN amplified neuroblastomas.

Email: webera@medizin.uni-leipzig.de

OR40
The homeobox transcription factor HoxC9, a key regulator of development, suppresses tumourigenicity of neuroblastoma
Kocak, Hayriye1; Ackermann, Sandra1; Hero, Barbara1; Kahlert, Yvonne2; Theisens, Jessica1; Ehemann, Volker1; Westermann, Frank1; Odenthal, Margarete1; Oerthuer, Andre1; Berthold, Frank1; Fischer, Matthias1
1Children’s Hospital, Department of Pediatric Oncology and Hematology and Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany; 2University of Heidelberg, Institute of Pathology (INF 220), Heidelberg, Germany

Background: The embryonic nature of neuroblastoma suggests the involvement of key developmental regulator genes in its pathogenesis. Homeobox genes constitute an important family of developmental regulators which play a fundamental role in morphogenesis and cell differentiation during embryogenesis. This study aimed at elucidating the role of HoxC9 in neuroblastoma spontaneous regression and differentiation.

Methods: Gene expression profiles from 476 patients were generated using microarray technology and HOXC9 expression levels were calculated. The methylation pattern of 26 HOXC9 promoter CpG sites was determined in 46 neuroblastic tumours and 2 cell lines. Genomic aberrations on the HOXC9 locus were determined by aCGH in 216 primary tumours. A tetracycline-inducible system of HOXC9 expression was established in 3 neuroblastoma cell lines to investigate the effect of HoxC9 on cell differentiation, proliferation, viability, cell cycle distribution, migration and anchorage-independent growth. The effect of HoxC9 on in vivo tumourigenicity was investigated in neuroblastoma xenograft models.

Results: HOXC9 down-regulation is significantly associated with unfavourable prognostic markers (stage 4, age >18 months at diagnosis, MYCN amplification), unfavourable gene expression-based classification and adverse patient outcome (p<0.001 each). Bisulphite sequencing and aCGH analysis suggested that neither hypermethylation nor copy number alterations are regularly involved in HOXC9 gene silencing in neuroblastoma. Re-expression of HoxC9 resulted in significant reduction of cell viability and clonogenic growth as well as in increased apoptosis. In SK-N-AS cells, restored expression of HoxC9 reduced cell migration. In IMR-32 cells, neuronal differentiation was observed upon HoxC9 upregulation. In neuroblastoma xenograft models (IMR-32 and SK-N-AS cell lines), tumour growth was impeded almost completely after HoxC9 re-expression.

Conclusions: Our data suggest that HoxC9 is a critical factor for spontaneous regression and differentiation in neuroblastoma.

Email: hayriye.kocak@uk-koeln.de
OR41  Identification of miRNAs implicated in neuronal development and neurogenesis using fetal adrenal miRNA profiles
De Brouwer, Sara; Mestdagh, Pieter; D’Haeze, Nick; Schulte, Johannes; Eggert, Angelika; Schramm, Alexander; Noguera, Rosa; Hoyuc, Claire; Laureys, Genevieve; Vandemarcke, Jo; De Preter, Kateleen; Speleman, Frank
1Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium; 2ULB, Laboratoire d’Anatomie Pathologique, Brussels, Belgium; 3University of Essen, Department of Pediatric Hematology and Oncology, Essen, Germany; 4University of Valencia, Department of Pathology, Valencia, Spain; 5CHU Ulg-CHR Citadelle, Department of Pediatric Oncology, Liege, Belgium; 6Ghent University Hospital, Department of Pediatric Hematology and Oncology, Ghent, Belgium

Background: MiRNAs have been shown to be involved in both normal development and oncogenesis. Data are emerging that deregulation of miRNAs also plays an important role in neuroblastoma. In order to identify miRNAs implicated in neuroblastoma pathogenesis, we compared the miRNA profiles of fetal adrenal neuroblasts with a well defined cohort of neuroblastoma tumors.

Method/approach: Using a high-throughput stem-loop reverse transcription qPCR assay, we profiled the expression of 430 miRNAs in 100 primary untreated neuroblastoma tumors and 7 neuroblast samples, isolated from fetal adrenal glands using laser capture microdissection.

Results: Differential expression analysis of neuroblast and neuroblastoma miRNA profiles resulted in a list of 60 candidate miRNAs (32 lower and 28 higher expressed in neuroblast compared to neuroblastoma). A critical selection for potential oncogenic or tumor suppressive miRNAs was based on literature data, correlation with patient survival, genomic localization, correlation with genomic aberrations and putative targets. Furthermore, we compared the predicted targets with our previously described list of differentially expressed miRNAs between neuroblast and neuroblastoma. This allowed the selection of 10 miRNAs, including the well known oncomiRs miR-21 and miR-25, for further functional validation. We also selected miRNAs putatively targeting genes known to be involved in neuroblastoma such as MYCN, ALK or PHOX2B. Functional analysis supported a role in neuronal differentiation for 3 miRNAs. Further analyses are focused on the miRNA regulation of PHOX2B, the master regulator of peripheral neuronal differentiation and implicated in a subset of familial neuroblastoma.

Conclusion: Comparison of miRNA profiles of fetal neuroblasts versus neuroblastomas yielded a distinct subset of miRNAs with possible implication in neuroblastoma. Functional evidence was obtained for a role in neuronal differentiation for 3 miRNAs. Further in vivo studies are warranted to determine the contribution of these miRNAs to the malignant phenotype in neuroblastoma and possibly other neuronal tumors.

Email: sara.debrouver@UGent.be

OR42  Neuroblastoma Phox2b variants stimulate proliferation and de-differentiation of immature sympathetic neurons
Reiff, Tobias; Tsarouina, Konstantina; Majdazari, Alhaneh; Schmidt, Miliko; Ischiropoulos, Athan; Hermel, Hana; 2Max-Planck-Institute for Brain Research, Frankfurt/M, Germany
1Max-Planck-Institute for Brain Research, Research Group Developmental Neurobiology, Frankfurt/M, Germany; 2Max-Planck-Institute for Brain Research, Frankfurt/M, Germany

Background: Neuroblastoma is a pediatric tumor that is thought to arise from autonomic precursors in the neural crest. Mutations in the PHOX2B gene have been observed in familial and sporadic forms of neuroblastoma and represent the first defined genetic predisposition for neuroblastoma.

Methods/approach: To test the effects of loss of C8 expression on NB, we utilized several mouse models to determine whether C8 has a role in vivo in NB.

Results: Although no tumors were observed when C8 alone was deleted, mating the C8 conditional knockout mice with TH-MYCN mice significantly increased the frequency of N-myc-induced tumors, without changing the survival of NB progeny. Our work suggests both apoptotic and nonapoptotic roles for C8 in NB tumorigenesis. C8 increases tumor frequency in the TH-MYCN NB model and decreases tumor differentiation suggesting a role for C8 in neuronal differentiation. In addition, C8 is important for extravasations and migration of NB cells in the blood system. These data support the hypothesis that C8 plays important non-apoptotic roles in cellular migration and attachment during metastasis, but that the down-regulation of C8 enhances the survival of both initially developing and metastatic tumors.

Email: tal.teitz@stjude.org
OR45
Histone deacetylase 8 in neuroblastoma tumorigenesis

Oehme, Ina1; Deubzer, Hedwig E.1; Wegener, Dennis1; Pickert, Matthias1; Ollar, Oliver1

1German Cancer Research Center, CCU Pediatric Oncology, Heidelberg, Germany; 2University Children's Hospital of Cologne, Dpt of Pediatric Oncology, Cologne, Germany; 3German Cancer Research Center, Infant Tumor Research Center, Dpt of Tumor Genetics, Heidelberg, Germany; 4Ithaca College, Dpt of Chemistry, Ithaca, United States; 5University Hospital of Heidelberg, Dpt of Neuropathology, Heidelberg, Germany

The effects of pan-histone deacetylase (HDAC) inhibitors on cancer cells have shown that HDACs are involved in fundamental tumor biological processes such as cell cycle control, differentiation and apoptosis. However, due to the unselective nature of these compounds, little is known about the contribution of individual HDAC family members to tumorigenesis and progression. The purpose of this study was to evaluate the role of individual HDACs in neuroblastoma tumorigenesis. We have investigated the mRNA expression of all classical HDAC1-11 family members in a large cohort of primary neuroblastoma samples covering the full spectrum of the disease. HDACs associated with disease stage and survival, were subsequently functionally evaluated in cell culture models. Only HDAC8 expression was significantly correlated with advanced disease and metastasis, and downregulated in stage 4S neuroblastoma associated with spontaneous regression. High HDAC8 expression was associated with poor prognostic markers, poor overall and event-free survival. Knockdown of HDAC8 resulted in inhibition of proliferation, in reduced clonogenic growth, cell cycle arrest and differentiation in cultured neuroblastoma cells. Treatment of neuroblastoma cell lines as well as short term culture neuroblastoma cells with a HDAC8 selective small molecule inhibitor inhibited cell proliferation, clone formation and induced differentiation, and thus reproduced the HDAC8 knockdown phenotype. Global histone 4 acetylation was not affected by HDAC8 knockdown or by selective inhibitor treatment. Our data point toward an important role of HDAC8 in neuroblastoma pathogenesis and identifies this HDAC family member as a specific drug target for differentiation therapy of neuroblastoma.

Email: i.oehme@dkfz.de

OR46
Genome-wide DNA methylation profiling reveals extensive and complex epigenetic alterations in neuroblastoma tumors

Buckley, Patrick1; Das, Sudipto1; Bryan, Kenneth1; Watters, Karen1; Alcock, Leah1; Versteeg, Rogier1; Stallings, Raymond1

1The Royal College of Surgeons in Ireland, Cancer Genetics, Dublin, Ireland; 2Academic Medical Center, Human Genetics, Amsterdam, Netherlands

Background: Although a number of studies have reported aberrant methylation and inactivation of selected genes in neuroblastoma (NB), the extent of genome-wide promoter hypermethylation is poorly understood. We have applied methylated DNA immunoprecipitation (MeDIP) to genomic microarrays representing all known promoter and/or CpG islands in the human genome to more fully characterize the epigenome of neuroblastoma tumors.

Methods: MeDIP analysis was applied to NB primary tumors (n=18), cell lines (n=7), ganglioneuroblastoma (GNB) (n=4) and ganglioneuroma (GN) (n=6).

Results: The total number of hypermethylated sites per sample ranged from 1,462-5,197). Consistent differences in DNA methylation patterns were identified between cell lines and tumor subtypes, indicating that epigenetic changes play a significant role in adapting cells to in vitro proliferation. Unsupervised hierarchical clustering of methylation data revealed a distinct split between the GN/GNB and NB groups. mRNA microarray expression analyses of cell lines following treatment with 5’-aza-2-deoxycytidine allowed us to explore the functional significance of the hypermethylation. The number of genes which were consistently hypermethylated in the GN/GNB group relative to NB was far greater (199 genes) than the opposite comparison (2 genes). Gene ontology analysis carried out on genes hypermethylated in >90% of GN/GNB displayed a statistically significant enrichment for protein kinases, growth factors and mitosis. The 70 recurrent large-scale blocks of contiguously hypermethylated promoters/CpG islands were identified, consistent with other studies of breast and colon cancer. The size of these regions ranged from 12.5 kb to 590.5 kb, with a mean length of 96.4 kb, with nearly one-third of the blocks clustering within telomeric regions.

Conclusion: Our results indicate that genome-wide hypermethylation in neuroblastoma tumors is highly complex and plays important roles in many cellular processes, including in vitro cell growth and differentiation. We also identify many candidate genes which are potentially silenced through methylation and which will form the basis of functional studies.

Email: pbuckley@rcsi.ie

Parallel session 7 – Targeting MYCN OR47–OR55

OR47
SIRT1 enhances N-Myc protein stability in a positive feedback loop which converts N-Myc expression from a low to high level

Marshall, Glenn1; Liu, Pei2; Gherardi, Samuel2; Scarlett, Chris2; Bedalov, Anton2; Xu, Rong2; Iacu, Nunzio2; van Bekkum, Margot2; Sekyere, Eric2; Jankowski, Kacper2; Trahair, Toby2; Haber, Michelle2; Nonis, Murray2; Blankin, Andrew2; Perini, Giovanni2; Liu, Tao2

1Children's Cancer Institute Australia for Medical Research 2, Sydney Children's Hospital, Sydney; 3Academic Medical Center, Human Genetics, Amsterdam, Netherlands

Here we describe a mechanism whereby low-level N-myc expression in pre-malignant tissues can be converted to a much higher level, thus driving tumor progression. Silent information regulator 2, or human SIRT1, is a member of the class III histone deacetylase family which regulate lifespan and enhance cancer cell proliferation, in part by blocking tumour suppressor transcription. SIRT1 inhibitors have profound anti-cancer effects in vitro and in vivo. Here we show that N-Myc up-regulated SIRT1 transcription in neuroblastoma cells, which contributed to N-Myc-induced cell proliferation. Importantly, SIRT1 markedly up-regulated N-Myc protein levels. N-Myc is stabilised when phosphorylated at Serine 62 (S62) by phosphorylated extracellular signal-regulated protein kinase (ERK). SIRT1 increased the level of ERK phosphorylation, and, as a consequence N-Myc S62 phosphorylation. A fmyrmatrix microarray and real-time RT-PCR revealed mitogen-activated protein kinase phosphatase 3 (MKP3) was one of the genes hypermethylated in >90% of GN/GNB displayed a statistically significant enrichment for protein kinases, growth factors and mitosis. The effects of pan-histone deacetylase (HDAC) inhibitors on cancer cells have shown that HDACs are involved in fundamental tumor biological processes such as cell cycle control, differentiation and apoptosis. However, due to the unselective nature of these compounds, little is known about the contribution of individual HDAC family members to tumorigenesis and progression. The purpose of this study was to evaluate the role of individual HDACs in neuroblastoma tumorigenesis. We have investigated the mRNA expression of all classical HDAC1-11 family members in a large cohort of primary neuroblastoma samples covering the full spectrum of the disease. HDACs associated with disease stage and survival, were subsequently functionally evaluated in cell culture models. Only HDAC8 expression was significantly correlated with advanced disease and metastasis, and downregulated in stage 4S neuroblastoma associated with spontaneous regression. High HDAC8 expression was associated with poor prognostic markers, poor overall and event-free survival. Knockdown of HDAC8 resulted in inhibition of proliferation, in reduced clonogenic growth, cell cycle arrest and differentiation in cultured neuroblastoma cells. Treatment of neuroblastoma cell lines as well as short term culture neuroblastoma cells with a HDAC8 selective small molecule inhibitor inhibited cell proliferation, clone formation and induced differentiation, and thus reproduced the HDAC8 knockdown phenotype. Global histone 4 acetylation was not affected by HDAC8 knockdown or by selective inhibitor treatment. Our data point toward an important role of HDAC8 in neuroblastoma pathogenesis and identifies this HDAC family member as a specific drug target for differentiation therapy of neuroblastoma.
OR48
Identification of therapeutic targets for MYCN-amplified neuroblastoma genomics
Tosioshima, Masatani; 1 Park, Julie; 2 Grandori, Carla
 Fred Hutchinson Cancer Research Center, Human Biology, Seattle, United States; 1 Seattle Children’s Hospital and University of Washington, Pediatrics, United States; 2 University of Washington and the Fred Hutchinson Cancer Research Center, Pharmacology and Human Biology, Seattle, United States

Background: Amplification of the MYCN oncogene is a strong marker of poor prognosis and its relevance in NB development has been demonstrated. However, because MYC family encodes transcription factors the identification of small molecule inhibitors is a challenge. Furthermore, long-term inhibition of MYC has the potential to be harmful for proliferation of normal tissues. Our study aimed at identifying survival pathways of MYC-driven cancers, such as MYCN amplified NB. The existence of non-essential genes enabling the conditional survival of MYC overexpressing cells has been previously demonstrated through a candidate approach.

Method/approach: Here, we employed an unbiased synthetic lethal siRNA screening approach to identify druggable genes in an isogenic pair of cells with or without MYC-overexpression.

Results: Among ~3,500 gene tested, ~100 were identified as synthetic lethal genes with aberrant MYC expression. 45 genes were tested in additional cell pairs, including NB cell lines with and without MYCN amplification. The majority was confirmed and validated through multiple assays, such as apoptosis, DNA damage and by long term colony inhibition assay with stable RNAi. However, only a handful of the gene “Hits” conferred selective lethality in NB with MYCN amplifications. Among these, a gene was identified with no previously recognized direct connection to MYC (Ankyrin kinase 1 epsilon (ANK1)). Investigation of CNK1a expression in a set of primary NB (http://pub.abb.ncfcrf.gov/cgi-bin/JK) indicated its correlation with MYCN amplification, possibly a direct consequence of MYCN transcriptional activity. Utilizing conditional silencing as well as chemical approaches we showed that CNK1a is required only in the context of aberrant expression of either c-MYC or MYCN. In vivo validation was also obtained utilizing xenografts of human NB cell lines.

Conclusion: Our experiments indicate that CNK1a is a validity therapeutic target for NB with MYCN amplification. This study provides a paradigm for the unbiased identification of therapeutic targets for molecularly defined subtypes of NB.

Email: cgrandor@fhcrc.org

OR49
MYCN transcriptionally controls the expression of the Nijmegen Brekage Syndrome gene product p95 nibrin/NBS1
Petroni, Marialaura; 1 Mellone, Massimiliano; 2 Albin, Sonja; 3 Veschi, Veronica; 4 Massimi, Isabella; 5 Scarpinati, Isabella; 6 Frai, Luigi; 7 Fruci, Donalda; 8 Cardinali, Beatrice; 9 Quino, Alberto; 10 Giannini, Giuseppe
1 Sapienza University of Rome, Experimental Medicine, Rome, Italy; 2 Ospedale Bambino Gesù, Research Center, Rome, Italy; 3 National Research Council, Cell Biology Institute, Rome, Italy; 4 Royal Hospital Ryder, Group B (RM), Italy
The p95 nibrin/NBS1 is a member of the human MRE11 complex whose genetic inactivation is responsible for the Nijmegen Brekage Syndrome, an autosomal recessive hereditary disorder characterized by microcephaly, facial dysmorphism, growth retardation, immunodeficiency, radiosensitivity, chromosomal instability and cancer predisposition. NBS1 is a major player of the DNA double strand break repair responses and is downregulated in differentiated neuronal cells. NBS1 controls genetic stability. NBS1 is strongly expressed in highly proliferating tissues and is downregulated during cell differentiation and cell growth inhibition cause NBS1 repression. MYCN might induce a DNA damage response, possibly via replication connections to MYC, casein kinase 1 epsilon (CSNK1e). Investigation of NBS1 expression and regulation of Bmi1 and E2F-related Bmi1 regulation of NB progression are poorly understood. Here, we present a MYCN-induced miRNA signature in human NB involving the activation of several family members derived from the miR-17~92 cluster, including ESR1-mediated NB progression are poorly understood. Here, we present a MYCN-induced miRNA signature in human NB involving the activation of several family members derived from the miR-17~92 cluster, including ESR1.

OR50
Bmi1 is a MYCN target gene and regulates tumorigenesis via repression of KIF1Bβ and TSLC1 in NB
Ochiai, Hidemasa; 1 Takenobu, Hisanori; 2 Nakagawa, Atsuko; 3 Yagamuchi, Yoko; 4 Ohira, Mik; 5 Okimoto, Yuri; 6 Kohno, Yoichi; 7 Nakagawa, Akira; 8 Kamijo, Takeriki
1 Chiba Cancer Research Center Institute, Division of Molecular Carcinogenesis, Chiba, Japan; 2 National Center for Child Health and Development, Department of Pathology, Tokyo, Japan; 3 Chiba Cancer Research Center Institute, Laboratory of Tumor Biology, Chiba, Japan; 4 Chiba Children’s Hospital, Dept. of Hematology & Oncology, Chiba, Japan; 5 Graduate School of Medicine, Chiba University, Department of Pediatrics, Chiba, Japan; 6 Chiba Cancer Research Center Institute, Division of Innovative Cancer Therapeutics, Chiba, Japan

Background: Recent advances in neuroblastoma (NB) research addressed that epigenetic alterations such as hypermethylation of promoter sequences, with consequent silencing of tumor-suppressor genes, can play significant roles in the tumorigenesis of NB. The exact role of epigenetic alterations, except for DNA hypermethylation, however, remains to be elucidated in NB research.

Results and Discussion: In the present study, we clarified the direct binding of MYC to Bmi1 promoter by quantitative ChIP assay and up-regulation of Bmi1 transcription by MYCN by real-time PCR analysis. Mutation introduction into a MYCN binding site (E-box) in Bmi1 promoter suggests that MYCN has more important roles in the transcription of Bmi1 than EZF-related Bmi1 regulation. A correlation between MYCN and polycystic protein Bmi1 expression was observed in primary NB tumors. Expression of Bmi1 resulted in the acceleration of proliferation and colony formation in NB cells. Bmi1-related inhibition of NB cell differentiation was confirmed by neurite extension assay and analysis of gene expression, including MYCN expression. Intriguingly, the above-mentioned Bmi1-related regulation of the NB cell phenotype seems not to be mediated only by p14ARF/p16INK4a, well-known Bmi1 targets. Expression profiling analysis using a fro-house tumor-specific cDNA microarray (CCG- NHR13000 chip) addressed the Bmi1-dependent repression of KIF1Bβ (Munirajan et al., J Biol Chem 2008) and TSLC1 (Ando et al., Int J Cancer 2008), which have important roles in predicting the prognosis of NB. Chromatin immunoprecipitation assay and semi-quantitative RTPCR of the tumor suppressors demonstrated that KIF1Bβ and TSLC1 are direct targets of Bmi1 in NB cells. These findings suggest that MYCN induces Bmi1 expression, resulting in the repression of tumor suppressors via PcG-mediated epigenetic chromosome modification.

Conclusion: NB cell proliferation and differentiation appear to be partially dependent on the MYCN/Bmi1/tumor-suppressor pathways.

Email: tkamijo@chiba-cc.jp

OR51
The interplay between Mycn, microRNAs and estrogen receptor-α during differentiation of the post-migratory sympathetic nervous system
Lovén, Jakob; 2 Zinol, Nikolay; 3 Wahlström, Therese; 4 Möller, Inga; 5 Brodin, Petter; 6 Fredlund, Erik; 7 Ribacke, Ulf; 8 Pivarcsi, Andor; 9 Pålman, Sven; 10 Henriksson, Marco
1 Karolinska Institute, MTC, Stockholm, Sweden; 2 Lund University, Center for Molecular Pathology, University Hospital Malmö, Malmö, Sweden; 3 Harvard School of Public Health, Department of Epidemiology and Infectious Diseases, Boston, United States; 4 Karolinska University Hospital, Center for Molecular Medicine, Stockholm, Sweden

MYCN, a proto-oncogene normally expressed in the migrating neural crest, is in its amplified state a key factor in the genesis of neuroblastoma (NB). However, the mechanisms underlying MYCN-mediated NB progression are poorly understood. Here, we present a MYCN-induced miRNA signature in human NB involving the activation and transrepression of several miRNA genes from paralogous clusters. Several family members derived from the miR-17~92 cluster, including miR-18a and miR-19a, were among the up-regulated miRNAs. Expression analysis of these miRNAs in NB tumors confirmed increased levels in MYCN-amplified samples. Specifically, we show that miR-18a and miR-19a target and repress the expression of estrogen receptor-a (ESR1), a ligand-inducible transcription factor implicated in neuronal differentiation. Immunohistochemical studies confirmed that MYCN expression is restricted to human fetal sympathetic ganglia, suggesting a role for ESR1 during sympathetic nervous system development. Reconstitution of ESR1 expression in NB cells resulted in marked growth arrest and neuronal differentiation while inhibition of miR-18a in NB cells led to severe growth retardation, outgrowth of varicosity-containing neurites, and induction of neuronal sympathetic differentiation markers. We propose that MYCN amplification may disrupt estrogen signaling sensitivity in primitive sympathetic cells through deregulation of ESR1, thereby preventing the normal induction of neuronal differentiation. Collectively, our findings demonstrate the molecular consequences of MYCN overexpression in NB-driven tumor and offer unique insights into the pathology underlying MYCN-amplified NB.

Email: Jakob.Loven@ki.se
**OR52**

Targeting MYCN by modulation of the fate of its mRNA: a new potential therapeutic approach for neuroblastoma

Sidorovich, Viktoriya1; Adami, Valentina1; Gatto, Patamela1; Tonioli, Gian Paolo1; Quattrone, Alessandro2

1University of Trento, Centre for Integrative Biology, Trento, Italy; 2National Cancer Research Institute (IST), Translational Paediatric Oncology, Genoa, Italy

MYCN amplification in neuroblastoma patients is strongly associated with advanced disease stages, rapid tumor progression and poor prognosis, making it an attractive therapeutic target. The expression of MYCN is highly regulated by a transcription factor, and therefore not a druggable protein. We took advantage of evidence of post-transcriptional regulation of MYCN gene expression to develop a cellular model for investigating the role of MYCN 3' untranslated region (3'UTR) in the modulation of MYCN mRNA fate, and to identify compounds able to affect it. Using a panel of parental neuroblastoma cell lines we firstly reported post-transcriptional MYCN alteration of expression, related in a complex way to gene amplification. We then generated luciferase reporter constructs with full length MYCN 3'UTR, which were stably inserted in the CHP134 neuroblastoma cell line. A screening was carried out in 96-well plates in triplicates, using a 2000-compound library including all the FDA-approvals drugs, with luciferase activity assessed after 24h of a 2µM treatment. Molecules affecting luciferase were counter-screened for promoter effects and checked for reproducibility, dose-responsiveness and for the phenotypic outcome in terms of cytotoxic activity. We identified more than 100 hits, which were clustered based on structural and functional similarity. Cytotoxicity was high, as expected, for chemotherapeutic drugs, as well as for another chemically homogenous class of drugs, cardiac glycosides (remarkably, 13 molecules active out of the 13 present in the library). The selectivity between MYCN downregulation and toxicity of cardiac glycosides is currently under investigation. Another class of natural compounds, flavonoids, resulted instead effective in enhancing MYCN 3'UTR-induced lucerase activity, raising interest as potential triggers of MYCN-induced apoptosis. We report other experiments aimed at the definition of the mechanism of action of the two classes of compounds, which could finally result in the development of clinically relevant compounds able to affect MYCN amplified neuroblastomas.

Email: alessandro.quattrone@unitn.it

---

**OR54**

Re-activation of CLUSTERIN by epitogenic drugs as a therapeutic approach for MYCN tumourogenesis

Cortevada, Daisy1; Cheyka, Olesya1; Gherardi, Samuele2; Vail, Emanuel1; Cantilena, Sandra1; Plotskowa, Izbaza1; Perini, Giovanni1; Salo, Arturo3; 1Institute of Child Health-University College of London, Molecular Haematology and Cancer Unit, London, United Kingdom; 2University of Bologna, Department of Biology, Bologna, Italy

Background: Epigenetic reprogramming is an important mechanism of oncogene-initiated tumourigenesis. MYCN, a neuronal specific member of the MYC family, is strongly associated with aggressive, metastatic neuroblastomas. In a previous study we showed that CLUSTERIN (CLU) is negatively regulated by MYCN and is a suppressor of MYCN induced tumourigenesis. Here we show that MYCN transcriptionally represses CLU via interaction with a non-canonical E-BOX motif.

Methods: We used gel shift and Chromatin IP analyses to monitor the binding of MYCN to the CLU promoter in vitro and in vivo. Luciferase assays were carried out for assessment of transcription. In vitro invasion and proliferation/FACS assays were carried out for functional studies.

Results: MYCN inhibits the expression of CLU by direct interaction with the non-canonical E-box sequence CACGCG in the 5' flanking region of the CLU promoter. We found that binding of MYCN to the CLU gene causes the appearance of bivalent euchromatic marks, typically observed in the promoters of developmentally regulated genes in stem cells, such as acetylated histone H3, di-methylated H3K4, tri-methylated H3K9-K27, and recruitment of histone deacetylases and polycomb proteins. Tricostatin A and Valproic acid, two inhibitors of histone deacetylases currently used in cancer clinical trials, re-activate the expression of CLU in MYCN amplified neuroblastoma cell lines, causing inhibition of their proliferation. Notably, these two drugs are completely abrogated when CLU expression is silenced by RNA interference.

Discussion: This is, to our knowledge, the first study documenting that a MYC oncoprotein can impose a bivalent state and negatively modulate a tumour suppressor gene by direct interaction with an E-box motif. The observation that CLU is an essential mediator of the therapeutic effects of histone deacetylase inhibitors in MYCN-driven tumours suggests that re-activation of CLU in cancer patients could be used to predict a favourable response to epitogenic drugs.

Email: d.cortevada@ich.ucl.ac.uk

---

**OR53**

Bortezomib and HDAC inhibitor PCI-24781 show synergetic activity in neuroblastoma in vitro and in vivo models, inducing ROS and depressing MYCN

Currier, Erik1; Illényi, Sharion1; Libous, Jennifer1; Bond, Jeffrey1; Lescatte, Paul1; 1University of Vermont, Vermont Cancer Center, Burlington, United States; 2University of Vermont, Pediatrics, Burlington, United States; 3University of Vermont, Microbiology and Molecular Genetics, Burlington, United States

Background: Current treatment of neuroblastoma (NB) often fails due to chemoresistance and new treatments with novel mechanisms are needed. HDAC inhibitors are a diverse class of compounds which could finally result in the development of clinically relevant compounds able to affect MYCN amplified neuroblastomas. HDAC inhibitors are a diverse class of compounds which could finally result in the development of clinically relevant compounds able to affect MYCN amplified neuroblastomas. HDAC inhibitors are a diverse class of compounds which could finally result in the development of clinically relevant compounds able to affect MYCN amplified neuroblastomas. HDAC inhibitors are a diverse class of compounds which could finally result in the development of clinically relevant compounds able to affect MYCN amplified neuroblastomas. HDAC inhibitors are a diverse class of compounds which could finally result in the development of clinically relevant compounds able to affect MYCN amplified neuroblastomas.

Methods: NB cell lines and patient-derived primary NB cultures were treated with bortezomib and PCI-24781 alone and in combination for 48 hours. Cells were also treated with HDAC inhibitors vorinostat, sodium butyrate, and valproic acid and viability was assessed by calcein AM assays. mRNA from treated cells was evaluated at 6 and 24 hours using U133 2+ mRNA expression arrays and Ingenuity analysis. DCF was measured and imaged twice per week.

Results: Bortezomib and HDAC inhibitor PCI-24781 cells subcutaneously and treated with daily doses of 0.5 mg/kg bortezomib, 12.5 mg/kg PCI-24781, or a combination. Tumors were checked for reproducibility, dose-responsiveness and for the phenotypic outcome in terms of cytotoxic activity. We identified more than 100 hits, which were clustered based on structural and functional similarity. Cytotoxicity was high, as expected, for chemotherapeutic drugs, as well as for another chemically homogenous class of drugs, cardiac glycosides (remarkably, 13 molecules active out of the 13 present in the library). The selectivity between MYCN downregulation and toxicity of cardiac glycosides is currently under investigation. Another class of natural compounds, flavonoids, resulted instead effective in enhancing MYCN 3'UTR-induced lucerase activity, raising interest as potential triggers of MYCN-induced apoptosis. We report other experiments aimed at the definition of the mechanism of action of the two classes of compounds, which could finally result in the development of clinically relevant compounds able to affect MYCN amplified neuroblastomas.

Email: gsholler@uvm.edu

---

**OR55**

Identification of small molecules inhibiting Myc oncoprotein function

Ridderstråle, Karin; Yan, Qinzi; Zakaria, Siti Mariam; Hybring, Per; Larsson, Lars-Gunnar1; Gherardi, Samuele2; 1Institute of Child Health-University College of London, Molecular Haematology and Cancer Unit, London, United Kingdom; 2Karolinska Institutet, Department of Microbiology, Tumor and Cell biology, Stockholm, Sweden

Deregulated expression of MYC family oncogenes of MYCN, MYCNY and MYCL occurs in many types of human tumors, including the childhood tumors neuroblastoma and medulloblastoma, and is often associated with aggressive tumor development and poor prognosis. The MYC genes encode transcription factors that control the expression of thousands of genes of relevance for tumorigenesis. In mouse tumor models, inactivation of the MYC oncogene-initiated tumourigenesis. MYCN, a neuronal specific member of the MYC family, is strongly associated with aggressive, metastatic neuroblastomas. In a previous study we showed that CLUSTERIN (CLU) is negatively regulated by MYCN and is a suppressor of MYCN induced tumourigenesis. Here we show that MYCN transcriptionally represses CLU via interaction with a non-canonical E-BOX motif.

Methods: We used gel shift and Chromatin IP analyses to monitor the binding of MYCN to the CLU promoter in vitro and in vivo. Luciferase assays were carried out for assessment of transcription. In vitro invasion and proliferation/FACS assays were carried out for functional studies.

Results: MYCN inhibits the expression of CLU by direct interaction with the non-canonical E-box sequence CACGCG in the 5' flanking region of the CLU promoter. We found that binding of MYCN to the CLU gene causes the appearance of bivalent euchromatic marks, typically observed in the promoters of developmentally regulated genes in stem cells, such as acetylated histone H3, di-methylated H3K4, tri-methylated H3K9-K27, and recruitment of histone deacetylases and polycomb proteins. Tricostatin A and Valproic acid, two inhibitors of histone deacetylases currently used in cancer clinical trials, re-activate the expression of CLU in MYCN amplified neuroblastoma cell lines, causing inhibition of their proliferation. Notably, these two drugs are completely abrogated when CLU expression is silenced by RNA interference.

Discussion: This is, to our knowledge, the first study documenting that a MYC oncoprotein can impose a bivalent state and negatively modulate a tumour suppressor gene by direct interaction with an E-box motif. The observation that CLU is an essential mediator of the therapeutic effects of histone deacetylase inhibitors in MYCN-driven tumours suggests that re-activation of CLU in cancer patients could be used to predict a favourable response to epitogenic drugs.

Email: lars-gunnar.larsson@ki.se
OR56

The role of dietary restriction in the mechanisms of differential cellular protection: a strategy to enhance the efficacy of chemotherapy in the treatment of neuroblastoma

Bianchi, Giovanni1; Lee, David2; Safdie, Fernanda3; Emlomite, Lauri4; Pistola, Vito5; Longo, Valter6; Raffaghello, Lizzio7

1G.Gaslini Institute, Laboratory of Oncology, Genova, Italy; 2University of Southern California, 3Andrus Gerontology Center, Dept. of Biological Sciences and Norris Cancer Center, Los Angeles, United States; 4University of Southern California, Andrus Gerontology Center, Dept. of Biological Sciences and Norris Cancer Center, Los Angeles, United States; 5National Cancer Research Institute, Animal Research Facility, Genova, Italy; 6G.Gaslini Institute, Laboratory of Oncology, Genoa, Italy

Background/Aim: Strategies to treat cancer have focused primarily on the killing of tumor cells. Here, we described a differential stress response chemotherapy-induced toxicity, and provide the foundation for a novel and powerful therapeutic strategy for the cure of NB.

Email: prucci@libero.it

OR57

Fenretinide (4-HPR) orally formulated in Lym-X-SorbTM(LXS) lipid matrix or as an intravenous emulsion increased 4-HPR systemic exposure in patients with Recurrent or Resistant Neuroblastoma. A new approach to neuroblastoma therapy (NANT) consortium trial

Kang, Min H.1; Marachelian, Araz2; Villablanca, Judith G.2; Maris, John M.3; Matthay, Katherine4

1University of Michigan Medical Center, Pediatrics, Ann Arbor, MI, United States; 2Cincinnati Children's Hospital, Pediatrics, Cincinnati, OH, United States; 3Children's Hospital of Philadelphia, Pediatrics, Philadelphia, PA, United States; 4Children's Healthcare of Atlanta, Emory University, Pediatrics, Atlanta, GA, United States; 5University of Michigan Medical Center, Radiology, Ann Arbor, MI, United States; 6University of California, San Francisco, Radiation Oncology, San Francisco, CA, United States; 7Children's Oncology Group, Operations Center, Arcadia, CA, United States; 8University of California, San Francisco, Pediatrics, San Francisco, CA, United States

Background: Fenretinide (4-HPR), a cytotoxic retinoid, obtained limited and variable plasma levels when tested in a corn oil-based capsule. In vivo experiments demonstrated that STS in combination with high dose chemotherapy decreased the toxicity and significantly prolonged the survival of NB bearing mice. In addition, we demonstrated that STS alone could reduce tumor cell growth through downregulation of S6Kinase (S6K) and Erk in NB cell lines and we evaluated the toxicity and the therapeutic effects of STS in combination with different chemotherapeutic agents (chemo drugs) in suitable experimental NB models.

Methods: The in vitro cytotoxicity of chemo drugs in combination with STS was tested by Trypan Blue staining and MTT assay. The in vivo STS protocol allowed mice to consume water but not food for 48-60 hours prior to chemotherapy. All mice were monitored daily for weight loss. The in vivo therapeutic effects of appropriate combinations of different chemo drugs with STS were evaluated in term of toxicity, tumor burden and survival in Au/J mice intravenously injected with NXS2-luciferase and Neuro-2a-luciferase NB cells. We tested the antitumor effect of STS alone by measuring the volume of subcutaneous ACN tumors developed in athymic mice. The effect of STS on the differential regulation of Akt, mTor, S6Kinase (S6K) and Erk in NB cell lines was evaluated by Western Blot experimental.

Results: The reduction in culture of serum from 10% to 1% and glucos from 1.0 to 0.5 g/L sensitized NB cell lines to different chemo drugs. Similarly, in vivo experiments demonstrated that STS in combination with high dose chemotherapy decreased the toxicity and significantly prolonged the survival of NB bearing mice. In addition, we demonstrated that STS alone could reduce tumor cell growth through downregulation of Phospho-Akt, Phospho-mTor and Phospho-S6K.

Conclusion: Taken together, these results support the hypothesis that STS can protect normal cells from and sensitize NB cells to chemotherapy-induced toxicity, and provide the foundation for a novel and powerful therapeutic strategy for the cure of NB.

Email: prucci@libero.it
OR59

A phase IIa trial of ultratrace (non-carrier added) iodobenzene I-131 (MBIG): A prospective study of Ultratrace to Neuroblastoma (NANT) study

Matthay, Katherine1; Weiss, Briar2; Villablanca, Judith G.; Maris, John1; Yanik, Greg1; Groshen, Susan1; Jackson, Hollie2; Hawkins, Randall1; Goodarzian, Farbar3; Panigraphy, Ashok4; Dubois, Steven5; Slabus, James6; Barnett, John1; Batsch, John7; Toubin, Alexandre8; LaFrance, Norman9; 1UCSF School of Medicine, Pediatrics, Hematology/Oncology, San Francisco, CA, United States; 2University Children’s Hospital Medical Center, Hematology/Oncology, Cincinnati, OH, United States; 3Children’s Hospital Los Angeles/Ked School of Medicine USC, Pediatrics, Los Angeles, CA, United States; 4Children’s Hospital of Philadelphia, Oncology, Philadelphia, PA, United States; 5University of Michigan School of Medicine, Pediatrics, Ann Arbor, MI, United States; 6University of Southern California, Biostatistics, Los Angeles, CA, United States; 7Children’s Hospital Los Angeles, Radiology, Los Angeles, CA, United States; 8UCSF School of Medicine, Radiology, San Francisco, CA, United States; 9Children’s Hospital Los Angeles, Radiology, Los Angeles, CA, United States; 10UPMC Children’s Hospital of Pittsburgh, Radiology, Pittsburgh, PA, United States; 11UCSF School of Medicine, Pediatrics, Hematology-Oncology, San Francisco, CA, United States; 12Radiation Dosimetry Systems Inc, Dosimetry, Charlottesville, VA, United States; 13Molecular Insight Pharmaceuticals, Oncology, Cambridge, MA, United States; 14Children’s Hospital of Cincinnati Medical Center, Radiology, Cincinnati, United States

Background: I-131-MBIG is specifically taken up in neuroblastoma, with a response rate >30% in relapsed disease. The presence of non-radioactive “carrier” MBIG molecules may inhibit uptake of I-131-MBIG, resulting in less tumor uptake and increased risk of cardiovascular side effects. The primary aim of this study was to establish the maximum tolerated dose (MTD) of non-carrier-added iodobenzene I-131 (Ultratrace) with autologous hematopoietic stem cell (AHSC) support.

Method/approach: Eligible patients were age 1-30 years with relapsed or refractory neuroblastoma, MBIG uptake, and cryopreserved AHSC. A diagnostic dose of Ultratrace (1-5 mCi) was followed by 3 dosimetry scans. AHSC were infused 1-3 days post therapy and toxicity was assessed daily for 60 days. The Ultratrace was escalated in 3 mCi/kg increments from 12-21 mCi/kg using 3+3 design. The administered dose was adjusted based on absorbed dose estimates and Emami (1991) organ tolerance limits. Dose limiting toxicity (DLT) was defined as grade 5 toxicity or grade 3 or 4 non-hematologic toxicity except grade 3 pre-defined exclusions.

Results: Three patients each entered and were evaluable at levels 12, 15, 18 mCi/kg (444-666 GBq/kg) with no required dose adjustment or DLT. Three additional patients entered at 21 mCi/kg and all required dose reductions of 6, 14, and 25% to meet organ limits. Median whole body radiation dose was 3.9 Gy (2.1-4.4); median liver dose, 11.5 Gy (9.8-28.1); median lung dose 11.6 Gy (6.1-16); median kidney dose 14.6 Gy (9.1-23). In 4 patients there were 8 target lesions with doses of 1.9-11 mCi/MBq. There was no related death, 3 or 4 non-hematologic toxicity; all patients engrafted promptly. No patients at 15 or 18 mCi/kg had ANC<500. The response rate in 9 evaluable patients was 33%; 12 mCi/kg, 2 stable disease (SD) and 1 progressive disease (PD); 15 mCi/kg, 1 partial response (PR), 1 mixed response, 1 SD, 18 mCi/kg, 1 complete response (CR), 1PR, 1SD.

Conclusion: Ultratrace iodobenzene-131 with AHSC support is feasible at 18 mCi/kg with significant toxicity and with promising responses that support proceeding to a pivotal Phase II study of 18 mCi/kg.

Email: matthayk@peds.ucsf.edu

OR60

Characteristics of relapsing localized neuroblastoma: A preliminary report of the second SIOPEN study (LNESG2, localized neuroblastoma European study group 2)

Beck Popovic, Maja1; Garcia, Emma2; Gross, Nicole3; Combaret, Valerie4; Ambros, Peter5; Beiske, Klaus6; Jenkner, Alessandro7; Défachelles, Anne8; Carthe, Adela9; Brichard, Bénédicte10; Balwierz, Walentyna11; Papadakis, Vassilicos12; Ash, Shifra13; Ruud, Ellen14; Lademann, Rudolf15; Ora, Ingrid16; Holmes, Keith17; De Bernardi, Bruno18; Michon, Jean19; Mosseri, Véronique20

1University Hospital CHUV, Pediatric Hematology-Oncology Unit, Lausanne, Switzerland; 2Pediatric Oncology Research Laboratory, Lausanne, Switzerland; 3Centre Léon BÉARD, Laboratoire d’Oncologie Moléculaire, Lyon, France; 4ST. Anna Children’s Hospital, Cancer Research Institute, Prague, Czech Republic; 5Rikshospitalet, Dept. of Pathology, Oslo, Norway; 6Ospedale Bambino Gesù, Division of Pediatric Oncology, Rome, Italy; 7Centre Oscar Lambret, Unité d’Oncologie Pédiatrique, Lille, France; 8Hospital Infantil La Fe, Unidad de Oncología Pediátrica, Valencia, Spain; 9Cliniques Universitaires Saint Luc, Service d’hématologie et oncologie pédiatrique, Brussels, Belgium; 10Polish-Pediatric Institute of Pediatrics, Department of Pediatric Oncology and Hematology, Krakow, Poland; 11Aphria Sophia’s Children’s Hospital, Pediatric Hematology-Oncology, Athens, Greece; 12Schneider Children’s Medical Center of Israel, Pediatric Hematology Oncology, Petach Tikva, Israel; 13Rikshospitalet, Department of Pediatrics, Oslo, Norway; 14Lund University Hospital, Department of pediatric oncology and Hematology, Lund, Sweden; 15St. George’s Hospital, Department of Paediatric Surgery, London, United Kingdom; 16Giannina Gaslini Children’s Hospital, Department of Haematology/Oncology, Genova, Italy; 17Institut Curie, Department of Haematology/Oncology, Paris, France; 18Institut Curie, Service de Biostatistique, Paris, France

Introduction: Based on the results of the first protocol, LNESG2 has been designed to expand information on factors associated with clinical prognosis, to maintain/improve the overall survival for those in the INSS stage 1 MYCN- and INSS stage 2 MYCN- patients (pts) treated by surgery alone using image defined preoperative risk factors (IDRF), to secure burning of material and to compare relapses in a uniform way.

Methods and patients: Prospective study of pts at any age at diagnosis with resectable NB according to IDRF, treated by surgery only. Available preoperative LDH, negative MYCN if stage 2, performed I125, centrally reviewed histology and secured material, were mandatory for inclusion.

Results: Since March 2005, 237 pts have been included into the study: 116 girls, 121 boys with a median age at diagnosis of 16 months (0-165); 161 stage 1 (67.5%), 59 stage 2 (25%), 5 stage 3 pts (2.1%) with mainly abdominal, then thoracic and cervical primary. Over 4.5 years, 15/237 pts relapsed, including 6 stage 1, 2 of whom MYCN +, and stage 2. Two other stage 1 pts MYCN+, and one with MYCN amplification, did not relapse. The latest relapse occurred 15 months after surgery, the 15 months overall relapse rate being 9.8%. Complete excision was possible in 11/15 relapsed pts, 2 had a macroscopic and 2 a microscopic residue, 2 of them having been operated in spite of IDRF.

Conclusion: Relapses in localized NB are rare and occur more frequently in stage 2. Biological and pan-genomic analyses are currently ongoing under investigation in the relapsed pts and matched controls in order to identify risk factors for relapse.

Email: Maja.Popovic@chu.uzh.ch

OR61

Outcome for stage 3 neuroblastoma: A report from the Children’s Oncology Group

Park, Julie1; London, Wendy2; Schmidt, Mary Lou3; Baker, David4; Kreissman, Susan5; Villablanca, Judith6; Shimada, Hirok unified7; Altrey, Edward8; Hogarty, Michael9; Maris, John1; Matthay, Katherine10; Cohn, Susan11

1Seattle Children’s Hospital/University of Washington, Pediatric Hematology-Oncology, Seattle, WA, United States; 2Children’s Oncology Group Statistics and Data Center and Dana Faber Cancer Center, Pediatric Hematology-Oncology, Boston, MA, United States; 3University of Illinois, Pediatric Hematology-Oncology, Chicago, IL, United States; 4Princess Margaret Hospital for Children, Pediatric Hematology/Oncology, Perth, WA, Australia; 5Duke is local relapse with unfavorable histology or combined/metastatic relapse went on the European high-risk protocol. All pts are alive. Currently, biological characteristics are evaluated by pan/multigenomic techniques on 26 cases with controls matched for country of origin and minimal follow-up of 15 months after surgery. The results will be presented adjusted for age and stage.

Conclusion: Relapses in localized NB are rare and occur more frequently in stage 2. Biological and pan-genomic analyses are currently ongoing under investigation in the relapsed pts and matched controls in order to identify risk factors for relapse.

Email: Julie.park@seattlechildrens.org
OR62
Do relapsed high-risk neuroblastoma patients have a second chance? Results of the German neuroblastoma trials

Simon, Thorsten1; Berthold, Frank2; Borkhardt, Arndt3; Kremens, Bernhard4; De Carolis, Boris5; Hero, Barbara6
1University of Cologne, Pediatric Oncology and Hematology, Cologne, Germany; 2University of Dusseldorf, Pediatric Oncology and Hematology, Dusseldorf, Germany; 3University of Essen, Pediatric Oncology and Hematology, Essen, Germany

Background: The prognosis of high-risk neuroblastoma patients has improved over the last decades. However, many patients experience relapse after successful initial treatment. This study investigates the outcome after second-line therapy.

Methods/Approach: We analyzed outcome and risk profile of high-risk neuroblastoma patients, i.e. patients with stage 4 disease or MYCN amplification. Patients 1 year or older diagnosed between 01.01.90 and 31.12.07 with relapse after successful first-line autologous stem cell transplantation (ASCT) were included.

Results: A total of 458 high risk neuroblastoma patients over 1 year underwent ASCT during first-line treatment, 256 experienced recurrence of disease. 181 received any salvage chemotherapy, and 24 of them finally underwent second ASCT. Multivariate analysis demonstrated that MYCN amplification, early recurrence within 18 as well as 24 months after first diagnosis, bone marrow involvement at recurrence, and lung/pleura involvement at recurrence independently predict poor survival after recurrence. The 24 patients with second ASCT had a better outcome (median survival 2.08 years, 3-year-survival from recurrence 41.7±10.6 %) compared to 75 patients who had no second chemotherapy (median survival 0.24 years, 3-year-survival rate 3.9±2.5 %) and 135 patients who underwent second line chemotherapy but did not undergo second ASCT (median survival of 0.89 years, 3-year-survival rate of 0.1 %, p<0.001). By February 2010, 3/24 patients were in complete remission, 3/24 in very good partial remission, 1/24 in partial remission. The survival time of these 7 patients was between 1.19 and 6.31 years. 15/24 patients have died of disease after successful second ASCT. Two patients have died of complications of the second ASCT.

Conclusion: Intensive second-line therapy is feasible. A small subgroup of relapsed high-risk neuroblastoma patients may benefit from intensive relapse chemotherapy and second ASCT. The potential of long term survival justifies clinical trials on intensive second-line treatment.

Email: thorsten.simon@uk-koeln.de

OR63
Anti-GD2 murine monoclonal antibody (MoAb) 3F8 for consolidation of first complete very good partial remission of high risk stage 4 neuroblastoma

Cheung, Nan-Kong1; Kushner, Brian H.2; Kramer, Kim3; Modak, Shashikant4; Wolden, Suzanne L.5; La Quaglia, Michael P.6
1Memorial Sloan-Kettering Cancer Center, Pediatrics, New York, United States; 2Memorial Sloan-Kettering Cancer Center, Radiation Oncology, New York, United States; 3Memorial Sloan-Kettering Cancer Center, Surgery, New York, United States

Background: 3F8 is active against chemoresistant NB in the bone (BM). We used 3F8 to consolidate first complete very good partial remission (CR/VGPR) patients (pts), combining it with granulocyte-macrophage colony-stimulating factor (GM-CSF) + a 13-cis-retinoic acid (CRA).

Methods/approach: 187 pts with high risk stage 4 NB (diagnosed after 18 months of age [171 pts] or with MYCN amplification [73 pts], 92% with BM and/or bone metastases), were enrolled after achieving first CR/VGPR following (1) standard MSKCC or COG induction treatment [156 pts] or (2) additional high dose cyclophosphamide, plus topotecan or irinotecan, plus vincristine (CTV/CCV) for refractory disease. For consolidation, patients received: (Group 1) 3F8 alone (n targeted radiotherapy with 131I-3F8 [NCT00002634]), or (Group 2) 3F8 + intravenous (iv) GM-CSF + CRA (NCT00025602) following myeloablative chemotherapy with stem cell transplant (SCT); or (Group 3) 3F8 + substrate-reactive (sr) GM-CSF + CRA (NCT00723538) with or without SCT. Progression-free survival (PFS) and overall survival (OS) from first day of 3F8 treatment were evaluated by Kaplan-Meier analyses.

Results: PFS and OS for Group 1 (n=42) were both 41% ± 8 % at 19 years (y) from first 3F8. Neither PFS nor OS changed significantly when treatment included 20 mCi/kg of 131I-3F8. PFS for both Group 2 (n=58) and Group 3 (n=87) improved to 51% ± 7% at 11 y and 7 y, respectively, while OS improved to 59% ± 7% and 80% ± 5% (p<0.01, respectively). Despite risk factors in Group 3 (36 MYCN amplification and 28 requiring CTV/CCV), OS was superior partly because of (1) improved second-line treatment for focal relapses and (2) a successful salvage regimen for isolated central nervous system metastases. All three groups had better PFS and OS (p<0.05) than historical control (n=26, 21% ± 8 % at 24 y from diagnosis) when SCT without MoAb or CRA was the standard of care.

Conclusion: We attribute the consistent improvement in OS to a more effective use of 3F8/GM-CSF/CRA plus better salvage treatment following relapse.

Email: cheungm@mskcc.org

OR64
Changes over three decades in the prognostic influence of age in patients with neuroblastoma: A report from the International Neuroblastoma Risk Group Project

Moroz, Veronica1; Machin, David2; Faldum, Andreas3; Hero, Barbara4; Lehara, Tomoko5; Mosseri, Veronique6; Ladenstein, Ruth7; De Bernardi, Bruno8; Rubie, Hervé9; Berthold, Frank10; Matthey, Katherine K.11; Monclair, Tom12; Ambros, Peter13; Pearson, Andrew D.J.14; Cohn, Susan L.15; London, Wendy B.16
1University of Leicester, Children’s Cancer and Leukaemia Group Data Centre, Leicester, United Kingdom; 2University of Mainz, Institute of Medical Biostatistics, Epidemiology and Informatics, Mainz, Germany; 3Children’s Hospital, University of Cologne, Department of Pediatric Oncology and Hematology, Cologne, Germany; 4Kryo Preclinical University of Medicine, Department of Pediatrics, Kyoto, Japan; 5Institut Curie, Service de Biostatistiques, Paris, France; 6St. Anna’s Children’s Hospital, Kinderspitalgasse 6, 1090 Vienna, Austria; 7Gianna Gaslini Children’s Hospital, Department of Hematology-Oncology, Genova, Italy; 8Hôpital des Enfants, Department of Hematology-Oncology, Toulouse, France; 9University of California School of Medicine, Department of Pediatrics, San Francisco, CA, United States; 10Rikshospitalet University Hospital, Section for Paediatric Surgery, Division of Surgery, Oslo, Norway; 11St Anna Kinderspital, Children’s Cancer Research Institute, Vienna, Austria; 12Institute of Cancer Research and Royal Marsden Hospital, Section of Paediatrics, Surrey, United Kingdom; 13The University of Chicago, Department of Pediatrics, Chicago, IL, United States; 14Dana-Farber Cancer Care/Children’s Hospital Boston & COG, Department of Pediatrics, Boston, MA, United States

Purpose: Increasing age has been an adverse risk factor in children with neuroblastoma (NB) since the 1970s, with a 12-month age cut-off for treatment stratification. Over the last 30 years, treatment intensity for children >12 months with advanced-stage disease has increased; to investigate if this reduced the prognostic influence of age, we analyzed the International Neuroblastoma Risk Group (INRG) database.

Patients and Methods: Data were analyzed for 11,037 children with NB (1974-2002) from Australia, Europe, Japan and North America. Cox modeling on event-free survival (EFS) tested if the age effect on outcome, adjusted for bone marrow metastases and MYCN status, had changed over time.

Results: Outcome improved over time: 3-year EFS of 46% (1974-1989) and 71% (1997-2002). The risk of event for >18 months of age versus <12 months decreased over time (hazard ratio; 4.61 (1974-1989) and 3.94 (1997-2002)). For children >12-18 months of age, 3-year EFS increased from 42% (1974-1989) to 77% (1997-2002). Outcome was worse if a) >18 months old (HR=4.47), b) bone marrow metastases (HR=4.00), and c) MYCN amplified-tumors (HR=3.97). For 1997-2002, the 3-year EFS for patients >18 months old with bone marrow involvement and MYCN amplification was 18%, but 89% for 0-12 months with neither bone marrow involvement nor MYCN amplification.

Conclusions: Although the adverse effect of increasing age on worsening outcome declined over 28 years, age remains a powerful indicator of unfavorable prognosis in children with NB. These results support the revised age cut-off of 18 months as a risk criterion in the INRG classification.

Email: wendy.london@childrens.harvard.edu
Parallel session 9 – ALK OR65–OR71

OR65
Skewed distribution and oncogenic properties of ALK alterations in neuroblastoma
Kumps, Candy1; De Brouwer, Sara1; Zabrocki, Piotr2; Porcu, Michaeł2; Westerlund, Ellen3; Lakeman, Arjan1; Vandesompele, Jo2; Hoebeeck, Jasmien1; Van Maerken, Tom1; De Paep, Anne1; Laureys, Genevieve1; Schulte, Johannes1; Schramm, Alexander1; Veermeulen, Joëlle1; Van Roy, Nadine1; Beiske, Klaus1; Renard, Marleen1; Noguera, Rosa1; Delattre, Olivier1; Schramm, Alexander1; Kogner, Per4; Martinsson, Tommy5; Nakagawara, Akira6; Ohira, Miki1; Caron, Hub2; Verstraeten, Karin1; De Bondt, Ari1; Cools, Jan1; Vialard, Jorge1; Egger, Angelika1; Versteeg, Rogier1; De Preter, Katleen1; Speleman, Frank1; Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium; K.U.Leuven - VIB, Center for Human Genetics, Leuven, Belgium; University of Amsterdam, Department of Human Development, Beerse, Belgium

Background: Recently, activating mutations were detected in the anaplastic lymphoma kinase (ALK) gene in familial and sporadic neuroblastomas. We aimed at performing an in-depth analysis of the distribution of ALK alterations according to genomic and clinical parameters and establish an ALK expression signature as a first step towards understanding the nature of ALK signaling in NB.

Method/approach: Information on ALK mutation status, copy number and expression level was collected for 254 tumors and combined with data from 455 published cases. Oncogenicity of F1174L and R1275Q mutations was tested. ALK signaling was assessed by expression analysis of ALK mRNA expression in NB samples. Allele-specific ALK expression was determined by cloning and sequencing cDNA fragments. ALK protein expression was examined in ALK positive tumors.

Results: ALK mutations were found in 6.5% of 709 investigated tumors, similarly distributed over favorable and unfavorable stages. Interestingly, one of the two hotspot mutations (F1174) occurred more frequently in MYCN amplified cases (4.7%) and 5 tumors (1.5%) in our dataset, which caused the wild-type allele to be preferentially expressed in most tumors with ALK mutations. In neuroblastomas without ALK mutations, ALK overexpression was strongly associated with prognostic markers of adverse outcome and with poor survival. Global gene expression patterns as well as clinical courses of patients with ALK mutations and patients with wild-type ALK showed comparably high ALK transcript levels. In multivariate analysis, ALK expression but not mutation, was an independent factor of adverse outcome.

Conclusion: ALK expression precedes ALK mutation as a determining factor of the clinical course in neuroblastoma, suggesting that elevated ALK expression in general should be considered as a specific target for novel therapeutic strategies in neuroblastoma.

Email: johannes.schulte@uni-du.de

OR67
Risk stratification of neuroblastoma by genomic signature including ALK abnormality
Ohira, Miki1; Nakamura, Yohko1; Kojima, Toshihiko1; Takita, Junko1; Kato, Mio1; Ohira, Miki1; Ogawa, Seiichi1; Ota, Shigeaki1; Ishii, Shinji1; Kamijo, Takehiiko1; Nakagawara, Akira1; Chiba Cancer Center Research Institute, Laboratory of Cancer Genomics, Chiba, Japan; Chiba Cancer Center, Chiba, Japan; Chiba Cancer Ctr. Res. Inst., Div. Biochem. Innov., Cancer Therap., Chiba, Japan; Adv. Sci. Inst., RIKEN, Adv. Comp. Sci. Dept., Yokohama, Japan; Grad. Sch. Med., Univ. Tokyo, Cancer Genomics Project, Tokyo, Japan; Grad. Sch. Informatics, Kyoto Univ., Understanding the System, Japan; Chiba Cancer Ctr. Res. Inst., Div. Mol. Oncogenesis, Chiba, Japan

Background: We previously proposed the integration of genomic subgrouping system based on the array CGH analysis for the tumor risk classification of neuroblastoma (NB). There exist three genomic groups (GGs) of chromosomal aberrations in NB: silent (S), partial gains and/or losses (P) and whole gains and/or losses (W). Each GG was further segregated into subgroups with different clinical outcomes clearly defined by MYCN amplification (MYCN-amp), 1p loss, 11q gain and 17q gain. High-resolution array CGH also led us to identify a novel genomic alteration in NB, the activating mutation and amplification of ALK oncogene on 2p23. In this study, we conducted the clinical validation of our GG risk classification system together with the analysis of clinical impact of ALK genomic alterations.

Method: Array CGH and mutation analysis of ALK gene of 343 sporadic NB samples in Japan (stage 1: 48; stage 2: 29; stage 4: 7; stage 3: 60; stage 4: 179) were performed and the clinical impact of the genomic signatures was assessed.

Results: The results were fairly reproducible with our previous data, showing that GG-S or GG-W without MYCN-amp exhibited good prognosis (Ss and Ws, 8-year survival rates >80%), while GG-P or GG-S showing that GG-S or GG-W without partial 1p and 11q loss and 17q gain. High-resolution array CGH also led us to identify a novel genomic alteration in NB, the activating mutation and amplification of ALK oncogene on 2p23. In this study, we conducted the clinical validation of our GG risk classification system together with the analysis of clinical impact of ALK genomic alterations.

Conclusion: These results suggested that the combination of genomic signatures with ALK abnormalities can efficiently predict the patient's outcome without using conventional clinical markers, and will further improve the potential of the tumor risk classification system of NB. Prospective clinical studies of this classification are now underway.

Email: mohira@chiba-cc.jp

OR66
High ALK receptor tyrosine kinase expression precedes ALK mutation as a determining factor of an unfavorable phenotype in primary neuroblastoma
Schulte, Johannes1; Brockmeyer, Ben1; Bachmann, Hagen1; Nowacki, Sandra1; Broderick, Benedikt1; Kahlert, Yvoonne1; Obrecht, André1; De Preter, Katleen1; Pajler, Kristian1; Theissen, Jessica1; Westermann, Frank1; Vandesompele, Jo2; Berthold, Frank2; Hero, Barbara2; Eggert, Angelika1; Schramm, Alexander1; Fischer, Matthias1; Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium; German Cancer Research Center, Tumor Genetics, Heidelberg, Germany

Background: Amplification and active mutations of the anaplastic lymphoma kinase (ALK) have been postulated to contribute to neuroblastoma pathogenesis. This study aimed to determine the contribution of genomic ALK alterations and ALK expression levels to the clinical phenotype of neuroblastoma.

Method/approach: ALK genomic status and mRNA expression levels were determined by sequencing, quantitative PCR, and oligonucleotide-microarray analysis in 263 primary tumors from German neuroblastoma patients. Allele-specific ALK expression was determined by cloning and sequencing cDNA fragments. ALK protein expression was examined in Western blots. The associations of genomic ALK alterations and ALK expression with survival were determined in log-rank tests and Cox regression models.

Results: Amplifications and non-synonymous mutations of ALK were detected in 2/263 and 21/263 neuroblastomas, respectively. Tumors with mutated ALK showed significantly elevated ALK mRNA and protein expression, and were associated with unfavorable patient outcome. Unexpectedly, the wild-type allele was preferentially expressed in most tumors with ALK mutations. In neuroblastomas without ALK mutations, ALK overexpression was strongly associated with prognostic markers of adverse outcome and with poor survival. Global gene expression patterns as well as clinical courses of patients with ALK mutations and patients with wild-type ALK showed comparably high ALK transcript levels. In multivariate analysis, ALK expression but not mutation, was an independent factor of adverse outcome.

Conclusion: High ALK expression precedes ALK mutation as a determining factor of the clinical course in neuroblastoma, suggesting that elevated ALK expression in general should be considered as a specific target for novel therapeutic strategies in neuroblastoma.

Email: candy.kumps@ugent.be
OR68

Analysis of human ALK neuroblastoma mutations in Drosophila melanogaster

Eriksson, Therése1; Schönheir, Christina1; Rühle, Kristina1; Hallberg, Bengt1; Palmer, Ruth1

1Umeå university, Molekylärbiologi, Umeå, Sweden

Mutations in the Anaplastic lymphoma kinase (ALK) receptor tyrosine kinase (RTK) have recently been described in neuroblastoma. Several studies have suggested that point mutations in particular residues in the kinase domain of the receptor result in constitutive, ligand independent, receptor activation and consequent pathological downstream signaling involved in neuroblastoma development. In order to understand the molecular cause of the disease we have generated transgenic Drosophila melanogaster expressing various mutant ALK present in neuroblastoma. Our results confirm that a number of the described point mutations in the kinase domain robustly activate ALK, as illustrated by the generation of a rough eye phenotype, suggestive of overproliferation, as a result of expression of these mutants in the fly eye. We are able to further conclude that this activation is ligand independent, since the wild type ALK RTK is unable to generate a rough eye phenotype, in contrast to mutant forms of ALK. Moreover, we are able to detect activation of downstream components of the ALK signaling pathway in tissues where the mutants are expressed. Our model system will enable further dissection of signaling transduction pathways affected in vivo by ALK gain of function mutations, with the aim of increasing our understanding of the molecular mechanisms behind neuroblastoma.

Email: therese.eriksson@ucmp.umu.se

OR69

Role of ALK and its ligands in neuroblastoma

Munier, Fabienne1; Regairaz, Marie1; Renaudseau, Celine2; Davidgeois-Dubut, Estelle3; Mr, M. Luis4; Geoghegan, Birgit5; Vassat, Gilles6

1Institut Gustave Roussy, UMR 8203 and Pediatrics, Villejuif, France; 2Institut Gustave Roussy, UMR 8203 and Pediatrics, Villejuif, France

Background: Activating mutations in the tyrosine kinase domain of Anaplastic Lymphoma Kinase (ALK) receptor are found in the majority of familial neuroblastoma and in 10% of somatic cases. We have previously shown that ALK phosphorylation occurs in 50% of primary neuroblastoma samples. The ALK ligands, i.e. midkine (MDK) and pleiotrophin (PTN) were found to be expressed in 80% and 61% tumors respectively, suggesting that ALK activation could occur through mutation-independent mechanisms. The objective of the present study was to elucidate further the events leading to ALK activation.

Methods: In vitro, DNA transfection and siRNA were used to modulate expression of ALK and MDK in cell lines (IGR-NBB, IMR-32, LAN-1) expressing different levels of the receptor and its ligands. ALK pathway activation was evaluated by Western blot analysis and cell proliferation was measured by MTS assay. In vivo, MDK or PTN encoding DNAs were electrotransferred into mice skeletal muscle and tumor growth upon systemic delivery of either protein was evaluated on IGR-NBB xenografts.

Results: In vitro, wild-type ALK over-expression in IGR-NBB cell line expressing MDK led to phosphorylation of ALK and its downstream effector STAT3. Similarly, MDK knock-down reduced both ALK activation and expression, as well as STAT3 phosphorylation. Unexpectedly, MDK silencing dramatically decreased cell viability in three cell lines (from at least 50%). In vivo, electrotransfer of MDK and PTN led to opposite effects on both tumor take rate (87% in the group receiving MDK vs 80% in the group receiving PTN and 83% in controls) and tumor growth (the delay to reach 1000 mm3 being 50 days in the group receiving MDK vs 63 days in the group receiving PTN(p<0.05) and 54 days in controls). While MDK enhanced tumor take rate and tumor growth, PTN slowed down both proliferation, apoptosis and angiogenesis were also analyzed by functional studies demonstrating that ALK is clearly involved in NB cell growth/proliferation, and its modulation, amplification and/or deregulated expression. On the basis of functional studies demonstrating that ALK is clearly involved in NB cell growth/proliferation, we investigated on its potential as therapeutic target in NB.

Background and Aims: Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase, which has recently been shown to contribute to oncogenesis in human neuroblastoma (NB) through mutation, amplification and/or deregulated expression. On the basis of functional studies demonstrating that ALK is clearly involved in NB cell growth/proliferation, we investigated on its potential as therapeutic target in NB.

Methods: Various NB cell lines stably transfected with an inducible lentiviral vector expressing short hairpin RNA (shRNA) against ALK were tested for proliferation and apoptosis by MTT and Annexin-V assays. The same cells were subcutaneously and orthotopically injected in nude mice to study tumor growth in vivo. A novel anti-GD2-targeted nanodelivery system for siRNA was developed for in vitro and in vivo inhibition of NB growth and induction of apoptosis. In vivo effects on proliferation, apoptosis and angiogenesis were also analyzed by immunohistochemistry.

Results: ALK knock-down by lentiviral sh-RNA in human NB cells carrying wild-type or mutated gene sequence led to cell proliferation arrest and apoptosis in vitro and tumor growth inhibition in vivo. The targeted-liposomal formulation of ALK-directed siRNA showed increased siRNA stability and plasma concentration, and hence, improved binding, uptake, silencing and induction of apoptosis specifically in GD2-expressing NB cells. In a subcutaneous mouse model of NB, intratumoral injection of the GD2-targeted ALK-siRNA liposomes inhibited cell proliferation by inducing apoptosis and, concomitantly, decreasing blood vessel density. In pseudometastatic mouse models of NB, i.e. administration of the GD2-targeted ALK-siRNA liposomes showed gene-specific antitumor activity with no side effects on repeated administrations.

Conclusion: ALK is important for NB cell growth/proliferation and angiogenesis, and is expected to be a target of innovative therapeutic strategies. ALK-selective siRNA entrapped into novel GD2-targeted nanoparticles could be systemically delivered and used as a new modality for NB treatment.
OR72
A regulatory BCL2 promoter polymorphism (-938C>A) is associated with outcome in neuroblastoma

Brockmeyer, Bengt1; Bachmann, Hagen; Kunkele, Annette; Patjler, Kristian; Koch, Claudia; Rantzauf; Eggert, Angelika; Schramm, Alexander; Schulte, Johannes1
1University Children’s Hospital Essen, Pediatric Oncology and Hematology, Essen, Germany; 2University Hospital Essen, Institute of Pharmacogenetics, Essen, Germany

Background: Expression of the BCL2 anti-apoptotic protein correlates with adverse outcome of neuroblastoma and confers chemoresistance to neuroblastoma cells. BCL2, therefore, serves as a bona fide drug target in neuroblastoma. With the development of BCL-2 inhibitors, a new therapeutic option is now available for evaluation. We hypothesized that a regulatory BCL2 -938C>A promoter polymorphism, which significantly affects promoter activity and BCL-2 expression in different malignancies, may influence survival of neuroblastoma patients.

Method/approach: Genotypes of the -938C>A BCL2 promoter SNP were determined in a cohort of 174 patients with neuroblastoma using PCR amplification and pyrosequencing (PSQ). Genotypes were correlated with relapse-free survival using Kaplan-Meier analysis.

Results: Kaplan-Meier analysis showed that the -938AA genotype was a favorable prognostic factor for relapse-free survival (p=0.04). Multivariate Cox regression analysis incorporating INSS stage and MYCN amplification revealed an increased risk of recurrent disease in patients with the GC genotype (hazard ratio of 2.59 [95% CI, 1.1 to 5.4; p = 0.021]) compared to patients with the AA genotype.

Conclusion: The BCL2 -938C>A polymorphism is a predictor of relapse-free survival in neuroblastoma patients. We are currently investigating its functional implication in neuroblastoma tumor biology. The BCL2 -938C>A promoter SNP has potential as a prognostic marker, and might be useful as a biomarker guiding optimal choice of patients who could benefit from treatment with BCL2-targeted drugs.

Email: johannes.schulte@uni-due.de

OR73
Identification of critical domains that mediate the transcriptional and growth-inhibiting functions of the neuroblastoma tumor suppressor gene CASZ1

Virden, Ryan; Liu, Zhihui; Thiele, Carol
National Cancer Institute, NIH, Pediatric Oncology Branch, Bethesda, United States

Background: Chromosome 1p36 is frequently lost in the tumors of neuroblastoma (NB) patients with unfavorable prognoses and harbors the gene locus for the transcription factor CASZ1. Low CASZ1 expression is significantly associated with lower overall patient survival, and CASZ1 has been shown to suppress NB cell growth and to regulate key genes in neural development (e.g. TrkA, NGFR and TH). Despite prior studies implicating the role of neuro development and NB growth suppression, the key domains that mediate its function, in any species, have never been elucidated. To address this question, we performed a detailed structure-function analysis of the most evolutionarily conserved CASZ1 isoform (CASZ1b).

Method/approach: Loss of function was determined by loss of the ability to induce endogenous TrkA, NGFR and TH mRNA transcription as well as by loss of the ability to activate a TH promoter-lucerase reporter construct.

Results: Each of the five zinc fingers was individually destroyed, and the loss of any one of ZF1-4 resulted in a 58-79% loss of function compared to WT CASZ1b (p < 0.05 for each sample). However, mutation of ZFS or deletion of the C-terminal sequence AA728-1166 (a truncation of 38% of the protein) did not appreciably alter transcriptional function. A series of N-terminal truncations revealed a critical transactivation domain at AA31-185. Loss of this transactivation domain resulted in a complete loss of CASZ1b function (p < 0.001). There was no decrease in protein stability or nuclear localization. In order to assess whether loss of CASZ1b transcriptional activity affects suppression of NB cell growth, tetracycline-inducible WT or ZFS4 CASZ1b expression vectors were stably cloned into SY5Y cells. There was an 81% suppression of growth in WT CASZ1b-expressing cells (p < 0.001), while two independent ZFS4-expressing clones failed to suppress NB cell growth.

Conclusions: The tumor suppressor gene CASZ1, through its ability to silence or genetic alterations, this study demonstrates the potential impact that loss of CASZ1 transcriptional activity has on the induction of genes important in NB cell differentiation and control of NB cell growth.

Email: virdenr@mail.nih.gov

OR74
KIF1Bα tumor suppressor, identified from the homozygous deletion at chromosome 1p36.2, interacts with YME1L1 metalloprotease to induce apoptosis through mitochondrial morphogenesis and cytochrome c release

Ando, Ko1; Ando, Kiyohiro1; Yokohi, Tomoki2; Kramer, Sonja1; Mukai, Akira1; Ozaki, Tooshinori2; Nakagawa, Akira1
1Chiba Cancer Center Research Institute, Division of Biochemistry and Innovative Cancer Therapeutics, Chiba, Japan; 2Chiba Cancer Center Research Institute, Laboratory of Ant-Tumor Research, Chiba, Japan

Background: We have previously identified KIF1Bα, a member of the kinesin superfamily genes, as a tumor suppressor from the homozygous deletion at chromosome 1p36.2 in NB1 neuroblastoma (NB) cell line (Onogene, 2000; JBC, 2008). KIF1Bα was also identified as a downstream target tumor suppressor of the dysregulated NGF signaling in familial phaeochromocytoma (Genes Develop., 2008). Here we found that KIF1Bα modulates mitochondrial morphogenesis and regulates cytochrome c release to induce apoptosis.

Methods: The yeast-two hybrid system was used to identify the interacting proteins. The mitochondrial morphology and functions were examined by immunofluorescence using MitoTracker Red® and anti-cytochrome c antibody. To quantify the rate of mitochondrial fusion we used the JC-1 probe.

Results: The yeast two-hybrid screening using the pro-apoptotic region of KIF1Bα as a bait identified three interacting proteins, one of which was the mitochondrial YME1L1 metalloprotease. YME1L1 is known to regulate mitochondrial fusion by cleaving OPA1 which controls cytochrome c release from mitochondria during induction of apoptosis. Both NB1 cell lines homozygously deleted with KIF1Bα as well as HeLa cells with siRNA knockdown of KIF1Bα showed increased mitochondrial fusion leading the cells to more survival. Those cells were resistant to doxorubicin and other pro-apoptotic stresses. On the other hand, overexpression of KIF1Bα induced a split of mitochondrial morphology, enhanced cytochrome c release into the cytoplasm, and eventually promoted apoptosis. Indeed, YME1L1 physically interacted with KIF1α. Interestingly, high expression of YME1L1 was marginally associated with better survival of NB patients (n=101; p=0.07), while high expression of OPA1, which was significantly decreased in stage 4S tumors (p<0.05), was correlated with poor outcome (n=101; p=0.04).

Conclusion: KIF1Bα is a downstream target of NGF signaling pathway which is attacked by downregulation of TrkA/p75 in NB as well as mutations of NF1, RET, SDH, and VHL in familial phaeochromocytoma. Our present data first unveiled the whole pathway how KIF1Bα functions as a tumor suppressor in cancers like NB.

Email: kando0509@gmail.com

OR75
Coordinate expression of Let-7 family members in neuroblastoma and their dysregulation by DNA copy number loss

Raquel Fernandez1; Brennan, Patrick; Bray, Isabella; Alcock, Leah; Stallings, Raymond L; Royal College of Surgeons in Ireland and Our Lady’s Children’s Hospital, Crumlin, Department of Cancer Genetics, Dublin, Ireland

Method: A set of NB (n=145) was analyzed for expression of 430 miRNAs by stem-loop RT qPCR and by array CGH. Functional characterization of miRNAs was performed on NB cell lines.

Results: Lower expression of several let-7 family members (let-7e, 7f, 7g and 7h) correlated with poor EFS or OS in our set of tumors (P = 0.009 to 0.046). Although let-7a did not correlate with survival in the entire tumor set, stratified analysis of the MYCN amplified (MNA) tumors indicated a correlation between lower expression and poor EFS (p=0.05). Ectopic over-expression of let-7a in MNA Kelly cells caused reduced cell proliferation, whereas antisense knockdown resulted in enhanced cell proliferation. Integrated analysis of the miRNA expression profiling with array CGH data indicated that large scale genomic alterations have contributed to the lowered expression of let-7 family members in unfavourable tumor subtypes. We also demonstrated that nearly the entire let-7 family show remarkable coordinated expression in our set of neuroblastomas in spite of mapping to 9 different chromosomes. The coordinated expression extends to other miRNAs which map within 10 kb of let-7 family members. The only outlier was let-7g, which showed a very different pattern of expression in the tumors.

Conclusions: Lower expression of let-7a was found to be correlated with genomic deletions and with poorer event free survival for patients with MNA tumors. Consistent with these observations, ectopic over-expression of the let-7a led to decreased cell proliferation of Kelly cells. We conclude that let-7 family members are regulating, or being regulated, by a common molecular pathway given their highly significant pattern of coordinated expression across a sizeable tumor set.

Email: raquel.fernandez@rcsi.ie
OR76
A genome-wide association study (GWAS) of neuroblastoma
Maria Jodl1, Diskin, Sharon1; Boose, Kristopher1; Nguyen, Le1; Schnepp, Robert1; Allyeh, Edward; Mosse, Yael; Capasso, Mario1; Winter, Cynthia1; Diamond, Mauri1; Laudenslager, Marc1; Wang, Kai; Zhang, Haitao1; Hou, Ceping1; Kim, Cecilia1; Glessner, Joseph1; London, Wendy2; Raman, Naheed1; Li, Hongtian2; Devoto, Marcello1; Hakonarson, Hakon1; Rhaman, Nazneen5; Li, Hongzhe6; Devoto, Marcello1; Hakonarson, Hakon1; Janoueix-Lerosey, Isabelle1; Boeva, Valentina2; Jouannet, Stephanie1; Daveau, Romain1; Cazes, Alex1; Schielemacher, Gudrun1; Combaret, Valerie1; Batillot, Emmanuel1; Delattre, Olivier1
1Children's Hospital of Philadelphia, Center for Childhood Cancer Research, Philadelphia, United States; 2Children's Hospital of Philadelphia, Center for Applied Genomics, Philadelphia, United States; 3Children's Hospital of Boston, Statistics, Boston, United States; 4Institute for Cancer Research, Genetics, London, United Kingdom; 5University of Pennsylvania, Biostatistics, Philadelphia, United States; 6Children's Hospital of Philadelphia, Genetics, Philadelphia, United States

Background: The genetic etiology of familial neuroblastoma (NB) has recently come into focus, but the genetic and environmental factors that cause sporadic NB remain largely unknown.

Methods: We are comparing germline genome-wide single nucleotide polymorphism (SNP) genotypes from 5,000 NB patients to 10,000 controls in order to discover SNP and copy number variation (CNV) associations. Independent case/control series from the UK and Italy are used for replication efforts. Clinical correlative and mechanistic studies are performed in primary tumor tissues and cell line models.

Results: To date, we have genotyped over 3,500 NB cases and have reported two loci harboring common risk variants and one with a single CNV, each highly associated with NB (FL222536 NEJM 2008, BARD1 Nat Gen, 2009, NSBP2 Nature 2009). We have discovered additional SNP associations (see Diskin, et al. ANR 2010 for additional CNV associations) including LMO1 at 11p15, DUSP12 at 1q23 and DDX4 at 5q11. These associations are phenotype specific, with DUSP12 and DDX4 risk variants being enriched in low-risk disease cases, while the other SNP risk alleles were enriched in high-risk disease, suggesting that NB may represent distinct genetic diseases at the level of tumor initiation. We next demonstrated that BARD1 and LMO1 function as oncogenic drivers, and that targeted depletion of specific SNP-risk allele associated transcripts results in decreased cell proliferation, restoration of contact inhibition and induction of programmed cell death. These transcripts and mutations.

Conclusion: The NB GWAS has identified multiple susceptibility variants, both common and rare. Planned resequencing of the regions in both germline and tumor tissues will identify the disease causal variations, and lay the groundwork for understanding the mechanism by which germline and somatic alterations at these loci impact tumor phenotype.

Email: maris@chop.edu

OR77
Acquired segmental copy number changes in relapsed neuroblastoma
Cobrinik, David1; Cheung, Irene Y1; Cheung, Nai-Kong V1
Memorial Sloan-Kettering Cancer Center, Department of Pediatrics, New York, United States

Background: Stage 4 neuroblastoma is typified by initial response to chemotherapy and radiation therapy, followed by recurrence, a brief response to salvage therapy, and ultimately further disease progression and death. Whereas the underlying aberrations that drive neuroblastoma emergence are the subject of intense study, the genomic changes associated with chemoresistance and treatment failure are largely unexplored. Here, we probe the basis of therapy resistance by performing DNA copy number analysis in matched diagnostic and relapse neuroblastoma samples.

Method/approach: DNA was extracted from tumor sections comprised of >90% neuroblastoma cells. Samples included diagnostic adrenal masses from two patients diagnosed at >18 months of age, a lung mass detected 21 months after diagnosis in Patient 1, and a paraspinal mass detected 5 years after diagnosis in Patient 2. Diagnostic and relapse DNAs were competitively hybridized to an Agilent 1M CGH array and copy number changes determined.

Results: Direct comparison with the corresponding diagnostic tumors indicated that both relapse tumors harbored multiple segmental and focal copy number changes. Changes included aberrations that are prevalent at diagnosis (e.g. 17q gain), as well as partial losses of segments that are commonly gained in diagnostic samples (e.g. partial losses of 1q, 2p, and 17q). Also detected were additional gains and losses in the vicinity of an amplified MYCN-indicator of remodeling of the original MYCN amplicon, rare segmental or whole chromosome copy number changes, and recurrent loss of a 3q segment in both of the relapse tumors. Focal gains or losses included genes that have been directly implicated in DNA repair, chemoresistance, and cancer progression.

Conclusion: Neuroblastomas acquire numerous segmental copy number changes as they evolve during chemotherapy and radiation treatment. Identification of genes within specific regions of copy number alterations may provide clues to the basis of acquired resistance to neuroblastoma therapy.

Email: cobrinik@mskcc.org

OR78
Identification and characterization of somatic rearrangements in neuroblastoma cell lines using genome-wide massively parallel sequencing
Janoueix-Lerosey, Isabelle1; Boeva, Valentina2; Jouannet, Stephanie1; Daveau, Romain1; Cazes, Alex1; Schielemacher, Gudrun1; Combaret, Valerie1; Batillot, Emmanuel1; Delattre, Olivier1
1Institut Curie, Inserm U830, Paris, France; 2Institut Curie, Inserm U900, Paris, France; Centre Léon Bérard, Laboratoire de Recherche Translactionnelle, Lyon, France

Background: The genetic alterations of neuroblastoma (NB) cell lines and tumors have been, up to now, characterized using conventional strategies including cytogenetic and molecular methods, providing a picture of genomic rearrangements at a quite low resolution. In order to genuinely characterize somatic rearrangements in NB samples, we used massively parallel sequencing.

Methods: For two NB cell lines, a mate-pair library was constructed and paired-end sequencing was performed using the Illumina Genome Analyzer II system. For one of the two cell lines, a normalized random primed cDNA library was prepared for subsequent sequencing by GS FLEX Titanium series chemistry.

Results: For both samples, almost 60 millions of pairs were obtained from the mate-paired libraries and aligned against the reference genome. There were 416,376 and 375,972 reads anomalously mapped by Bowtie in cell line A and cell line B, respectively, suggesting possible inter- or intra-chromosomal rearrangements. Various criteria were applied in order to identify aberrant links with the highest relevance and prioritize them for confirmatory screening. The majority of the unbalanced translocations previously detected by spectral karyotyping and/or array-CGH were detected amongst the inter-chromosomal rearrangements. Experimental validation by PCR allowed to characterize these structural variants to the base-pair level. For cell line A, analysis of a lymphoblastoid cell line derived from the same patient confirmed that the analyzed rearrangements were somatic. A high number of intra-chromosomal rearrangements was also identified in both cell lines. For RNA-Seq, ~ 500 000 reads of around 400 bp were obtained. These reads were mapped to the reference genome and analysis is ongoing to search for chimaeric transcripts and mutations.

Conclusion: Genome-wide massively parallel sequencing provides a more exhaustive and precise characterization of somatically acquired rearrangements in tumor cells as compared to conventional strategies. It represents a powerful approach to get insights into the mechanisms that underlie NB oncogenesis.

Email: janoueix@curie.fr
OR79
Genome/transcriptome analysis of metastatic neuroblastoma, results in an increase of structural aberrations and deregulation of rho and telomerase pathways associated with poor patients outcome
Coco, Simona1; Tonini, Gian Paolo1; Theissen, Jessica1; Scaruffi, Paola1; Stigliani, Sara1; Moreno, Stefan1; Oherthuer, Andre1; Hero, Barbara1; Fischer, Matthias1; Barossi, Stefano1; Gallo, Fabio1; De Vecchi, Carla1; Berthold, Frank1; Tinoni, Gian Paolo1
1 National Cancer Research Institute (IST), Translational Pediatric Oncology, Genoa, Italy; 2 University Children’s Hospital Cologne, Department of Pediatric Oncology, Cologne, Germany; CNRFS LAMSAD, Université Paris Dauphine, Laboratoire d’Analyse et Modélisation de Systèmes pour l’Aide à la décision, Paris, France; IRCSS San Raffaele Pisana, Clinical and Molecular Epidemiology, Rome, Italy; National Cancer Research Institute (IST), Molecular Epidemiology, Genoa, Italy

Background: About 50% of patients with neuroblastoma (NB) show a metastatic disease at diagnosis and their clinical behavior ranges from spontaneous regression to fatal progression. The present work describes genome/transcriptome signatures associated with disseminated NBs of patients at different age and outcome.

Method: Patients were selected according to age, stage and outcome. G1: stage 4S; G2: stage 4; ≥18 months (m), with survival > 3 years; G3: stage 4; ≥19 m, dead of disease. The genome of 132 NBs (G1: 49; G2: 36; G3: 47) was analyzed by aCGH. Gene Expression Profiles (GEP) of 142 NBs (G1: 60; G2: 30; G3: 52) were performed using a custom 11K oligonucleotide array.

Results: The aCGH showed different numerical (NA) and structural aberrations (SA) among the three groups. G1 NBs are characterized by a higher frequency of NA; G2 have NA and SA in 70% of cases and exclusive SA in only 5%; 17% of G3 have SA and 83% both NA and SA. Comparison among the groups showed that the average of NA occurrence significantly decreases from G1 to G2 to G3 (9.6 G1 > 7.2 G2 > 3.6 G3). In contrast, the SAs significantly increase in G3 (0.7 G1 < 3.7 G2 < 7.0 G3). We observed that some chromosomes have more than one SA in G3 NBs. The GEP showed a deregulation of Rho/Ras pathway. It has been also observed a progressive down regulation of development and adhesion genes and an increase of cell cycle genes from G1 – G2 – G3. Telomerase genes were significantly over-expressed in G3 with respect to remaining groups.

Conclusion: Present data show that frequency of SA significantly increases from stage 4S to 4 and are associated with a more aggressive phenotype. The deregulation of Rho/Ras pathway genes may explain the increase of tumor aggressiveness from G1 – G2 – G3. The increase of cell cycle and telomerase genes expression associated with G3 provides a possible explanation for the unlimited growth potential of NB and may be responsible for the increase of SA. Finally, tumor progression in older patients in relation with SA accumulation and gene regulation is discussed.

Email: paola.scaruffi@istge.it

OR80
Irregular chromosome segregation by tripolar divisions; mechanism for heterogeneity in neuroblastoma
Kasai, Fumi1; Kobayashi, Hirofumi2; Rens, Willem3; Ferguson-Smith, Malcolm A1; Kaneko, Yasuhiro4
1 Saitama Cancer Center, Research Institute for Chromosome, Komura, Ina, Saitama, Japan; 2 Saitama Cancer Center, Department of Hematology, Komura, Ina, Saitama, Japan; 3 University of Cambridge, Department of Veterinary Medicine, Madingley Road, Cambridge, United Kingdom; 4 University of Cambridge, Department of Veterinary Medicine, Madingley Road, Cambridge, United Kingdom; 5 Saitama Cancer Center, Research Institute for Clinical Oncology, Komuro, Ina, Saitama, Japan

Neuroblastoma (NB) shows a distinct characteristic in ploidy and can be classified into diploid and triploid tumors, which are closely related with respect to prognosis. Diploid tumors are found in both infants and children; however, the prognosis is favorable in infants but not in children. MYCN amplification is a poor prognostic factor in diploid tumors and usually accompanied by 1p terminal deletion. Triploid tumors are found mainly in infants and show good prognosis despite 50% increase of DNA contents. The range of chromosomes in triploid cells is defined as 58-80 chromosomes and karyotypes of triploid tumors vary in individuals. These triploid cells have gains or losses of certain whole chromosomes and do not show a complete set of 3 of each chromosome. A hypothesis describes that triploid cells are derived from tripolar divisions of tetraploid cells and this tripolar division produces two triploid and one diploid cells. In this study, we confirmed that tripolar divisions were observed in 3% of anaplasia in a NB cell line and the chromosome complement in daughter cells was examined by fluorescence in situ hybridization (FISH) using centromeric probes. The number of FISH signals for chromosomes 1, 2 and X in the daughter cells was different depending on each chromosome, indicating that two identical triploid cells could not be formed through tripolar division. Chromosome complements based on these three chromosomes in daughter cells formed by a tripolar division varied between mitotic cells. Some of the daughter cells did not contain X chromosome, however no metaphases without X were found. Our results suggest that tripolar division is not a perfect cell division and might involve in chromosome losses or gains, and all the 3 daughter cells cannot always survive because of lack of certain chromosomes. Tripolar divisions change chromosome complements in triploid cells, and could be a cause of heterogeneity in NB.

Email: k-230@umin.ac.jp

Parallel session 11 –
Prognostic factors and markers OR81–OR89

OR81
Is subtotal resection sufficient for treatment of ganglioneuroma and localized ganglioneuroblastoma intermixed?
De Carolis, Boris1; Simon, Thorsten2; Leuschnner, Ivo3; von Schweinitz, Dietrich3; Kingsbiiel, Thomas4; Erttmann, Rudolf5; Schweigerer, Lothar6; Kaatsch, Peter1; Berthold, Frank1; Hero, Barbara7
1 University of Cologne, Department of Pediatric Hematology and Oncology, Cologne, Germany; 2 University of Kiel, Department of Pathology and Molecular Epidemiology, Kiel, Germany; 3 University of Cambridge, Department of Pediatric Surgery, Munich, Germany; 4 Johann Wolfgang Goethe-University, Clinic for Pediatric Hematology and Oncology, Frankfurt, Germany; 5 University Medical Center Hamburg-Eppendorf, Department of Pediatric Hematology and Oncology, Hamburg, Germany; 6 Helios Klinikum Berlin-Buch, Clinic for Pediatrics, Berlin, Germany; 7 Johannes Gutenberg University Mainz, German Childhood Cancer Registry, Institute for Medical Biostatistics, Epidemiology and Informatics, Mainz, Germany

Background: Ganglioneuroma (GN) and ganglioneuroblastoma intermixed (GNBNI) form the mature end in the range of neuroblastomas (NT). We studied the clinical characteristics of GN/GNBNI and the impact of resectability on long term outcome.

Methods: Clinical characteristics of patients with GN and GNBNI were analyzed retrospectively and compared to patients with immature localizing neuroblastoma (NB) and ganglioneuroblastoma nodular (GNBN) registered between 2000 and 2009.

Results: Of 874 consecutive registered patients with localized NT, 790 were reviewed centrally according to the International Neuroblastoma Pathology Classification. Of 790 tumors 159 (20%) were classified as GN (10% GN mature, 90% GN maturing) and 52 as GNBNI (6.6%). Patients with GN/GNBNI had more often stage 1 disease (69% vs. 37%, p<0.001) and presented less frequently an adrenal tumor (31% vs. 41%, p=0.02), positive mIBG-uptake (33% vs. 96%, p<0.001) and elevated urine catecholamine metabolites (HVA 38% vs. 63%, p<0.001, VMA 26% vs. 66%, p<0.001) than patients with more immature NT. The median age at diagnosis is increased with the grade of differentiation (NB/GNBN; 9; GNBNI; 62; GN mature: 70; GN mature: 120 months, p<0.001). Of 211 patients with GN/GNBNI, the tumor was completely resected at diagnosis in 150 patients (71%; 116/159 GN, 34/52 GNBNI) and in delayed surgery after 4 to 33 months in 11 patients (5 GN, 6 GNBNI). Chemotherapy was given to 11 patients (3 GN, 8 GNBNI). In 45 patients (34 GN, 11 GNBNI) a residual tumor is currently under observation (Median observation time: 40 months). Only 2 patients (1 GN, 1 GNBNI) showed local progression so far. Two patients died of treatment related complications (surgery: 1, chemotherapy: 1), but none of tumor progression.

Conclusions: GN and GNBNI account for about one quarter of neuroblastoma tumors and differ from more immature tumors in age at diagnosis, stage, localization, mIBG-uptake and catecholamine metabolism. Surgery alone seems to be sufficient for the treatment of GN and most patients with GNBNI and does not need to be radical.

Email: boris.de-carolis@uk-koeln.de
**OR82**

**Survival variability by race and ethnicity in neuroblastoma: A Children’s Oncology Group (COG) Study**

Pinto, Nayan1; Henderson, Tara1; Bhatia, Smita2; London, Wendy1; McGady, Patrick3; Crotty, Catherine1; Sun, Can-Lan1; Cohn, Susan L1

1University of Chicago, Section of Pediatric Hematology, Oncology and Stem Cell Transplantation, Chicago, United States; 2City of Hope National Medical Center, Population Sciences, Duarte, United States; 3Children’s Hospital of Boston/Dana Farber Cancer Institute, Hematology/Oncology, Boston, United States; 4Children’s Oncology Group, Data Center, Gainesville, United States

**Background:** Survival probabilities according to race and ethnicity have been reported in a number of cancers, but little is known about outcome disparities in neuroblastoma.

**Methods:** We compared disease presentation and survival probabilities among white, black, Hispanic, Asian, and Native American children enrolled on the COG NBL protocol ANB08B1. Disease outcome was measured as overall OS and event-free survival (EFS), and was adjusted for prognostic factors.

**Results:** 3,923 children with NBL consented and enrolled between 2001 and 2009 and were included. Non-Hispanic whites (“white”) constituted 73% of the cohort; 11% were Hispanic, 11% non-Hispanic black (“black”), 4% Asian, and 1% Native-American. When compared with whites, blacks were more likely to present >18 months of age (blacks: 62% vs. 48%, p<0.001), have stage 4 disease (blacks: 53% vs. 46%, p=0.003), unfavorable histology (blacks: 49% vs. 40%, p<0.001), and high-risk disease (blacks: 56% vs. 43%, p<0.001). Native Americans also had a higher prevalence of high-risk disease than whites, but the incidence of high-risk disease in Asian and Hispanic children was similar to that of white children. Overall three-year EFS was: Hispanics, 74%; whites, 69%; blacks, 63%; Asians, 62%; and Native-Americans, 38% (p<0.001). When compared with whites, EFS was worse for blacks (HR 1.28; p=0.01) and Native-Americans (HR 2.33; p=0.003). However, adjustment for risk group abrogated these differences. Examining EFS by follow-up time (<2 years vs. >2 years from diagnosis) revealed a higher prevalence of late-occurring events among blacks. This was confirmed in the multivariate analyses restricted to >2 year survivors, where blacks had significantly worse EFS (HR=1.5, p=0.04) as compared with whites.

**Discussion:** Black and Native-American children have a higher prevalence of high-risk NBL and worse outcome than other ethnic groups. The propensity for delayed events in blacks suggests that this population may be more resistant to chemotherapy. Studies are being planned to delineate the role of genetic predisposition in the racial differences in prevalence of high risk NBL and survival.

**Email:** npintot1@peds.bsd.uchicago.edu

**OR83**

**Stable incidence of neuroblastoma during 28 years in Sweden with significant sex differences and improved survival, in particular for children with high-risk disease with MYCN amplification**

Träger, Catarina1; Vernby, Åsa1; Caren, Helena1; Kryh, Hanna1; Hedborg, Fredrik1; Martinsson, Tommy1; Gustafsson, Göran1; Kogner, Per1; 1Karolinska Institutet and Uppsala University Hospital, Department of Women and Child Health, Stockholm and Uppsala, Sweden; 2Karolinska Institutet, Department of Women and Child Health, Stockholm, Sweden; 3University of Gothenburg, Department of Clinical Genetics, Gothenburg, Sweden; 4Uppsala University, Dept of Genetics and Pathology and Dept of Women’s and Children’s Health, Uppsala, Sweden

**Background:** Over 28 years, 1982-2009, 384 children with neuroblastoma were included in the population based Swedish Childhood Cancer Registry.

**Methods:** All children were characterized according to age at diagnosis, sex, chromosomal aberrations with FISH/CGH, INRG stage, treatment and overall survival.

**Results:** 207 children had localized stage (L), 153 metastatic stage (M) and 21 infants metastatic special stage (MS) (three missing stage, 2 girls). Standardized annual incidence was 1/100000 children <15 years and did not change over time with boys:girls ratio 1.15:1. Boys had worse outcome than girls (61.3% vs. 70.2% 5y survival) due to more stage M (ratio 1:39:1, 1:5.1 >18 months). Sex ratio for stage L was 0.99:1 with boys significant younger (p<0.01). 17q-gain was more common in boys (p=0.04) but no sex difference for MYCN-amplification (MNA) or 11q-deletion. Age (>12 or >18 months), stage M, MNA, 1p- or 11q-deletion and 17q gain were all related to worse outcome. Children with 11q-deleted tumors were older (median 41 months; boys and girls equal) than those with MNA (median 22.5, boys older than girls, median 28 vs. 20). Five year survival for all children improved from 57% to 62% for those diagnosed 82-90 to 62.1% for 91-99 and 78.6% for 00-09 (p<0.001). The most improved prognosis was achieved in high-risk patients (stage M >18 months or MNA, n=148) from 11.1% (82-90, n=36) to 17.9% (91-99, n=56) and 61.6% (00-09, n= 56, p=0.001). Among outcome improved for children with MNA (from 11.1% for 82-95 to 48.9% for 96-09) but not for those with 11q-deletion (37.5% for 82-95, and 42.9% for 96-09).

**Conclusion:** Population-based neuroblastoma incidence was stable over 28 years with significant sex differences with respect to stage, age and biology. Outcome improved over time related to risk-based therapy, in particular for children with high-risk disease where the majority now can be long-term survivors. However, children with MYCN amplification benefit most from current intensified multimodal treatment, whereas our results identify the need of reconsidered therapy for children with 11q-deleted neuroblastoma.

**Email:** catarina.trager@ki.se
OR85
Exon-Level gene expression analyses of primary neuroblastoma improves risk prediction and identifies MYCN status as major determinant of alternative transcript use
Schramm, Alexander1; Schowe, Benjamin1; Marschall, Tobias2; Martin, Marcel1; Vermeulen, Joelle3; Vandesompele, Jo4; Theissen, Jessica5; Hero, Barbara2; Schleiermacher, Gudrun4; Combaret, Valerie1; Brejon, Stephanie1; Iacono, Isabelle1; Pariente, Yann1; Schlamn, Johann1; Sch半月, Johannes1
1University Clinic Essen, Childrens Hospital, Essen, Germany; 2TU Dortmund, LS Informatik 8, Dortmund, Germany; 3TU Dortmund, LS Informatik 11, Dortmund, Germany; 4Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium; 5University Clinic Cologne, Childrens Hospital, Cologne, Germany

Background: Assignment of patients to adequate treatment regimens according to their individual risk is a major goal in cancer therapy. On the route to clinical application of array-based classifiers to predict the risk profile of a patient with neuroblastoma (NB), significant progress has been made over the past years.

Method/approach: We here present data on a cohort of 138 primary NB using a novel approach including information for all human coding exons described to date (Affymetrix ExonST Array). Prediction of outcome and identification of alternative transcript use was achieved using machine learning algorithms and conventional statistics, respectively. Validation of alternative transcript use was performed using PCR- and siRNA-based approaches.

Results: Using a classifier trained on 100 NB tumor samples and then used to predict the outcome of the remaining 38 NB patients, we were able to achieve prediction accuracies >80% in cross-validation using support vector machine (SVM) learning algorithms, which is superior to the current clinical risk stratification. Interestingly, exon-based prediction proved to be superior to gene-based prediction. Of all clinical and biological parameters analysed, the status of the MYCN oncogene was most tightly linked to the number of alternatively used transcripts. Classification of these transcripts revealed an association of MYCN amplification with up-regulation of genes involved in cell cycle control and down-regulation of genes involved in DNA repair. These findings could be verified in independent primary NB samples as well as in NB cell lines. Using siRNA-based approaches, transcript-specific knock-down could be verified for CCNB1/Cyclin B1, for which a longer and shorter isoform were identified in varying ratios in NB cell lines with single copy or amplified MYCN. Conclusion: Exon-level analysis turned out to be a powerful tool for prediction of outcome in NB. This technique also facilitates the identification of alternative transcript use. The knowledge gained in NB should be readily translatable to other tumor entities and offers a deeper understanding of gene regulation in embryonal tumors.

Email: alexander.schramm@uni-due.de

OR86
High expression of KIF1Bα-interacting protein MAP1A and its family member MAP1B significantly correlates with favourable prognosis of neuroblastoma
Kramer, Sonja1; Ohiya, Mikiko2; Tsuchiya, Tomoko2; Ando, Koji3; Makai, Akira2; Eggen1, Angelika1; Nakagawa, Akira2
1Chiba Cancer Center Research Institute, Division of Biochemistry and Innovative Cancer Therapeutics, Chiba, Japan; 2Chiba Cancer Center Research Institute, Laboratory of Cancer Genomics, Chiba, Japan; 3Institut National de Recherche pour le Développement, Unité de Génétique Somatique, Paris, France

Background: Previously we identified KIF1Bα as a haploinsufficient tumour suppressor gene at the 1p36.2 locus in neuroblastoma (NB). To better understand the regulation of KIF1Bα at the molecular level we performed a yeast two-hybrid screening assay using its unique apoptosis inducing ROD-region as a bait and identified the light chain 2 (LC2) of MAP1A (Microtubule Associated Protein 1A) as a new binding partner. MAP1 family members consist of 3 proteins, MAP1A, MAP1B and MAP1S and they are almost exclusively expressed in neuronal cells. They stabilize the neuronal cytoskeleton and play an important role in neuronal development.

Method/approach: In this study we investigated the mRNA-expression levels of MAP1A and MAP1B in 25 NB cell lines and 106 primary NBs by RT-PCR and real-time PCR, respectively.

Results: Our RT-PCR data showed that both genes are highly expressed in all examined NB cell lines. Furthermore, our quantitative real-time PCR analysis has illustrated that high levels of MAP1A expression are significantly associated with the favourable prognostic indicators, such as low tumour stages (p<0.001), high ThA4-expression (p<0.0005), MYCN single copy number (p<0.0001), Shimada histology of favourable NBs (p<0.014) and the tumour origin (p=0.004). High MAP1B expression correlated with MYCN single copy number (p=0.029), ploidy (p=0.0013) and Shimada histology of favourable NBs (p=0.028). Furthermore, high expression of both genes markedly prolonged the overall survival of NB patients (MAP1A p<0.018, MAP1B p=0.031). Additionally, in the multivariate analysis, MAP1B expression was identified as an independent prognostic parameter (p=0.048) for NB.

Conclusion: Our results demonstrate that high expression of MAP1A and MAP1B are significantly correlated with a better overall survival of NB patients. Moreover, MAP1B was identified as an independent prognostic parameter for NB. The differential expression of both genes may contribute to differentiation and/or enhanced regression of favourable NBs. Further experiments will be needed to elucidate their functional roles in NB development.

Email: Sonja.Kramer@gmx.net

OR87
Determination of 17q gain in neuroblastoma patients by analysis of circulating DNA
Combaret, Valérie1; Brejon, Stephanie1; Iacono, Isabelle1; Schleiermacher, Gudrun1; Pierron, Gaelle1; Ribéo, Agnes2; Bergeron, Christophe2; Marabelle, Aurelie1; Pluieux, Alain3
1Centre Leon Berard, Laboratoire de Recherche Translationnelle, Lyon, France; 2Inserm U830, Laboratoire de Génétique et Biologie des Cancers, Paris, France; 3Institut Curie, Unité de Génétique Somatique, Paris, France

Purpose: Retrospective studies have demonstrated the prognostic impact of genomic profiles in neuroblastoma. Segmental chromosome alterations have been found useful for identifying tumors with a high risk of relapse. As the gain of chromosome arm 17q is the most frequent chromosome alteration reported in neuroblastoma primary tumors, we attempted to evaluate the presence of this 17q gain in the peripheral blood of neuroblastoma patients.

Patients and Methods: Using duplex quantitative real-time PCR we quantified simultaneously on the one hand, MPO (17q23.1) and a reference gene, p53, and on the other hand, Survivin (17q25) and p53. Then MPO and Survivin copy numbers were evaluated as MPO/p53 and Survivin/p53 ratios in 143 serum or plasma samples in which 17q gain had been determined by array-based comparative genomic hybridization (aCGH) or Multiplex ligation-dependent probe amplification (MLPA).

Results: In patients less than 18 months of age, serum-based determination of 17q gain in DNA sequences had good specificity (93%) and 55% sensitivity (p=0.001). In contrast, for patients over 18 months of age the approach exhibited low specificity (69%) and 51% sensitivity (p= not significant). Similar results were observed in neuroblastoma tumors without MYCN amplification.

Conclusion: Our results show that 17q gain determination in circulating DNA is possible and suggest that this non invasive test could be useful for infants and toddlers with neuroblastoma for whom the "wait and see" strategy is often recommended. This test is complementary to previously developed techniques for detecting circulating MYCN DNA sequences.

Email: combaret@lyon.fncc.fr
OR88
Phox2B but not TH mRNA detected by QRT-PCR in peripheral blood stem cell harvest predicts time to relapse in randomised children with high risk neuroblastoma: a SIOPEN molecular monitoring group study

Dallorso, Sandro1; Corrias, Maria1; Viprey, Virginie2; Vicha, Alex3; Swerts, Katrien4; Tchirkov, Andrei5; Gregory, Walter6; Luksch, Roberto7; Brock, Penelope8; Malis, Josef3; Laureys, Genevieve9; Valteau-Couanet, Dominique10; Ladenstein, Ruth11; Burchill, Susan2
1Gaslini Institute, Paediatric Oncology, Genoa, Italy; 2Leeds Institute of Molecular Medicine, Children’s Cancer Research Group, Leeds, United Kingdom; 32nd Medical Faculty Charles University, Paediatric Oncology, Prague, Czech Republic; 4University Hospital Gent, Haematology, Gent, Belgium; 5Centre Jean Perrin, Radiotherapy, Clermont-Ferrand, France; 6University of Leeds, Clinical Trials Research Unit, Leeds, United Kingdom; 7Istituto Nazionale Tumori di Milano, Paediatric Oncology, Milano, Italy; 8Great Ormond Street Hospital, Paediatric Oncology, London, United Kingdom; 9University Hospital Gent, Paediatric Oncology, Gent, Belgium; 10Institut Gustave Roussy, Paediatric Oncology, Villejuif, France; 11CCRI/St. Anna Children’s Hospital, Children’s Cancer Research Group, Vienna, Austria

Aim: The aim of this study was to determine whether the presence of neuroblastoma cells detected by quantitative reverse transcriptase polymerase chain reaction (QRT-PCR) for Phox2B or tyrosine hydroxylase (TH) mRNA in peripheral blood stem cell (PBSC) harvest from children with high risk neuroblastoma entered on the HR-NBL1/SIOPEN trial predicts outcome.

Methods: PBSC (0.5ml) were collected into PAXgene™ Blood RNA tubes (PreAnalytix) after induction therapy and processed according to standard operating procedures (Viprey et al, 2007, EJC, 43, 341-350; Viprey et al, 2008, J. Pathol, 216, 245-252). Blinded quality control was utilised to ensure the sensitivity, specificity and reliability of data across participating reference centres and countries. QRT-PCR data are reported on PBSC samples from 133 children for TH mRNA and from 119 children for Phox2B mRNA. RNA extraction and QRT-PCR results are recorded in the SIOPEN-R-NET database and reviewed by the central reference laboratory in Leeds.

Results: The frequency of Phox2B and TH mRNA detection by QRT-PCR in PBSC is 13% (16/119) and 49% (65/133) respectively. Of these children 74 were randomised to high dose therapy; TH was measured in all 74 and Phox2B in 65/74. Eighty-eight % (14/16) of PBSC samples positive for Phox2B were also positive for TH mRNA. The level of Phox2B and TH mRNA was low in all positive PBSC samples with Ct values of 38.9 (34.9-39.9) and 37.7 (31.0-39.8) respectively. In children randomised for high dose therapy Phox2B predicted time to relapse; HR 4.39 (95% CI 1.32-14.61; p =0.02). There was no significant correlation between TH mRNA in PBSC and overall survival or time to relapse.

Conclusions: Phox2B mRNA detected by QRT-PCR in the PBSC harvest (n=65) from children with high risk neuroblastoma randomised for high dose therapy predicts time to relapse. This requires further investigation in a larger patient group.

Email: S.A.Burchill@leeds.ac.uk

OR89
Clinical utility of minimal residual disease marker panel during sequential phases of a multi-modality treatment of high-risk neuroblastoma

Cheung, Irene; Kushner, Brian; Kramer, Kim; Modak, Shakeel; Cheung, Nai-Kong
Memorial Sloan-Kettering Cancer Center, Pediatrics, New York, United States

Background: At MSKCC, induction for high-risk neuroblastoma (NB) patients (pts) included dose-intensive chemotherapy, surgery and local radiation. Consolidation consisted of either (1) anti-GD2 monoclonal antibody 3F8 followed by I-131-3F8 radioimmunotherapy (RIT) [NCT00002634] and autologous marrow/stem cell rescue, or (2) 3F8 plus granulocyte-macrophage colony-stimulating factor (GM-CSF)+13-cis-retinoic acid (CRA) [NCT00002560], or (3) myeloblastic chemotherapy plus autologous marrow/stem cell transplant (SCT)+ 3F8/GMCSF/CRA. Since minimal residual disease (MRD) in the bone marrow (BM) portends poor outcome, we utilized a four-marker panel to measure MRD during different phases of this multi-modality treatment in 149 high risk pts to assess when the presence of MRD would correlate with clinical relapse.

Method/approach: BM samples from 4 groups of pts: (1) at diagnosis and after induction (43 pts), (2) before and following RIT (42 pts), (3) before and after SCT (57 pts), (4) before and after 2 cycles of 3F8/GMCSF (63 pts), were tested for MRD by qRT-PCR using a marker panel consisting of CCND1, GD2 synthase, PHOX2B, and TH. MRD analyses were based on a logistic regression model using marker panel positivity as a predictor of relapse. MRD response was scored by the marker panel.

Results: Except for the BM at diagnosis, all other BM tested in this study were histologically negative in 2/2 biopsies and 4/4 aspirates. Marker panel positivity correlated significantly with progression-free survival (PFS) in only 3 time points: after induction (p=0.03) in group 1; after 4 cycles of 3F8 (without GM-CSF) and right before RIT (p=0.002) in group 2; and after 2 cycles of 3F8/GMCSF, measured at a median time of 2.8 months from first day of 3F8 treatment (p=0.03) in group 4. MRD response scored by the marker panel following 2 cycles of 3F8/GMCSF was also strongly correlated with PFS (p=0.004).

Conclusion: MRD marker panel has clinical utility in the management of high risk NB pts. It can measure NB in histologically-negative BM, and on the time of BM sampling, the presence of MRD correlates with eventual clinical relapse.

Email: cheungi@mskcc.org
SEL1

Omics analysis and evolution for identification of candidate genes in progression of neuroblastoma

Hiyaum, Eizo1; Kamei, Naomi1; Kimamitauke, Anata1; Hiyaum, Keiko1; Hirai, Yukimasa2, Teatomi1; Hiroshioma University Hospital, Pediatric Surgery, Hiroshima, Japan; 2Hiroshioma University, Research Institute for Radiation Biology and Medicine, Hiroshioma, Japan; 3Hiroshioma University, Graduate School of Biomedical Science, Hiroshioma, Japan

Background: Neuroblastoma (NBL) is biologically heterogeneous and demonstrates both favorable and unfavorable outcomes. Genome-wide genetic aberrations and expression using microarray have already been reported. In this study, proteome and messenger RNA (mRNA) data were combined to evaluate the mechanism of tumor progression.

Methods: From 200 NBL samples analyzed by Affymetrix SNP and expression arrays, 10 NBL cell lines and 40 tumor samples were selected, including 20 favorable cases which regressed or matured spontaneously and 20 unfavorable cases who died of tumor progression. The miRNA expression levels were examined by Agilent microarray, using total RNA and LC-MS analysis using cell extracts. This was performed by mass spectrometers (QSTAR Elite or LTQ Orbitrap XL) with ESI module. MS/MS data of the specific peaks was matched with the data in the MassBank.

Results: About 2,500 peaks were extracted from the LC-MS data. The comparison between the gene expression and LC-MS data showed that MYC-induced and cholinergic pathways are activated in unfavorable tumors, while apoptosis pathways, including neuro-differentiation and glycopolypid metabolites were activated in the favorable ones. The data of SNP array showed that the genes including DX1, NAG, NME1 located in the amplified region gained loci activated this pathway in unfavorable tumors. The data of miRNA showed that the activated pathways in favorable tumors were mainly down-regulated by miRNA located in the genetic aberrated loci in unfavorable tumors. Gene discrimination was correlated to located gene and miRNA expression levels, consequently regulating the products of these cell lines. Pathways showed the specific activation of miRNA and genes, which might correlate with in vitro proliferation.

Conclusions: Omics analysis provided important candidates of indicators for risk assessment and of therapeutic targets for unfavorable NBLs.

Email: eiso@hiroshioma-u.ac.jp

SEL2

Impaired activation of the tumor suppressor p14^ARF impedes its oncosuppressive impact in neuroblastoma

Dreidix, Daniel1; Gogolin, Sina1; Muth, Daniel1; Zapata, Marc1; Berthold, Jossif1; Heinberg, Christian1; Schwat, Manfred1; Westermann, Frank1; 1German Cancer Research Center, Department of Tumor Genetics, Heidelberg, Germany; 2German Cancer Research Center, Department of Theoretical Bioinformatics, Heidelberg, Germany; 3University Children’s Hospital of Cologne, Department of Pediatric Oncology and Center for Molecular Medicine Cologne (CMMC), Cologne, Germany

Background: The p14^ARF promoter as indicated by activating/inactivating histone modifications. Functional characterizations were carried out by conditional overexpression in NB cell lines. The frequency of mutation or p13^INK4a inactivation in NB, whereas a functional loss of the p53 path during malignant NB progression is indicated. Low expression levels of the p53 stabilizer p14^ARF led us to analyze its role and regulation in NB.

Method/approach: Gene expression was measured via real-time quantitative RT-PCR. The genomic status of CDKN2A (p16^INK4a/p14^ARF) was determined via array-based comparative genomic hybridisation (A-CGH). Methylation specific PCR served to assess p14^ARF promoter methylation. Chromatin immunoprecipitation on chip (ChIP) chip detected the activity of the p14^ARF promoter as indicated by activating/inactivating histone modifications. Functional characterizations were carried out by conditional overexpression in NB cell lines.

Results: Expression of p14^ARF mRNA was low but differential in NB cell lines and in a set of 81 NB tumors. One homozygous CDKN2A deletion was found in 194 primary NBs. Heterozygous losses either by whole (28 cases) or by partial chromosome loss (14 cases) occurred in 22% of 194 NBs. No evidence was found for p14^ARF promoter methylation in 80 NBs. ChIP chip analyses revealed weak p14^ARF promoter activities in NB cell lines. Promoter activity states differed among cell lines and were positively correlated with p14^ARF expression. Conditional p14^ARF overexpression in SH-EP and IMR-5 cells, partially reduced cell viability. Expression and cell cycle analyses revealed an induction of the p53 machinery and a doubling of the apoptotic cell fraction after 48 h of p14^ARF overexpression.

Conclusion: Low expression levels indicate that activation of p14^ARF in NB cell lines is reined to determine intervention points for targeted activation of the gene in NB.
SEL5
A SPI/MIZ1/MYCN ternary complex induces repression of TRKA and p75NTR neurotrophin receptors and affects neuroblastoma malignancy through inhibition of the cell apoptotic response to NGF
Vali, Emmanuël1; Dellà, Valeria1; Iacoviello, Loredana1; De Giorgio, Lorenzo1; Salerno, Giovanni1; Mancini, Giuseppe1
1University of Insubria, Varese, Italy; 2University of Insubria, Varese, Italy
Background: Overexpression of the TRKA and p75NTR neurotrophin receptors is associated with poor outcomes in neuroblastoma. The aim of the current study was to investigate the potential involvement of the SPI/MIZ1/MYCN complex in the regulation of TRKA and p75NTR expression in neuroblastoma.

Method: The SPI/MIZ1/MYCN complex interacts with the TRKA/p75NTR genes in neuroblastoma cell lines. The expression of SPI and MIZ1 in neuroblastoma cell lines was assessed using quantitative RT-PCR and western blotting. The expression of SPI and MIZ1 was found to be significantly higher in neuroblastoma cell lines compared to normal controls. The expression of SPI and MIZ1 was also found to be inversely correlated with the expression of TRKA and p75NTR in neuroblastoma cell lines.

Results: The expression of SPI and MIZ1 was found to be significantly higher in neuroblastoma cell lines compared to normal controls. The expression of SPI and MIZ1 was also found to be inversely correlated with the expression of TRKA and p75NTR in neuroblastoma cell lines.

Conclusion: The SPI/MIZ1/MYCN complex may play a role in the regulation of TRKA/p75NTR expression in neuroblastoma, and its expression may be a potential therapeutic target for the treatment of neuroblastoma.

SEL6
NCYM, a protein product of an antisense MYCN gene co-amplified with MYCN, targets MYCN for functional modulation and affects the prognosis of neuroblastoma
Yukiko Nakamura1; Yoshikawa, Akira1; Nakamura, Nao1; Nakagawara, Masanobu1; Nakamura, Yohiho1; Yamada, Shun1; Nakamura, Hiroyuki1; Nakagawara, Akira1; Nakagawara, Masanobu1; Nakamura, Nao1; Nakamura, Yohiho1; Yamada, Shun1; Nakamura, Hiroyuki1; Nakagawara, Akira1
1Chiba Cancer Research Center, Laboratory of Cancer Genomics, Jichi, Japan; 2Chiba Cancer Research Center, Laboratory of Cancer Genomics, Chiba, Japan
Background: Neuroblastoma (NB) is a pediatric malignancy that affects the sympathetic nervous system. Therapy-resistant NB is associated with the presence of MYCN gene amplification. The MYCN gene is located on chromosome 22q11.2 and is involved in the regulation of cell proliferation and apoptosis.

Method: The expression of MYCN mRNA was examined by quantitative RT-PCR in NB samples. The expression of MYCN mRNA was found to be significantly higher in therapy-resistant NB samples compared to responsive NB samples. The expression of NCYM mRNA was also found to be higher in therapy-resistant NB samples compared to responsive NB samples.

Results: The expression of MYCN mRNA was found to be significantly higher in therapy-resistant NB samples compared to responsive NB samples. The expression of NCYM mRNA was also found to be higher in therapy-resistant NB samples compared to responsive NB samples.

Conclusion: The expression of MYCN and NCYM mRNAs was found to be higher in therapy-resistant NB samples compared to responsive NB samples. The expression of MYCN and NCYM mRNAs may be useful as a prognostic marker for therapy-resistant NB.

SEL7
Identification of a new fusion gene on 11q23 in neuroblastoma tumor samples
Molenaar, J.1; Molenaar, J.1; Abrahamsson, J.2; Jegerås, E.1; Sjöberg, M.1
1UMC Utrecht, Department of Human Genetics, Amsterdam, Netherlands; 2University Children's Hospital, Gothenburg, Sweden; 3University of Gothenburg, Institute of Biomedicine, Gothenburg, Sweden; 4Karolinska Institutet, Children's Cancer Research Unit, Stockholm, Sweden
Background: Neuroblastoma is a pediatric malignancy that affects the sympathetic nervous system. Therapy-resistant NB is associated with the presence of MYCN gene amplification. The MYCN gene is located on chromosome 22q11.2 and is involved in the regulation of cell proliferation and apoptosis.

Method: The MYCN gene was amplification in 106 NBs (p=0.035). Our further analyses found that the expression of 15 kDa NCYM protein in human NB cell lines, particularly in those with MYCN amplification.

Results: The MYCN gene was amplification in 106 NBs (p=0.035). Our further analyses found that the expression of 15 kDa NCYM protein in human NB cell lines, particularly in those with MYCN amplification.

Conclusion: The expression of MYCN and NCYM mRNAs was found to be higher in therapy-resistant NB samples compared to responsive NB samples. The expression of MYCN and NCYM mRNAs may be useful as a prognostic marker for therapy-resistant NB.
SEL9
Causal inference, a novel approach to disentangle the effects of off-protocol therapy from the primary effects of interest in COG protocol P9462: Topotecan vs. Topotecan+cyclophosphamide in relapsed neuroblastoma
London, Wendy B; Frantz, Christopher N; Campbell, Laura A; Seeger, Robert C; Brummelkamp, Babette A; Cohn, Susan L; Matthay, Katherine K; Castleberry, Robert P; Diller, Lisa
1Dana-Farber Harvard Cancer Care/Children's Hospital Boston & Children's Oncology Group, Pediatrics, Boston, MA, United States; 2A.I. DuPont Hospital for Children, Pediatrics, Wilmington, DE, United States; 3Kaiser Permanente Medical Group, Pediatrics, Oakland, CA, United States; 4Children's Hospital Los Angeles, Pediatrics, Los Angeles, CA, United States; 5University of Florida, Epidemiology and Biostatistics, Gainesville, FL, United States; 6University of Chicago, Pediatrics, Chicago, IL, United States; 7University of California San Francisco School of Medicine and UCSF Children's Hospital, Pediatrics, San Francisco, CA, United States; 8University of Alabama, Pediatrics, Birmingham, AL, United States; 9Dana-Farber Harvard CancerCare/Children's Hospital Boston, Pediatrics, Boston, MA, United States

Background: Responders to Phase II therapy for relapsed disease often undergo subsequent off-protocol therapies. Novel statistical methods are needed to elucidate the effect of protocol therapy on long-term outcome. Using such methods, outcome for single agent topotecan (TOPO) and combination topotecan and cyclophosphamide (TOPO/CTX) was compared after completion of a Phase II randomized trial in relapsed/refractory neuroblastoma.

Patients and Methods: Children with refractory/recurrent neuroblastoma were randomized to daily 5-day TOPO (2 mg/m^2) or combination TOPO (0.75 mg/m^2) and CTX (250 mg/m^2). A randomized two-stage group sequential design enrolled 119 eligible patients. Patients could go on to other therapies, and some underwent ASCT. If ASCT results in increased survival, and if patients exposed to TOPO/CTX are more likely to undergo ASCT, then our ability to test for the hypothesized benefit of TOPO/CTX was confounded by ASCT. Long-term outcome of protocol therapy and surgery. 177 children were not randomised but were treated according ENSG5, 36 were long-term survivors, seven with persistence of disease were included in the analysis. Seventeen children (median age 2.49 years) had persistent primary tumour after induction chemotherapy. Two patients had persistent bone marrow disease (up to 9 years after diagnosis) and six had persistent MIBG skeletal positivity (up to 16 years after diagnosis). Seven children had persistent primary tumour during follow-up with residual masses (up to 16 years after diagnosis).

Results: Out of 262 children randomized in ENSG5, 62 were alive at five years and 19 of them showed persistence of disease after induction therapy and surgery. 177 children were not randomised but were treated according ENSG5, 36 were long-term survivors, seven with persistence of disease were included in the analysis. Seventeen children (median age 2.49 years) had persistent primary tumour after induction chemotherapy. Two patients had persistent bone marrow disease (up to 9 years after diagnosis) and six had persistent MIBG skeletal positivity (up to 16 years after diagnosis). Seven children had persistent primary tumour during follow-up with residual masses (up to 16 years after diagnosis).

Conclusions: There was no obvious difference between the characteristics of these groups and the whole ENSG5 cohort.

Discussion: Some patients can be long-term survivors despite of persistent disease after end of treatment (large primary tumours, bone or bone marrow metastases). Our findings show that a clinical course similar to infants with stage 4 disease is possible for this group of older patients. This may be explained in the future by more knowledge of tumour biology.

Email: lucas.moren@icr.ac.uk

SEL10
Persistence of disease in long-term survivors of high-risk neuroblastoma. Analysis of long term ENSG5 cooperation
Moreno, Luca; Vaidya, Sucheta; Pinkerton, Ross; Lewis, Ian J; Imeson, John; Ellenershaw, Caroline; Machin, David; Pearson, Andrew DJ
1The Royal Marsden Hospital, Institute of Cancer Research, Paediatrics, Sutton, United Kingdom; 2Royal Children’s Hospitals, Paediatrics, Brisbane, Australia; 3St.James’ University Hospital, Paediatrics, Leeds, United Kingdom; 4University of Leicester, CCLG Data Centre, Leicester, United Kingdom

Background: There is no evidence available regarding the long-term outcome of children that do not achieve remission, especially for a small group of patients whose disease persists after several lines of treatment and have persistence of neuroblastoma during follow-up. We reviewed remission status and presence of active disease after End of Treatment (EOT) in long-term survivors of ENSG5 trial that treated high-risk neuroblastoma in Europe from 1990 to 1999.

Methods: Patients were randomised to receive the same induction drug doses but in one arm the dose intensity was 1.8 times greater (OPEC/OJEC vs. COJEC), surgical removal of primary tumour and high-dose melphalan with haemopoetic stem cell rescue. We identified children that were alive at five years from diagnosis who were not in remission after induction therapy and surgery. Patients were grouped in: 1) Persistent metastatic disease, 2) Persistent primary disease. Data were verified by sending questionnaires to registering centres (82.9% responses).

Results: Out of 262 randomized children in ENSG5, 62 were alive at five years and 19 of them showed persistence of disease after induction therapy and surgery. 177 children were not randomised but were treated according ENSG5, 36 were long-term survivors, seven with persistence of disease were included in the analysis. Seventeen children (median age 2.49 years) had persistent primary tumour after induction chemotherapy. Two patients had persistent bone marrow disease (up to 9 years after diagnosis) and six had persistent MIBG skeletal positivity (up to 16 years after diagnosis). Seven children had persistent primary tumour during follow-up with residual masses (up to 16 years after diagnosis).

Conclusion: Persistent metastatic disease and Persistent primary disease are verified by sending questionnaires to registering centres (82.9% responses).

Email: lucas.moren@icr.ac.uk

SEL11
High dose MIBG and haploidentical stem cell transplantation with cell therapy in therapy resistant neuroblastoma
Toporski, Jacek; Turkiewicz, Dominik; Tennywall, Jan; Garkavík, Michael; Dykes, Josefina; Le Blanc, Katarina; Lenhoff, Stig; Juliusson, Gunnar; Scheding, Stefan; Ora, Ingrid; Bekassy, Albert
1Skånes University Hospital, Department of Pediatric Oncology, Lund, Sweden; 2Skånes University Hospital, Department of Oncology, Lund, Sweden; 3Institute of Laboratory Medicine, Division of Hematology and Transfusion Medicine, Lund, Sweden; 4Karolinska Institutet, Division of Clinical Immunology Karolinska University Hospital Huddinge, Stockholm, Sweden; 5Skånes University Hospital, Department of Hematology, Lund, Sweden

Background: The prognosis for relapsing/refractory neuroblastoma (RRNB) remains dismal and no effective salvage treatment has been identified so far. We evaluated the feasibility and efficacy of using high-dose 131-I-MIBG (HDMIBG) followed by reduced-intensity conditioning (RIC) and transplantation of T cell-depleted haploidentical peripheral blood stem cells (haplo-SCT) to treat RRNB.

Methods: Ten children with RRNB were enrolled: 6 with relapse (4 after autologous SCT) and 4 with primary resistant disease. The preparative regimen included HD-MIBG on day –20, followed by fludarabine (Flu), thiotepa, and melphalan (Mel) from day –8 to –1. Granulocyte-colony stimulated factor (G-CSF)-mobilized, T cell-depleted haploidentical parental stem cells were infused on day 0 together with donor (n=7) or third party (n=3) mesenchymal stem cells. A single dose of rituximab was given on day +1. After cessation of short immunosuppression (MMF, OKT3), 7 children received donor lymphocyte infusion (DLI).

Results: Treatment was well tolerated. Two children developed primary acute graft-versus-host disease (aGVHD). Five children developed aGVHD after DLI and were successfully treated. Analysis of immunologic recovery showed fast reapparance of potentially immunocompetent natural killer (NK) and T cells, which might have acted as effector cells responsible for the graft-versus-tumor effect. Eight children are alive. Four patients are doing well with no evidence of disease 53, 52, 8 and 5 months haplo-SCT. Four other children are alive 52, 17, 5 and 4 months after haplo-SCT having stable/ slowly progressing disease. There were no transplant related mortalities. Two children died because of progression 5 and 12 months after haplo-SCT.

Conclusion: HD MIBG followed by RIC and haplo-SCT for RRNB is feasible and promising. Eight of 10 children on that regimen are alive, four of them in complete remission. Large number of patients and a longer observation time are needed to evaluate the role of this approach including immune mediated graft versus neuroblastoma effect in the treatment of high-risk therapy resistant neuroblastoma.

Email: jacek.toporski@med.lu.se
SEL12
Combined radioimmunotherapy and anti-angiogenic therapy for resistant neuroblastoma
Modak, Shakti1; Kreuzer, Brian H.2; Kramer, Kim1; Pandi-Taskar, Neeta2; Carraquillo, Jorge A.3; Zanonzico, Pat3; Smith-Jones, Peter4; Larson, Steven5; Cheung, Na-Kang V.6; 1Memorial Sloan-Kettering Cancer Center, Pediatrics, New York, United States; 2Memorial Sloan-Kettering Cancer Center, Pediatics, New York, United States; 3Memorial Sloan-Kettering Cancer Center, Radiology, New York, United States; 4Memorial Sloan-Kettering Cancer Center, Radiation and Medical Physics, United States

Background: Using preclinical mouse models, we demonstrated synergy between 131I-3F8-mediated radioimmunotherapy and bevacizumab (BV)-mediated anti-angiogenesis (ANR 2006 Abs 193). We translated our findings into a phase I study for patients with resistant NB.

Method/approach: Heavily pretreated (median of 4 prior therapeutic regimens; 10 with progressive disease [PD]) patients (pts) with recurrent or refractory stage 4 NB were treated on an IRB-approved study (NCT01450827) investigating the toxicity and effectiveness of the combination of 131I-3F8 and BV. Each cycle consisted of a single dose of 131I-3F8 on day 0 and a fixed dose of BV at 15mg/kg on days 1 and 15. 13.13I-3F8 was escalated at 4.8-mCi/kg in cohorts of 6 pts. Patients could receive a maximum of 4 cycles in the absence of grade-2 non-hematopoietic toxicity, human antinouse antibody response, severe myelosuppression and/or PD.

Results: 6 pts each received 4.5, 5.6 and 8-mCi/kg 131I-3F8. 39 cycles were administered. 2, 3, 3 and 16 pts completed 4, 3, 2 and 1 cycles respectively. All were evaluable for toxicity and response. 131I-3F8 targeting to NB was demonstrated in all pts in PET/CT scans. BV was well tolerated (MTD) for 131I-3F8 was not reached. All pts developed grade 4 myelosuppression. 9 pts required apheresis. 2 pts had a non-hematologic toxicity leading to withdraw from study: anaphylaxis (1), grade 3 BV-related intestinal perforation (1) and sepsis requiring ASCR (2). Response to 131I-3F8 alone: 1 complete response, 1 mixed response, 16 no response and 6 PD. Objective responses were observed in 13/24 patients. 3-year event-free survival was 39±12%; median 7 months.

Conclusion: Multicenter trials of 131I-3F8 and BV resulted in no unexpected side-effects and showed anti-NB activity. MTD for 131I-3F8 was not reached. BV did not impair 131I-3F8 targeting to NB or bone marrow recovery after ASCR.

Email: modaks@msk.cc

SEL13
MYCN amplified neuroblastoma differs in clinical features at initial presentation
Heró, Barbaro1; Simon, Thorsten1; Spitz, Ruediger1; Theissen, Jessica1; Christiansen, Holger2; De Carolis, Boris1; Berthold, Frank1; Niggli, Hero, Barbara1; Kogner, Per4; Douglas, Lena3; Guler, Lindan1; Kneff, Per5; Gudin, Linda5; Proust-Houdemont, Stephanie1; Benhamou, Etel1; Dubor, Christelle1; et al.
1Institut Gustave Roussy, Pediatrics Department, Villejuif, France; 2Institut Gustave Roussy, Biostatistics Department, Villejuif, France; 3Karolinska University Hospital, Dept of Pediatric Radiology, Stockholm, Sweden; 4Karolinska University Hospital, Dept. of Radiology, Stockholm, Sweden; 5Karolinska University Hospital, Dept of Medical Physics, Stockholm, Sweden; 6Karolinska Institutet, Childhood Cancer Research Unit, Stockholm, Sweden

Background: For oncology patients repeated imaging scans are necessary for diagnosis, staging and treatment response monitoring. This may lead to considerable ionizing radiation doses to the patient, depending on the imaging method. Minimizing or avoiding ionizing radiation is especially important regarding pediatric patients. Diffusion-weighted whole-body imaging with background body suppression (DWIBS) is a free-breathing diffusion weighted (DWI) whole-body sequence with multiple acquisitions, resulting in images resembling Position Emission Tomography (PET) images. The potential value of this method in oncology imaging as a substitute for PET has been suggested in the literature. We propose that the method should be evaluated in pediatric oncology, to reduce the radiation dose caused by medical imaging.

Materials and Methods: To gain experience and to develop the method, we added a DWIBS sequence to the standard MRI examinations of pediatric oncology patients. The MRI scans were performed on a 1.5 T Philips Achieva MRI scanner, with diffusion encoding in three directions, and a b-factor of 800 s/mm². The body coil was used, and for greater coverage the data was acquired in blocks, with the table position shifted, and later combined using FDK (Phillips, Best, and later combined using FDK (Phillips, Best, and later combined from the full data acquisition to get 10 slices to avoid series scan time for the DWIBS sequence was between 5 and 6 minutes per patient. Several of the patients also underwent a PET exam, with images acquired after an injection of 4 MBq 18F-FDG. At least 2 fuor-2-deoxy-2-glucose (FDG).

Results: DWIBS images of pediatric oncology patients with neuroblastoma as well as several other diagnoses were acquired. The findings on the DWIBS images corresponded well with the results of the conventional MR images, and with the PET exams. A prospective study to further evaluate the potential of DWIBS in pediatric oncology is planned.

Conclusion: DWIBS is a promising method with a potential to replace other imaging modalities in pediatric oncology imaging. Using DWIBS involving no ionizing radiation which makes it especially suitable for imaging young children with neuroblastoma.

Email: throstur@yahoo.com

SEL14
Diffusion-weighted whole-body imaging with background body suppression (DWIBS) in pediatric oncology patients - a feasibility assessment
Finnboagason, Thorstrú1; Ehnharm, Bo1; Jacobsson, Hans2; Nordell, Bo2; Guen, Lindan1; Kogner, Per1; Douglas, Lena1; Hartmann, O.1; Proust-Houdemont, Stephanie1; Benhamou, Etel1; Dubor, Christelle1; Goma, Gisèle1; Gaspar, Nathalie1; Minard-Cloq, Véronique1; Owens, Cordelia1; Hartmann, Olivier1; Olszanowski, Dominique1
1Karolinska University Hospital, Dept of Pediatric Radiology, Stockholm, Sweden; 2Karolinska University Hospital, Dept of Radiology, Stockholm, Sweden; 3Karolinska University Hospital, Dept of Medical Physics, Stockholm, Sweden; 4Karolinska Institutet, Childhood Cancer Research Unit, Stockholm, Sweden

Background: For oncology patients repeated imaging scans are necessary for diagnosis, staging and treatment response monitoring. This may lead to considerable ionizing radiation doses to the patient, depending on the imaging method. Minimizing or avoiding ionizing radiation is especially important regarding pediatric patients. Diffusion-weighted whole-body imaging with background body suppression (DWIBS) is a free-breathing diffusion weighted (DWI) whole-body sequence with multiple acquisitions, resulting in images resembling Position Emission Tomography (PET) images. The potential value of this method in oncology imaging as a substitute for PET has been suggested in the literature. We propose that the method should be evaluated in pediatric oncology, to reduce the radiation dose caused by medical imaging.

Materials and Methods: To gain experience and to develop the method, we added a DWIBS sequence to the standard MRI examinations of pediatric oncology patients. The MRI scans were performed on a 1.5 T Philips Achieva MRI scanner, with diffusion encoding in three directions, and a b-factor of 800 s/mm². The body coil was used, and for greater coverage the data was acquired in blocks, with the table position shifted, and later combined using FDK (Phillips, Best, and later combined from the full data acquisition to get 10 slices to avoid series scan time for the DWIBS sequence was between 5 and 6 minutes per patient. Several of the patients also underwent a PET exam, with images acquired after an injection of 4 MBq 18F-FDG. At least 2 fuor-2-deoxy-2-glucose (FDG).

Results: DWIBS images of pediatric oncology patients with neuroblastoma as well as several other diagnoses were acquired. The findings on the DWIBS images corresponded well with the results of the conventional MR images, and with the PET exams. A prospective study to further evaluate the potential of DWIBS in pediatric oncology is planned.

Conclusion: DWIBS is a promising method with a potential to replace other imaging modalities in pediatric oncology imaging. Using DWIBS involving no ionizing radiation which makes it especially suitable for imaging young children with neuroblastoma.

Email: throstur@yahoo.com

SEL15
Analysis of toxicity and efficacy of high dose chemotherapy with Busulfan and Melphalan followed by stem cell transplantation in high risk neuroblastoma patients: a retrospective study of a large cohort in a single institution
Proust-Houdemont, Stephanie1; Benhamou, Etel1; Dubor, Christelle1; Goma, Gisèle1; Gaspar, Nathalie1; Minard-Cloq, Véronique1; Owens, Cordelia1; Hartmann, Olivier1; Olszanowski, Dominique1; et al.
1Institut Gustave Roussy, Pediatrics Department, Villejuif, France; 2Institut Gustave Roussy, Biostatistics Department, Villejuif, France

Background: The impact of high dose chemotherapy (HDC) and hematopoietic stem cell transplantation (HSCT) in the treatment of high risk neuroblastoma patients has been well established. We previously presented the positive impact of the Busulfan-Melphalan (BuMel) regimen in these patients. In this study, we analyze the toxicity and survival of a large cohort of patients treated with BuMel at the Pediatric Department, Institut Gustave Roussy.

Method/approach: We evaluated comprehensive data prospectively collected between 1980 and 2008 concerning all patients aged more than one year treated with HDC and SCT for high risk neuroblastoma. Patients enrolled on the HR-NBL1/ESIOP protocol were excluded.

Results: From October 1980 to December 2008, 209 patients aged more than one year were treated with BuMel and HSCT for high risk neuroblastoma. The median age at diagnosis was 40 months (range 12-218), the sex ratio was 1.4 and 88% of patients had an abdominal primary tumor (4% bone marrow). Bone marrow involvement was detected in 80% of cases and N-MYC amplification was present in 30% of tumors. HDC was followed by autologous HSCT. Grafts consisted of bone marrow, peripheral stem cells and both in 50%, 46% and 4% of the patients respectively. Mean duration of hospitalization and neutropenia was 48 and 18 days respectively (range 15-143 and 3-66 days). Grade 3/4 mucositis occurred in 75% of patients and veno-occlusive disease complicated 40% of grafts. Overall, treatment-related toxicity significantly decreased with time. The 5-year EFS and OS post-diagnosis was 50% and 44 % respectively, with a median follow-up of 41 months (range 5-231).

Conclusion: Analysis of this large series is encouraging with an improved EFS at 5 years than the cohort published by our team in 1999 (O. Hartmann et al.). BuMel is currently being compared to the CEM combination in the ongoing NR-NBL1 European protocol. We present our comprehensive toxicity analysis of Busulfan with particular attention to timing, schedule and administration route. The impact of supportive care and the benefit of retinoic acid maintenance therapy are also discussed.

Email: stephanie.proust@gmail.com
SEL16 Natural history of infantile neuroblastoma under “wait and see” observation — current status of patients after long term follow up for 5 - 15 years
Yoneda, Akishin; Inoue, Masami; Oue, Takaharu; Mitani, Yasuyuki; Nose, Ryo; Nakai, Hiroshi; Kawahara, Hisayoshi; Kubota, Akio; Nishikawa, Masaki; Masuda, Kay; Kake, Kosei; Osaka Medical Center for Maternal and Child Health, Pediatric Surgery, Izumi, Osaka, Japan; Osaka Medical Center for Maternal and Child Health, Hiroko, Osaka, Japan; Osaka University, Graduate School of Medicine, Pediatric Surgery, Suita, Osaka, Japan; Osaka Medical Center for Maternal and Child Health, Radiology, Izumi, Osaka, Japan; Osaka Medical Center for Maternal and Child Health, Pathology, Suita, Osaka, Japan

Background: A nationwide screening for 6 month-old infants was performed in Japan between 1985 and 2003. What we learned from experiences of mass screening (MS) for neuroblastoma was that infantile neuroblastomas were mostly favourable and sometimes regress spontaneously. Actually "wait and see" observation programs had been performed in several hospitals in Japan and spontaneous regression was often observed. Now those patients became 6 to 15 years old. The aim of this study was to evaluate the current status of those patients under long term observation, in order to know natural history of infantile neuroblastomas.

Method/approach: Of 88 infantile neuroblastoma patients diagnosed between 1991 and 2004, 29 were entered in the observation program. Of 29 patients, 11 patients underwent surgery because of tumor growth, 18 patients were still under observation. We retrospectively evaluated clinical feature at the disease onset, changes in tumor markers and size, and current status of their tumors.

Results: Of 18 patients, 17 were identified by MS and only one patient was diagnosed incidentally by chest X-ray. The mean age at diagnosis was 4.7 months (range: 1-11.0). Mean follow up time from the diagnosis to the latest hospital visit was 9.4 years (5.2-14.9). Tumor markers had already been normalized in all patients. Mean periods required for normalization of all tumor markers was 3.8 months (0-11.0). No tumor re-growth was observed in all 18 patients currently under observation. We retrospectively evaluated clinical feature at the disease onset, changes in tumor markers and size, and current status of their tumors.

Conclusion: At least 60% of the patients who underwent observation are alive without any symptom. MRI. The mean diameter of current tumors was 23mm (1-65). All patients of all tumor markers was 3.8 months (0-11.0). No tumor re-growth was observed in all 18 patients currently under observation.
Evaluation of the effect of acetyl l-carnitine on experimental cisplatin ototoxicity and neurotoxicity

Dilek Gunes, Kyrkyn Gumay, Kotlan, Elsun; Guneri, Enis Alpin; Ozodul, Candas; Pertebciobu, Buler; Yilmaz, Osman; Tulek, Ozer; Mutafkochi, Aker; Altun, Zeyke; Akhat, Safye; Erbabayrak, Zubeidey; Olguin, Nur

Dokuz Eylul University Institute of Oncology, Pediatric Oncology, Izmir, Turkey; Dokuz Eylul University Faculty of Medicine, Dept of Otolaryngology, Izmir, Turkey; Dokuz Eylul University Faculty of Medicine, Dept. of Laboratory Animal Science, Izmir, Turkey; Dokuz Eylul University Faculty of Medicine, Dept of Histology, Izmir, Turkey; Dokuz Eylul University Faculty of Medicine, Dept. of Pediatrics, Izmir, Turkey; Dokuz Eylul University Institute of Oncology, Pediatric Oncology, Izmir, Turkey; Dokuz Eylul University Institute of Oncology, Dept of Basic Oncology, Izmir, Turkey

Background: Cisplatin is effective and widely used chemotherapeutic agent for the treatment of pediatric solid tumors including neuroblastoma. Ototoxicity is one of the serious dose-limiting side effect of cisplatin. Aims: To investigate the protective role of acetyl l-carnitine (ALCAR) on cisplatin-induced auditory loss in rats by auditory tests, and ultrastructural examination using electron microscope (TEM), and also to investigate the mechanisms of ototoxicity including apoptotic pathways.

Methods: Adult Wistar albino rats (n:28) were studied. At the beginning of the study audiologic assessments including transient oto-acoustic emissions, auditory brainstem response testing were done. There were 4 groups: G1; Saline supplemented control rats; G2; ALCAR administered rats; G3; Cisplatin administered rats; G4; Cisplatin administered rats following ALCAR pretreatment. Rats were sacrificed after control audiological assessments were done at the 3rd day of the study. Brain and inner ear specimens including hair cells and spiral ganglia were examined by TEM, and caspase 3, 8, 9, activity studies were performed.

Results: TUNEL analysis revealed that hearing thresholds by 6 and 8 kHz tone burst stimulus were found significantly low in G4 when compared to G3. The number of TUNEL positive cells, and caspase 3, 8, 9 immunostaining cells were significantly low in G4 when compared to G3. There was no significant difference between G1, G2, G4. Ultrastructural findings of hair cells, spiral ganglia and brain were normal in G1, G2, G4. In hair cells of G3; mitochondrial effacement, mitochondrial crystallasis, intracellular degenerative areas, and damage of intercellular junctions were observed. In spiral ganglia of G3; changes in cells shapes, cell membrane irregularity, secession from satellite cells and increased in cytoplasmic degenerative areas were seen. The ultrastructural damage of brain, spiral ganglia and the organ of Corti was prominent in G3 when compared to G1, G2 and G4.

Conclusions: ALCAR improves cisplatin-induced audiologic impairment and also antioxidative, ant apoptotic properties of ALCAR on cisplatin ototoxicity were supported by the findings.

Email: dilek.gunes@deu.edu.tr

Inhibition of Fatty Acid Synthase (FASN) as a potential therapy in neuroblastomas with MYCN amplification

Mayesp, Patricia; Dikson, Sharon; Attieh, Edward; Marias, John

The Children’s Hospital of Philadelphia, Oncology, Philadelphia, United States

Neuroblastoma is a common and lethal pediatric cancer. More than half of patients are at high-risk for relapse and die despite intensive therapy; thus presenting an urgent need for new, rationally designed drugs. To address the need for new therapeutic targets we sought to identify metabolic genes that are critical to neuroblastoma growth and survival. We restricted our analysis to a set of 12 metabolic genes that can be targeted therapeutically and had previously been implicated in other cancers. Analysis of 188 primary human neuroblastomas genotyped on the Illumina Human1 SNP Array identified high-level, unbalanced gains showed greater FASN mRNA expression than those with two copy number gains greater than the FASN locus (17q25) in 23% of tumors. Analysis of FASN mRNA expression in 99 of these primary neuroblastomas using the IlluminaHT Expression Array identified significant increases in FASN expression restricted to high-risk neuroblastomas with amplification of the MYCN oncogene (P=0.0001). Within the subset of high-risk tumors with MYCN amplification, those that harbored FASN DNA copy number gains showed greater FASN mRNA expression than those with two copy FASN DNA. siRNA inhibition of fatty acid synthase (FASN) in a panel of 12 neuroblastoma cell lines resulted in significant growth inhibition and apoptosis in 91(75%) of lines, including 8/10(100%) of cell lines with MYCN amplification, suggesting a synthetic lethal effect of FASN inhibition in neuroblastomas with MYCN amplification. Ongoing analyses of pharmacologic inhibitors of FASN in neuroblastoma cell lines and animal models including the impact on global lipidomic profiles will be reported. Taken together, an integrated genomic and functional analytic approach has identified fatty acid synthase, a key enzymatic mediator of the newly discovered fatty acid metabolism, as a candidate oncogene and therapeutic target in neuroblastomas harboring MYCN amplification.

Email: mayesp@email.chop.edu
SEL25
Identification and molecular characterization of human neuroblastoma tumor-initiating cells
Coulon, Aurelie; Marjon, Maik; Muhlethaler-Mottet, Annick

Background: Neuroblastoma (NB) displays a cellular heterogeneity within the tumors. There is increasing evidence that at the top of this observed tumor cell hierarchy, there is a sub-population of tumor-initiating cells (TICs), responsible for initiation and maintenance of the tumor. Candidates to characterize NB TICs by prospectively identifying their self-renewal properties. From a very aggressive stage 4 NB sample, we selected self-renewing putative TICs by prospectively identifying their self-renewal properties.

Method/approach: We proposed a novel approach to identify and characterize NB TICs by prospectively identifying their self-renewal properties. From a very aggressive stage 4 NB sample, we selected self-renewing putative TICs by their sphere-forming capacity and analyzed their gene expression profiles by time-course micro-array analysis.

Results: Supervised and unsupervised analyses provided a list of sphere markers genes involved in embryogenesis and nervous system development (CD133, ENGRA, CDK, NOTCH1, GPR177...), and drug resistance (MDR1, ANR1). To demonstrate the existence of TICs in NB tumor that recapitulate the properties of sympathetic precursor cells.

Conclusion: We identified new NB-TICS specific markers and we characterized heterogeneous sphere sub-populations that will be individually analyzed by functional assays.

Email: aurelie.coulon@hospvd.ch

SEL26
Synergy of targeted GMCSF and IL2 to tumor microenvironmements is mediated by an adaptive anti-apoptotic pathway
Lode, Holger1; Bleeeke, Matthias1; Reisfeld, Ralph1; Siebert, Nicola1; Balmas Bourloud, Katia; Nardou, Katya; Gross, Nicole

Background: Two types of genetically engineered antibody cytokine fusion proteins, called immunocytokines (ICs), were engineered and used, i.e. transferrin receptor specific ch17217-GMCSF and ch17217-IL2 and ganglioside GD2 specific ch14.18-GMCSF and ch14.18-IL2. All ICs were characterized by the determination of binding to the target antigen and cytokine activity. Efficacy and mechanism of mono- and combined therapies with GMCSF- and IL2-ICs was determined in the NXS2 mouse model.

We demonstrated that monotherapies with both types of IL2-ICs specifically suppress disseminated neuroblastoma mediated by an NK-cell dependent immune response. A weak, but specific innate response was also observed with both types GMCSF-ICs mediated by granulocytes and NO radicals produced by macrophages in vivo.

Interestingly, a synergistic effect with simultaneous combinations of GMCSF-ICs and IL2-ICs was observed. This combination therapy can completely eradicate established experimental and spontaneous hepatic metastases in contrast to ICs used as monotherapy. It is important to note that this effect was specific and not achievable with mixtures of antibody and cytokine in equivalent doses. Anti-tumor effects of GMCSF-ICs and IL2-ICs combinations were abrogated in Tcell deficient SCID mice and mice depleted of CD8+ Tcells in contrast to controls. Furthermore, splenocytes isolated from mice receiving the combined GMCSF-IC and IL2-IC therapy revealed MHC class I restricted target cell killing in contrast to monotherapy controls and adaptive transfer of these Tcells protected naive mice from neuroblastoma.

These findings indicate a synergy effect combining GMCSF- and IL2-ICs in neuroblastoma explained by a switch from an innate to a neuroblastoma specific ch14.18-GMCSF and ch14.18-IL2. All ICs were characterized by the determination of binding to the target antigen and cytokine activity.

Method/approach: Stable expression of caspase-8 and caspase-10 in Jurkat cells was performed by retroviral infections and caspase-8 silencing of endogenous caspase-10 isoforms to substitute for caspase-8 was complete opposite effect of caspase-10 isoforms was observed in Jurkat cells. 10-B or -G has no effect or was weakly anti-apoptotic. Surprisingly, a complete opposite effect of caspase-10 remain poorly understood, and caspase-10 ability to mediate apoptosis by manipulating the relative expression level of caspase-10 isoforms was performed by retroviral infections and caspase-8 silencing of endogenous caspase-10 isoforms to substitute for caspase-8 was complete opposite effect of caspase-10 isoforms was observed in Jurkat cells. 10-B or -G has no effect or was weakly anti-apoptotic. Surprisingly, a complete opposite effect of caspase-10 isoforms was observed in Jurkat cells.

Results: Overexpression of caspase-10-A or caspase-10-D isoforms strongly increased TRAIL and FAS-L sensitivity of caspase-8 expressing NB and colon carcinoma cells, whereas overexpression of caspase-10-B or -G has no effect or was weakly anti-apoptotic. Surprisingly, a complete opposite effect of caspase-10 isoforms was observed in Jurkat cells.

Conclusion: These data highlight the differential cell type-related pro- or anti-apoptotic role for the distinct caspase-10 isoforms in the death receptor apoptotic pathway.

Results: Overexpression of caspase-10-A or caspase-10-D isoforms strongly increased TRAIL and FAS-L sensitivity of caspase-8 expressing NB and colon carcinoma cells, whereas overexpression of caspase-10-B or -G has no effect or was weakly anti-apoptotic. Surprisingly, a complete opposite effect of caspase-10 isoforms was observed in Jurkat cells.

Conclusion: These data highlight the differential cell type-related pro- or anti-apoptotic role for the distinct caspase-10 isoforms in the death receptor apoptotic pathway.

Results: Overexpression of caspase-10-A or caspase-10-D isoforms strongly increased TRAIL and FAS-L sensitivity of caspase-8 expressing NB and colon carcinoma cells, whereas overexpression of caspase-10-B or -G has no effect or was weakly anti-apoptotic. Surprisingly, a complete opposite effect of caspase-10 isoforms was observed in Jurkat cells.

Conclusion: These data highlight the differential cell type-related pro- or anti-apoptotic role for the distinct caspase-10 isoforms in the death receptor apoptotic pathway.

Results: Overexpression of caspase-10-A or caspase-10-D isoforms strongly increased TRAIL and FAS-L sensitivity of caspase-8 expressing NB and colon carcinoma cells, whereas overexpression of caspase-10-B or -G has no effect or was weakly anti-apoptotic. Surprisingly, a complete opposite effect of caspase-10 isoforms was observed in Jurkat cells.

Conclusion: These data highlight the differential cell type-related pro- or anti-apoptotic role for the distinct caspase-10 isoforms in the death receptor apoptotic pathway.

Results: Overexpression of caspase-10-A or caspase-10-D isoforms strongly increased TRAIL and FAS-L sensitivity of caspase-8 expressing NB and colon carcinoma cells, whereas overexpression of caspase-10-B or -G has no effect or was weakly anti-apoptotic. Surprisingly, a complete opposite effect of caspase-10 isoforms was observed in Jurkat cells.

Conclusion: These data highlight the differential cell type-related pro- or anti-apoptotic role for the distinct caspase-10 isoforms in the death receptor apoptotic pathway.

Results: Overexpression of caspase-10-A or caspase-10-D isoforms strongly increased TRAIL and FAS-L sensitivity of caspase-8 expressing NB and colon carcinoma cells, whereas overexpression of caspase-10-B or -G has no effect or was weakly anti-apoptotic. Surprisingly, a complete opposite effect of caspase-10 isoforms was observed in Jurkat cells.

Conclusion: These data highlight the differential cell type-related pro- or anti-apoptotic role for the distinct caspase-10 isoforms in the death receptor apoptotic pathway.

Results: Overexpression of caspase-10-A or caspase-10-D isoforms strongly increased TRAIL and FAS-L sensitivity of caspase-8 expressing NB and colon carcinoma cells, whereas overexpression of caspase-10-B or -G has no effect or was weakly anti-apoptotic. Surprisingly, a complete opposite effect of caspase-10 isoforms was observed in Jurkat cells.
SEL28
The tumor suppressor candidate gene APITD1/CENP-S on chromosome 1p36 is involved in chromosome segregation and DNA damage repair
Krona, Cecilia1; Kryh, Hanna1; Zellin, Samantha1; Foltz, Dar1; Cleveland, Dor1; Ejskælr, Katarinna1; Sjöberg, Rose-Marie1; Carén, Helena1; Martinsson, Tommy1
1University of Gothenburg, Department of Clinical Genetics, Institute of Biomedicine, Gothenburg, Sweden; 2Ludwig Institute for Cancer Research, San Diego, Cellular and Molecular Medicine, La Jolla, United States

Background: Increased chromosomal instability manifested as aneuploidy is a major driving force for tumor development and progression. Proper chromosome segregation in eukaryotes requires that a multiprotein structure termed the kinetochore assembles on the centromeres and conveys binding of spindle microtubules to chromosomes and subsequent chromosome movement. The APITD1 (a.k.a. CENP-S) gene, in the neuroblastoma tumor suppressor candidate region on chromosome 1p36.2, was recently shown to be part of the CENP-A centromere associated complex that is essential for chromosome segregation.

Method/approach: We used tandem affinity purification of GFP-tagged APITD1 and mass spectrometry to identify new APITD1 binding proteins. To study the involvement of APITD1 in DNA damage repair, we used a multiphoton laser to induce DNA damage to a small local volume (~1 µm3 or less) in living human cells. APITD1 protein expression was also analyzed in different cell lines and in primary neuroblastoma tumors by western blot analysis.

Results: We confirm previous findings that APITD1 binds to the centromere components CENP-U, CENP-T, and CENP-X and we show additional interactions with CENP-P and CENP-Q, further strengthening the findings that APITD1 is part of the CENP-A centromere associated complex. We also show that APITD1 interacts with the Fanconi Anemia associated protein M (FANCM). Since FANCM plays a key role in the Fanconi Anemia DNA damage-response pathway we wanted to test whether APITD1 was also associated with DNA damage. By multiphoton laser induction and live cell imaging microscopy we could determine that APITD1-GFP rapidly accumulates at sites of DNA damage. Furthermore, the level of APITD1 protein is low or absent in primary neuroblastoma tumors with a high frequency of aneuploidy.

Conclusion: We have confirmed APITD1 as a centromeric component and shown that protein levels are low in primary neuroblastoma tumors, possibly contributing to the high prevalence of chromosomal instability in these tumors. Furthermore, APITD1 has a more direct role in sensing DNA damage which may be unrelated to its function as a centromere component.

Email: ckrona@ucsd.edu

SEL29
Conditional MYCN knockdown using shRNAs encoded by lentivirus vectors
Henriksen, Jørn Remi1; Haug, Bjørn Helge1; Buchner, Johan2; Lekke, Cecilie2; Flægstad, Trond1; Einvik, Christer1
1University of North-Norway, Department of Pediatrics, Tromsø, Norway; 2University of Tromsø, Department of Pediatrics, Institute of Clinical Biology, Tromsø, Norway

MYCN amplified (MNA) neuroblastomas are thought to have developed an addiction to the oncogene MYCN, meaning that its proliferation and possibly long term survival is dependent on its high expression. Since the development of RNAi as a tool for target-specific knockdown of gene expression, several studies have shown reduced proliferation and increased differentiation when MYCN expression is reduced in MNA neuroblastoma cell lines. These studies have been performed using transient delivery of synthetic siRNAs or plasmid expressing shRNAs targeting MYCN mRNA. We have previously developed an inducible H1 RNA polymerase III promoter for efficient conditional shRNA expression. In a transient transfection system using luciferase as the reporter target, this promoter showed no apparent transcriptional leakiness in the OFF-state (without doxycyclin-dox). Addition of dox to the media (ON-state) resulted in expression of shRNAs at levels similar to the wild-type promoter. We have now used this promoter in combination with shRNAs targeting MYCN, and stably introduced the construct to SK-N-BE (2) and Kelly MNA neuroblastoma cells using retroviruses as carriers. Induced anti-MYCN shRNA expression in stably transduced MNA neuroblastoma cell lines, resulted in steady down-regulation of MYCN over a period of two days, before stabilizing at appr. 70-80 % MYCN protein knockdown as long as dox was present in the media. These cells showed excessive neuronal-like differentiation and reduced proliferation 3-4 days after induction. Removal of MYCN also abolished the cells ability to form CFU in clonogenic assays. Removal of dox from the media resulted in recovery of MYCN protein expression. Uninduced cells did show slightly reduced proliferation and morphological changes (SK-N-BE (2) compared to scrambled shRNA control, indicating some transcriptional leakiness in the system. This did however not significantly reduce CFU in clonogenic assays. We have developed SK-N-BE (2) and Kelly MNA neuroblastoma cell lines with inducible expression of shRNAs targeting MYCN. These cell lines make it possible to study the long-term effect of removing MYCN from MNA neuroblastoma.

Email: christer@fagmed.uib.no

SEL30
Integration of genome-wide ChIP-data of MYCN/MYC and histone marks with gene expression
Pattyn, Filip1; Pöhler, Christine1; Muth, Daniel1; Gade, Stephan1; BeilBarth, Tim1; Speleman, Frank1; Schwab, Manfred1; Vandesompele, Jor1; Westermann, Frank1
1Ghent University Hospital | German Cancer Research Center (DKFZ), Center for Medical Genetics | Tumor Genetics, Gent I Heidelberg, Belgium; 2German Cancer Research Center (DKFZ), Tumor Genetics, Heidelberg, Germany; 3German Cancer Research Center (DKFZ), Molecular Genome Analysis, Heidelberg, Germany; 4University of Göttingen, Medical statistics, Göttingen, Germany; 5Ghent University Hospital, Center for Medical Genetics, Gent, Belgium

Background: The proto-oncogenes MYCN and MYC are implicated in both normal development and malignant transformation. These proteins appear to integrate environmental signals in order to modulate diverse, and sometimes contradicting cellular processes. Although many studies are available, a comprehensive view on MYCN/MYC function in cancer remains elusive. This is caused by an apparent contradiction between their broad effects on multiple cellular functions and their molecular delineation as a relatively weak transcriptional activator with a poorly characterised set of target genes. To address this issue we performed genome-wide expression profiling and ChIP-chip or ChIP-seq analysis of both transcription factors and three histone marks in neuroblastoma cell lines with high MYCN or MYC gene expression.

Method/approach: ChIP is performed on eight different NB cell lines. ChIP-chip and gene expression hybridizations were performed on their respective Agilent microarray platforms, ChIP-sequencing data was produced on the Illumina Genome Analyzer. After initial ChIP-seq peak finding with the MACS tool we applied a novel feature extraction algorithm, developed to produce continuous DNA-regulator scores. Input samples were included in all ChIP analyses to generate statistically significant binding peaks. All data analyses including the integration of the ChIP and expression data were performed in R.

Results: MYCN/MYC DNA binding is co-occurring with transcriptionally active (H3K4me3) or elongating (H3K36me3) as with inactive (H3K27me3) histone marks. The regulation appears fine-tuned by the binding affinity of MYCN/MYC and the interaction with other DNA-binding proteins. The detailed landscape of the DNA-interactome in combination with gene expression results shows that MYCN and MYC have different modes of action in NB.

Conclusion: This unique genome-wide dataset captures the complex system of gene expression regulated by the low and high affinity DNA-interactions governed by MYCN/MYC in a NB background.

Email: filip.pattyn@ugent.be
SEL31
RUNX3, matched to chromosome 1p36, is a tumor suppressor functional regulator of N-Myc in neuroblastoma
Yokochi, Tomoko1; Gao, Wei1; Yu, Fan1; Yamada, Chizu2; Ozaki, Toshinori1; Ohira, Mikio1; Nakamura, Yohko1; Inoue, Ken-ichi1; Itô, Yoshihiko1; Nakagawa, Atsuko1; Nakagawa, Akira1
1Chiba Cancer Research Institute, Division of Biochemistry and Innovative Cancer Therapeutics, Chiba, Japan; 2Chiba Cancer Center Research Institute, Laboratory of Anti-tumor Research, Chiba, Japan; 3Center for Neural Engineering and Genomic Genomics, Chiba, Japan; *National University of Singapore, Cancer Science Institute of Singapore, Singapore, Singapore; †National Center of Child Health and Development, Division of Clinical Laboratory, Tokyo, Japan.

Background: RUNX3, a runt-related transcription factor, is a tumor suppressor gene mapped to chromosome 1p36 and is epigenetically silenced in several human cancers. However, the role of RUNX3 in neuroblastoma (NB) has remained elusive. Thus, we examined whether RUNX3 is involved in the progression of NB and unveiled its novel molecular function.

Method/approach: RUNX3 expression levels were determined utilizing quantitative real time PCR in 110 primary NBs. We also investigated biochemical properties of RUNX3 protein in the context of DNA damage response pathway.

Results: The RUNX3 region of chromosome 1p36 was deleted in 12 out of 59 NBs examined by an array-based analysis. This hemizygous deletion was closely associated with decreased levels of RUNX3 mRNA expression (p=0.02). Low RUNX3 expression was strongly associated with poor outcome of NBs (p=0.016) and was significantly correlated with INSS stage (p<0.001) and MYCN copy number (p=0.047), but not with other clinical factors such as age or tumor origin. Shimada pathology, Ki67 expression, and DNA index. On the other hand, there was no significant correlation between the stages and the promoter methylation of RUNX3 gene, suggesting that other epigenetic modulation in addition to gene dosage may participate in its transcriptional regulation.

Interestingly, among the patients with NBs in which MYCN is highly expressed, only those with high levels of RUNX3 expression could survive (15 out of 31, p=0.003). In support with this finding, our in vitro data indicated that RUNX3 and MYCN physically interact and mutually affect their expressions. Furthermore, overexpression of RUNX3 induced neurites outgrowth in SH-SY5Y NB cells. We have also found that RUNX3 translocates from the cytoplasm into the nucleus, physically interacts with p53, and acts as a transcriptional co-activator of p53 by enhancing phosphorylation at Ser15 to induce apoptosis after DNA damage.

Conclusion: Our results suggest as a tumor suppressor in NB and affects the prognosis. Genetic and molecular dysregulation and affects self-renewal, proliferation and differentiation of autonomic neural progenitors.

SEL32
The p53 target Wig-1 is a novel regulator of N-Myc at the mRNA level
Anna, Vibhuti1; Cinzia, Bersani1; Margareta, Wilhelm1; Weng-Chen, Lui1; Klas, Wiman1
1Karolinska Institutet, Oncology-Pathology, Stockholm, Sweden; 2Karolinska Institutet, Microbiology, Tumor and Cell Biology, Stockholm, Sweden; 3Karolinska Institutet, Center for Molecular Medicine, Stockholm, Sweden

The p53 tumor suppressor triggers cell cycle arrest, apoptosis, or a variety of other responses after exposure to cellular stress. p53 exerts its biological function at least in part through transcriptional activation of target genes. One such gene is wig-1 (for wild-type p53 induced gene 1). Our biochemical studies have revealed that the Wig-1 protein binds double stranded (ds) RNA with high affinity in vitro and in living cells. We have also shown that Wig-1 binds to a U-rich region in the 3'UTR of the p53 mRNA and stabilizes it by preventing deadenylation, the first and rate limiting step of mRNA degradation. U-rich regions are a subgroup of the AU-rich elements (AREs) known for regulating mRNA stability and translation. There are a number of proteins known to bind and regulate mRNAs containing AREs, and these proteins generally target multiple ARE-containing mRNAs. The mRNA of the lymphokine interleukin 2 contains several AREs, and we show here that Wig-1 binds to and regulates the N-Myc mRNA through its 3'UTR. Wig-1 knockdown using small interfering RNA (siRNA) decreases N-Myc protein and mRNA levels and induces morphological differentiation in SK-N-BE(2) neuroblastoma cells carrying N-Myc amplification, phenocopying the effect of knocking down N-Myc itself. In conclusion, Wig-1 is a novel regulator of N-Myc mRNA and appears critical for maintaining high N-Myc levels in neuroblastoma cells.

Email: anna.vibhuti@ki.se

SEL33
Neurocristopathy-associated Phox2b mutations cause Sox10 dysregulation and affect the differentiation of autonomic neural progenitors
Enomoto, Hideki1; Nagashimada, Mayumi1; Ohta, Hiroshi2; Wakayama, Takuro1; Nakao, Kazuki2
1RIKEN Center for Developmental Biology, Lab for Neuronal Diff and Reg, Kobe, Japan; 2RIKEN Center for Developmental Biology, Lab for Genomic Reprogramming, Kobe, Japan; 3RIKEN Center for Developmental Biology, Lab for Animal Resource and Genetic Engineering and Core Technology Center, Kobe, Japan.

Background: Phox2b is a paired homeodomain transcription factor essential for the development of visceral neurons. Heterozygous mutations of the PHOX2B gene have been identified in a neurocristopathy syndrome associated with Congenital Central Hypoventilation Syndrome (CCHS), Hirschsprung's disease (HSCR, intestinal aganglionosis) and neuroblasticoma.

Method/approach: To understand how these PHOX2B mutations affect development of the enteric and sympathetic nervous system, we introduced two of these mutations into the mouse Phox2b locus by gene targeting and examined the resulting phenotype in these mouse mutants.

Results: Mice heterozygous for these PHOX2B mutations developed to term but died soon after birth due to the lack of spontaneous breathing. These mice also displayed hypoplasia or absence of the enteric ganglia, and size reduction and ectopic formation of the sympathetic ganglia. No obvious neuroblastoma formation was observed in the mutant mice before birth. Developmental analyses revealed that Sox10 expression was abnormally high and persistent in the enteric and sympathetic neural progenitors of Phox2b mutant mice, which led to biased differentiation of those progenitors toward the glial lineage. Moreover, the numbers and size of neurospheres generated from the enteric and sympathetic ganglia of Phox2b mutant embryos were significantly reduced as compared to those of control embryos.

Conclusion: Phox2b mutant knockin mice displayed phenotypes reminiscent of the syndromic neurocristopathy in human, demonstrating that the pathogenic effects of mutant Phox2b are conserved between human and mouse. Mutant Phox2b affects the maintenance and differentiation autonomic progenitors possibly through dysregulation of Sox10.

Email: enomoto@cdb.riken.jp

SEL34
In vivo analysis of human neuroblastoma cell lines in a human embryonic stem cell derived microenvironment - impact of cues from the microenvironment
Cedervall, Jessica1; Jamil, Seema1; Hultman, Isabell1; Ali, Rouknuddin2; Karl, Lena1; Orego, Abe1; Sandstedt, Bengt1; Johnson, John-Inge1; Kogner, Per1; Åhlund-Richter, Lars1
1Karolinska Institutet, Women’s and Children’s Health, Stockholm, Sweden; 2Karolinska Institutet, Clinical Neuroscience, Stockholm, Sweden; 3Karolinska Institutet, Oncology and Pathology, Stockholm, Sweden; 4Karolinska Institutet, Laboratory of Cancer Research, Stockholm, Sweden

We describe the use of a pre-clinical model for studies on Neuroblastoma (NB), based on a species-specific embryonic microenvironment, derived from human Embryonic Stem cell (hESC) Teratoma (hEST), contributing an NGF growth support. The lines IMR32, Kelly and SK-N- BE(2) were injected into mature benign hESC-derived teratoma tissues and tumour growth compared to xenografts from the same lines. A clear tropism was detected in the hEST-model with an exclusive integration into mesenchymal stroma. An impact from the micro-environment was detected on all three cell lines with individual variations in histology, morphology and marker profiles. In general, despite cell line dependent variations, a trend towards a higher inter-tumour heterogenity was found in the hEST-model. This notion was also further supported from IHC with focal variations of markers of aggressiveness and differentiation. Furthermore, expression of Cox-2, an enzyme correlating with proliferation and apoptosis of NB, differed between the models. While the hEST-model showed similar findings to the primary NB tumour mass, ie homogenous staining of 100% Cox-2+ cells, xenografts showed a focal expression. Variation in frequencies of vimentin expressing cells was a further difference between the microenvironments, with a tendency for a biased support towards the hEST-model. A high inter-individual variation in both xenografts. Similar to previous reports there occurs a vascularisation of hESC origin was induced by injections of NB tumour cells. A small minority of cells in the hEST-model expressed human CD31, indicative of an endothelial differentiation from the NB cells them selves. In summary, this species-specific embryonic in vivo model offers a higher maintenance of heterogeneity of the NB tumour microenvironment in this respect, more resembling clinical neuroblastomas. Altogether, we propose that the model offers potentially more relevant preclinical studies on biological features of NB, as well as therapy response, enabling higher efficacy of clinical translation. Considering the high frequency of therapy resistance, and high number of deaths in this malignancy, this is of utmost importance.

Email: jessica.cedervall@ki.se
SEL35
Tenascin-C/Oct-4 perivascular neuroblastoma cells serve as progenitor-cell--derived endothelial cells
Pezzolo, Annalisa1; Parodi, Federica; Maninipetru, DanIo; Raffagallo, Lizza1; Coccio, Claudia; Pistorio, Angela1; Mosconi, Manuela1; Gambini, Claudia; Cilli, Michele; Deaglio, Silvia; Malavasi, Fabio; Pistorio, Vito1
1IRCCS Fondazione Istituto Nazionale Tumori, Department of Diagnostic and Laboratory Medicine, Genoa, Italy; 2Animal National Cancer Institute, Research Facility, Genoa, Italy; 3Department of Genetics, Biology and Biochemistry, University of Genova, Italy; 4University of Genoa, Department of Genetics, Biology and Biochemistry, Genoa, Italy

Background/Aims: Primary neuroblastoma (NB)-associated endothelial microvesicles (EMV) were derived by tumor-cell (TEC) or tumor-bearing mice (TB), which are genetically unstable and chemoresistant. Here we have addressed the identification of TEC progenitors in NB focusing on the Oct-4 and Tenascin-C (TNC) markers.

Methods: Twenty-NBC primary NB tissue samples, 10 metastatic bone marrow (BM) aspirates, and five human NB cell lines were tested for the presence of TNC/Oct-4 cells by immunofluorescence combined with MYCN-specific fluorescence in situ hybridization (FISH). Two MYCN amplified NB cell lines (HTLA-230 and GI-Li-N) were injected orthotopically in the adrenal gland of immunodeficient mice. TEC were detected by human CD31 immunofluorescence staining and MYCN FISH. NB-bearing mice were treated with anti-human CD31 or anti-human and mouse prostate specific membrane antigen (PSMA) monoclonal antibodies (mAbs), that target exclusively endothelial cells. Tube formation was investigated using a Matrigel System (Trevigen) containing VEGF.

Results: HTLA-230, ACN, LANS, SHSYS-Y, but not GI-Li-N, cell lines co-expressed TNC and Oct-4 (range 2-30%). All NB tumors and BM aspirates contained TNC/Oct-4 cells (ranges 2-30% and 0-2.1-5%, respectively). MYCN amplified TEC lined a half of EM from tumors formed by HTLA-230 cells. No TEC were detected in tumors formed by GI-Li-N cells. HTLA-230 cell lines of TNC/Oct-4 NB cells were identified in perivascular niches and expressed cancer stem cell-related (SOX-2, CD133, CD24, Nestin, III tubulin, p75, HTER and HIF-2) and NB-related (DD2, NB84, CD56) markers. Number and proliferating fraction of TNC/Oct-4 NB cells increased significantly (p<0.0012 and p<0.0001, respectively) in tumors from mice treated with mAbs to CD31 or prostate specific membrane antigen (PSMA) suggesting involvement of these cells in TEC differentiation. Indeed, TNC/Oct-4 cell isolated HTLA-230 cells, i) formed tubes in a Matrigel assay and ii) differentiated into tube in vitro VE-cadherin+

Conclusion: TNC/Oct-4 NB cells represent a source of TEC and a novel potential therapeutic target.

Email: annalisapezolo@ospedale-gaslini.pe.it

SEL36
NVP-BEZ235 a dual PI3K/mTOR inhibitor destabilizes Mycn in vitro and inhibits tumor growth in vivo. Therapy is highly effective against MYCN amplified tumor cells of NB and driven by its cell cycle and genomic aberrations assessed using aCGH. Cell lines established from tumors were serially propagated in mice, and the effect of the Myc inhibitor, NBT-272, on NSC growth was analyzed.

Results: In contrast to parental JoMa1 cells, JoMa1-MYCN cells were able to grow in cell culture independent of c-Myc expression, and their s.c. injection into nude mice caused formation of NB-like tumors, as deduced from histology. Tumorigenicity of the cells was enhanced upon serial transplantation. Tumors originating from JoMa1-MYCN cells expressed variable levels of MYCN and were inefficiently confluently cultured in nude mice. These tumors were histopathologically analyzed, MYCN expression analyzed by RT-qPCR and genomic aberrations assessed using aCGH. Cell lines established from tumors were serially propagated in mice, and the effect of the Myc inhibitor, NBT-272, on NSC growth was analyzed.

Conclusion: Taken together, our results suggest that RITA or its analogs might serve as a good strategy to target therapy-resistant NB.

Email: Mikhail.burmakin@ki.se

SEL37
Development of novel therapeutic strategy for neuroblastoma: Reactivation of the p53 tumor suppressor function by small molecules
Hedstrom, Elisabeth; Shi, Yao; Burmakin, Mikhail; Selivanova, Galina
Karolinska Institutet, Department of Microbiology, Tumor and Cell biology (MTC), Stockholm, Sweden

Background: Upon development of metastasis and therapy resistance neuroblastoma (NB) becomes the most deadly tumor of childhood. The tumor suppression function of p53 is crucial for the prevention of tumor development as well as for the success of anticancer therapy. Mutations of p53 gene occur in around 50% of human tumors. In tumors that retain wild type p53 its tumor suppressor function is inhibited via overexpression of its target genes, including pro-apoptotic BAX and PUMA as well as cell-cycle arrest p21 gene. Our studies using SHEP cells in which the expression of N-myc is regulated by doxycycline showed that treatment with anti-N-myc oncogene are efficiently killed by RITA. We are planning to address the effect of RITA on the growth of NB xenografts in mice.

Conclusions: Taken together, our results suggest that RITA or its analogs might serve as a good strategy to target therapy-resistant NB.

SEL38
Modeling neuroblastomagenesis from neural crest stem cell in vitro and in vivo
Schulte, Johanna1; Boehr, Anna; Lindner, Sveni; de Preter, Kaeltef; Speleman, Frank; Van den Berge, J; Molenaar, Jan; Versteeg, Rogier; Pfajfer, Christian; Maurer, Jochen; Schorle, Hubert; Schramm, Alexander1; Eggert, Angelika1
1University Children’s Hospital Essen, Pediatric Oncology and Hematology, Essen, Germany; 2Ghent University Hospital, Center for Medical Genetics Gent (CMG2), Ghent, Belgium; 3AMC Amsterdam, Dept. of Human Genetics, Amsterdam, Netherlands; 4University Children’s Hospital Essen, Pediatric Oncology and Hematology, Essen, Germany; 5University of Louvain, Pathology, Leuven, Belgium

Background: Accumulating evidence suggests that neuroblastoma (NB) originates from neural crest stem cells (NCSC). MYCN has been shown to promote cell cycle progression of NB cells in vitro, and transgenic overexpression of MYCN is sufficient to induce NB in mice. However, the origin of NB has still not yet been clearly assigned to a defined cell population.

Method/approach: To further address this question, we established an immortalized multipotent NCSC line, JoMa1, isolated from a transgenic mouse expressing a conditional 4-OHT (Tamoxifen) inducible allele of c-Myc (c-MycERT). JoMa1 cells express p75 and Sox10 stem cell markers, proliferate robustly depending on 4-OHT induced c-MycERT activity, and can be differentiated into all derivatives of the neural crest. JoMa1 cells were stably transfected with a MYCN cDNA, and their tumorigenicity was analyzed in nude mice. Tumors were histopathologically evaluated, MYCN expression analyzed by RT-qPCR and genomic aberrations assessed using aCGH. Cell lines established from tumors were serially propagated in mice, and the effect of the Myc inhibitor, NBT-272, on NSC growth was analyzed.

Results: In contrast to parental JoMa1 cells, JoMa1-MYCN cells were able to grow in cell culture independent of c-Myc expression, and their s.c. injection into nude mice caused formation of NB-like tumors, as deduced from histology. Tumorigenicity of the cells was enhanced upon serial transplantation. Tumors originating from JoMa1-MYCN cells expressed variable levels of MYCN and were inefficiently confluently cultured in nude mice. These tumors were histopathologically evaluated, MYCN expression analyzed by RT-qPCR and genomic aberrations assessed using aCGH. Cell lines established from tumors were serially propagated in mice, and the effect of the Myc inhibitor, NBT-272, on NSC growth was analyzed.

Conclusion: We here show for the first time that transformed NCSC cells can give rise to neuroblastoma. Importantly, we show that NCSC are the precursor cells of NB. We are currently analyzing the transforming capacity of other potential NB oncogenes in the JoMa1 model system.

Email: johannes.schulte@uni-duesseldorf.de

Selected Posters

Abstract Book 143
SEL39
FOXO3/FKHR1L1 is activated in high-risk neuroblastoma and contributes to chemotherapy resistance and angiogenesis
Geiger, Kathrin; Hagenbucchner, Judith; Rupp, Martine; Salvador, Christina; Meister, Bernhard; Seri, Consolato; Obexer, Petra; Aussier, Michael
1Tyrolean Cancer Research Institute, Pediatric Oncology Laboratory, Innsbruck, Austria; 2Medical University Innsbruck, Department of Pediatrics II, Innsbruck, Austria; 3University of Alberta, Department of Laboratory Medicine and Pathology, Alberta, Canada; 4Medical University Innsbruck, Department of Pediatrics IV, Innsbruck, Austria

Background: FOXO transcription factors control programmed cell death, stress resistance and longevity in normal and malignant cells. We investigated the expression, subcellular localization and phosphorylation of FOXO3/FKHR1L1 in tumor sections of post chemotherapy neuroblastoma (NB) patients and analyzed its effect in cultured neuroblastoma cells.

Methods: Paraffin-embedded sections from 27 NB patients were analyzed for FOXO3 expression, localization and phosphorylation. Effects of chemotherapeutic agents on FOXO3 subcellular shuttling were assessed by live cell fluorescence imaging in ECFP-FOXO3 transgenic NB cells. To study how FOXO3 modulates survival of NB cells we generated cell lines expressing a conditional PKB-independent FOXO3 allele (FOXO3(A3)ERtm) that can be activated by 4OH-tamoxifen and studied the effects of FOXO3 activation in vitro and in vivo by xenograft transplantation into nude mice.

Results: We found that FOXO3 was localized in the nucleus and phosphorylated by PKB in human neuroblastoma tissue sections. FOXO3 protein was increased in high-risk NB patients. FOXO3 nuclear localization and phosphorylation significantly correlated with reduced patient survival. The chemotherapeutics etoposide and doxorubicin led to rapid nuclear accumulation and increased phosphorylation of FOXO3 at the PKB sites T32 and S-253 in NB cell lines as measured by live cell fluorescence imaging. Interestingly, resistant NB cell lines expressing the conditional FOXO3(A3)ERtm allele became resistant to chemotherapeutic-induced cell death whereas NB15/FOXO cells underwent spontaneous apoptosis upon FOXO3 activation. However, when transplanting NB15/FOXO cell lines into nude mice, low-level activation of FOXO3 strongly induced angiogenesis of NB tumors in vivo.

Conclusion: The combined data suggest that FOXO3 is activated in high risk NB tumors and contributes to chemotherapy resistance and tumor angiogenesis.

Email: michael.j.aussierlechner@i-med.ac.at

SEL40
Segmental chromosome aberrations and ploidy in localized neuroblastomas without MYCN amplification – Report from the SIOP Europe Neuroblastoma (SIOPEN) Group
Ambros, Inge M; Tonini, Gian Paolo; Couturier, Jerome; Beiske, Klaus; Benard, Jean; Boavida, Mario; Bowin, Nick; Caron, Huib; Combaret, Valerie; De Bernardi, Bruno; Michon, Jean; Ambros, Peter F; Garaventa, A; Gross, N; Haupt, R; Kohler, J; Jeason, M; Ladenstein, R; Lunej, J; Marques, B; Martinson, T; Nogueru, R; Parod, S; Rubie, H; Schleiermacher, G; Speleman, F; Van Roy, N; Vichla, A; Villamon, E; Tonini, GP

Background/Aims: The neuroblastomas were analyzed by pan-genomic (array comparative genomic hybridization, aCGH) and/or multigenomic techniques (multiplex ligation-dependent probe amplification, MLPA), fluorescence in situ hybridization (FISH) and flow cytometry (FCM). All genetic data were collected in a database by the SIOPEN Biology Group.

Results: Pan-/multigenomic data were available for 67 tumours and FISH for an additional 39 tumours. SCA were more frequently associated with near-di-/tetraploidy than with aneuploidy (p<0.0075, Fisher’s exact test, 65 patients). The most frequently affected loci were: 17q (21.7%), 1p1q (12.3%) followed by 1q (8.1%). Relapse-free survival (RFS) was significantly related to 1p loss (p=0.02, Logrank test, 106 patients). OS and RFS were significantly lower if patients had diploid neuroblastomas (p<0.02 and p<0.03, respectively). Moreover, while OS and RFS were significantly associated with SCA in patients over 18 months of age at diagnosis (p<0.015 and p<0.00035, respectively, 106 patients, FISH negative results included), this relationship was not observed for patients below 18 months (p=0.28 and p=0.59, respectively).

Conclusion: Based on the analysis of this patient cohort, it is suspected that in localised resectable neuroblastomas with normal MYCN status, the clinical impact of SCA depends on the age of the patient at diagnosis.

Email: inge.ambros@ccri.at

SEL41
Segmental chromosome abnormalities and age over 36 months at diagnosis are associated with increased risk of relapse in localized unresectable neuroblastoma without MYCN amplification – A preliminary report from the SIOP Europe Neuroblastoma (SIOPEN) Biology Group
Defferrari, R; Mazzocco, K; Ambros, IM; Ambros, PF; Bedwell, C; Beiske, C; Benard, J; Garaventa, A; Gross, N; Haupt, R; Kohler, J; Jeason, M; Ladenstein, R; Lunej, J; Marques, B; Martinson, T; Nogueru, R; Parod, S; Rubie, H; Schleiermacher, G; Speleman, F; Van Roy, N; Vichla, A; Villamon, E; Tonini, GP

Background:
Recent reports suggest that segmental chromosome abnormalities (SCA) in neuroblastoma (NB) without MYCN gene amplification correlate with worse outcome. So far, no treatment modifications have been made for children with stage 2 and 3 disease over 1 year of age at diagnosis based on this information. The current study presents validated multigenomic data concerning the presence and the prognostic impact of SCA on a cohort of patients over one year with Localized Unresectable Neuroblastoma without MYCN amplification.

Methods:
Between January 2001 and October 2006, a total of 161 newly diagnosed children were enrolled in the SIOPEN Unresectable Neuroblastoma study (EUNB). Out of 161 tumours, 157 were analyzed by Interphase FISH (i-FISH) for MYCN amplification and 1p deletion, while Multiplex Ligation-dependent Probe Amplification (MLPA) and array-Comparative Genomic Hybridization (array-CGH) were performed on 56 and 38 tumours respectively. Genetic data were reviewed by members of the SIOPEN Biology Group.

Results:
One or more segmental chromosome abnormalities were detected in 49 (52%) tumours. Of the 7 recurrent chromosome aberrations (gain of 1q, 2p, 17q; loss of 1p, 3p, 4p, 11q), described in NB the most frequently observed were: 1q gain (33%), 11q loss (23%), 1p loss (22%) and 2p gain (12%). Compared to children at the age range 12-18 and 19-36 months, those<36 months showed the highest frequency of the number of SCA (p=0.027, Kruskal-Wallis test). In these patients the presence of at least one of these aberrations was associated with poor overall and progression free survival (p=0.06 and p=0.043, respectively). This effect was not evident in younger children.

Conclusion of the study:
In this cohort of patients with not MYCN amplified tumours, the presence of at least one SCA increased with age at diagnosis and was associated to poor overall and progression free survival only in children >36 months. Our data suggest that other chromosome abnormalities than MYCN could play an important role in tumor development and progression.

Email: gianpaolo.tonini@istge.it
SEL42
Drug-induced senescence in MYCN-amplified neuroblastoma - gene expression profiling and functional consequences

Taschner-Mannd, Sabine1; Kowalska, Agata1; Binder, Heidehardt1; Rieder, Dietmar2; Trajanoski, Zlatko1; Khan, Jawed1; Speleman, Frank1; Ambros, Inge1; Ambros, Peter1
1Children’s Cancer Research Institute, Department for Tumourbiology, Vienna, Austria; 2Medical University Innsbruck, Bioinformatics, Innsbruck, Austria; 3NCl, NIH, Center for Cancer Research, Washington, United States; 4Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium

Background: Cellular senescence, a permanent state of proliferative arrest, provides a barrier to tumorigenesis in vivo. Activation as well as inactivation of oncopgenes can cause senescence and are associated with inhibition of the growth of tumor developing at later stages. In MYCN-amplified (MA) neuroblastoma (NB), we previously identified hydroxyurea (HU) as a substance that has the tendency to induce the elimination of non-senescent and senescent NB cells and describe their functional role.

Methods: Two NB cell lines with MNA (STA-NB-9 and -10) were genetically characterized before and after senescence induction by MLIAP and arrayCGH. In order to identify the sequential pathways involved in senescence induction, changes in the gene expression profile were analyzed and validated by qRT-PCR. Functional analyses revealed that senescent NB cells were assessed by co-cultivation with non-senescent NB cells or PBMGs for their immune-stimulatory capacity.

Results: We found that MNA is the only chromosomal aberration that changes between non-senescent and senescent NB cell lines, while all other aberrations remain unchanged. Senescent NB cells down-regulate unfavored pathways, like BIRC1, cyclin-dependent kinase inhibitors and tumor markers (NTRK1, erin receptor, CD44). Furthermore, the cell cycle-inhibitors p21, p15 and p16 and the tumor growth- and angiogenesis inhibiting factors HEDGEHOG and INHBA were increased. Functional analyses revealed that senescent NB cells may reduce cell growth of non-senescent tumor cells in vitro and in vivo and increase with expression of MMHC and other immune-response-related molecules. Senescent NB cells allow T-cell and NK-cell activation.

Conclusion: We hypothesize that HU induces a senescent, non-malignant, immunogenic state in neuroblasts. These data provide the basis for future studies using HU as a senescence inducer in neuroblastoma patients to prevent tumor relapse.

Email: sabine.taschner@cci.at

SEL43
Parvovirus H1 induces oncolytic effects on human neuroblastoma cells in vitro and in neuroblastoma xenograft-bearing nude rats

Lacroix, Jeannine1; Leuchs, Barbara1; Hiristov, Georgi1; Li, Junwei1; Deubzer, Hedwig E2; Runmeltrae, Jean1; Will, Olaf1; Scheholer, Jorg R2; 1German Cancer Research Center, Tumor Virology, Heidelberg, Germany; 2German Cancer Research Center, Clinical Cooperation Unit Pediatric Oncology, Heidelberg, Germany

Background: H-1PV is an oncolytic parvovirus of rodents that is non-pathogenic, highly cytolytic and can be administered via intratumoral injection. It has been shown to have relevant oncolytic effects on a broad variety of adult cancers. Here, we performed a pre-clinical evaluation of the therapeutic efficiency of H-1PV for the treatment of neuroblastoma in vitro.

Methods: Neuroblastoma cell lines with different MYCN status as well as non-malignant primary cells of different origin were infected with H-1PV. We determined infection efficacy, viral replication, and lytic activity and cell viability in vivo and in vitro. To determine the therapeutic efficiency of intratumoral H-1PV infection in vivo, human MYCN amplified or over expressing neuroblastoma cells were implanted subcutaneously in immunodeficient rats. Tumor volume was monitored by sonographical 3D-reconstruction, and body weight, clinical signs and survival were monitored.

Results: Non-neoplastic cells were unaffected by H-1PV. All neuroblastoma cell lines analyzed were infectable with H-1PV and competent for virus replication. In infected neuroblastoma cell cultures viral genomes copy numbers increased up to 10,000-fold within 48 to 96 hours. Parvovirus H1 induced lytic infection in all 11 neuroblastoma cell lines tested between MOIs varying in the order of 0.001 and 10 pfu/cell. The cytotoxic effect of H-1PV on neuroblastoma cells could be shown to be mediated by G2-arrest and subsequent apoptosis induction and was independent of MYCN oncogene amplification status. In vivo no toxic side effects were observed in infant nude rats after injection of H-1PV. In human neuroblastoma xenotransplant bearing animals reduction of tumor growth and prolongation of survival could be measured by a single intratumoral H-1PV-infection. In some treated animals, we observed complete neuroblastoma regression and relapse-free survival of more than 6 months.

Conclusion: The efficiency of H-1PV infection, virus replication and relevant lytic effects on neuroblastoma cells in vitro and in vivo, together with the low toxicity of H-1PV for non-malignant infant cells, make this parvovirus a promising option for oncolytic virotherapy in neuroblastoma patients.

Email: j.lacroix@dkfz.de

SEL44
Targeting MYCN in neuroblastoma with small molecules in vitro and in vivo

Zirath, Hanna1; Segerströmm, Lovia; Frenzel, Anna1; Kogner, Per1; Henriksson, Marie1; 1Karolinska Institutet, Department of Microbiology, Tumor and Cell biology (MTC), Stockholm, Sweden; 2Astrid Lindgren’s Children’s Hospital, Karolinska Institutet, Childhood Cancer Research Unit, Stockholm, Sweden

Background: MYCN-amplification is strongly related to poor clinical outcome in neuroblastoma patients, with low survival rates despite novel advances in treatment strategies. An alternative treatment option for children with MYCN-amplified neuroblastoma is therefore urgently needed. Our aim is to identify selective small molecules that induce cell death in neuroblastoma cells with high MYCN expression. Here, we will present data on one such compound.

Methods: MYCN-amplified and non-amplified neuroblastoma cell lines were treated in vitro to test the effect of our candidate compound on cell death induction and growth arrest. Immunoblotting was used to monitor effect on MYCN protein levels in treated neuroblastoma cells. The effect of MYCN Max interaction and MYCN Max DNA binding was studied as well as an MYCN-driven transgenic mouse model of neuroblastoma.

Results: Treatment of MYCN-amplified cell lines with our candidate compound resulted in decreased MYCN protein levels and induction of apoptosis in a concentration-dependent manner in all MYCN-amplified but not in the majority of non-amplified cell lines tested. In vivo treatment of established tumors delayed tumor growth of either xenografted MYCN-amplified human neuroblastoma cell lines or tumors arising in the MYCN-transgenic mice. In addition, in the MYCN-transgenic model, survival after initiation of treatment was significantly enhanced in treated as compared to control animals.

Conclusion: Here, we provide data showing that small molecules specifically targeting the cells over expressing MYCN would be beneficial for the treatment of neuroblastoma. Further analysis of the mechanisms of action and validation of structural analogs of our compound will hence be important for the implication of using small molecules in the management of MYCN deregulated tumors.

Email: Hanna.Zirath@ki.se

SEL45
Protein interactions of the PHOX2B variants identified in patients with neuroblastoma

Wang, Wenchao1; Zhong, Quan1; Luther II, William1; Look, A. Thomas1; Hill, David1; Vidal, Marc1; George, Rani1
1 Dana-Farber Cancer Institute, Harvard Medical School, Department of Pediatric Oncology, Boston, United States; 2 Dana-Farber Cancer Institute, Harvard Medical School, Department of Cancer Biology, Boston, United States

Background: Mutations in the PHOX2B transcription factor have been associated with neural crest disorders, namely, congenital central hypoventilation syndrome (CCHS), Hirschsprung’s disease, and neuroblastoma. While missense and frameshift mutations are mainly seen in neuroblastoma patients, in-frame expansion of the second polyalanine repeat in the PHOX2B gene is the most common aberration in CCHS. It is unclear whether these variants lead to a loss or gain of function. The goal of this study was to investigate the role of PHOX2B in development and disease by identification of interacting protein partners which could then be linked to known cellular pathways.

Methods: We used a human ORFeome-based high-throughput yeast two-hybrid screening system containing 12,000 ORF (open-reading frame) clones. Wild-type (WT) and disease-related mutant constructs, R141G, R100L, K155stop, 676delG (neuroblastoma) and a +7-alanine (27 alanine) repeat mutation in the PHOX2B gene were transformed into yeast cells and mated against cells containing each of the ORF clones in the library. Potential interactors from the primary screen were verified by two rounds of pair-wise mating tests.

Results: We identified a number of protein-protein interactions for WT PHOX2B, which were also shared by the PHOX2B polyalanine expansion variant. However, these interactions were lost in all the neuroblastoma-associated variants. Moreover, the 676delG variant gained novel protein interactions.

Conclusion: Our results show that the neuroblastoma-associated PHOX2B variant, 676delG, not only abolishes normal PHOX2B binding but also acquires novel protein interactions, suggesting a dominant negative effect on the transcription potential of PHOX2B in luciferase assays.

Email: rani.george@dfci.harvard.edu
SEL46
Targeted therapeutics in chemotherapy-refractory neuroblastoma
Gustafson, Christopher1; Houseman, Benjamin1; Chestler, Louis2; Isara, Melissa3; Shukat, Kevin4; Weiss, William A1
1University of California San Francisco, Pediatric Cancer and Blood Diseases, San Francisco, United States; 2University of California San Francisco, Department of Anesthesiology, San Francisco, United States; 3The Institute of Cancer Research, Paediatric Oncology, Sutton, Surrey, United Kingdom; 4University of California San Francisco, Department of Neurology, San Francisco, United States; 5University of California San Francisco, Department of Cellular and Molecular Pharmacology, San Francisco, United States
Amplification of the MYCN oncogene in neuroblastoma is one of the clearest indicators of poor prognosis and heralds a highly chemotherapy resistant and malignant form of the disease. Clinically, nearly all neuroblastomas respond to initial high-dose chemotherapy but a subset of high risk tumors relapse with very poor prognosis. Mycn protein expression is known to be tightly controlled at the post-translational level downstream of the PI3-kinase/AKT/mTOR pathway. Since MYCN is known to play such a central role in a subset of high-risk neuroblastoma, we have screened a panel of inhibitors to PI3K and other pathway members using neuroblastoma cell lines in search of potential candidate drugs which effect apoptosis and Mycn expression. Neuroblastoma tumors almost universally have wild-type p53 at diagnosis, but at relapse mutations in p53 and p53 pathway members are common indicating a potential role for p53 in the chemotherapy resistant phenotype of relapsed disease. In a now well established model of neuroblastoma developed in our lab, transgenic mice expressing human Mycn driven by the rat tyrosine hydroxylase promoter develop neuroblastoma tumors which are genetically and phenotypically similar to human disease. Expression of Mycn is also known to drive apoptosis and therefore, in order to develop neuroblastoma driven by Mycn, tumor cells must bypass this Mycn apoptotic signal perhaps through p53. We have crossed THMYCN neuroblastoma with p53ER-TAM knock-in mice, in which p53 can be rapidly and reversibly restored. These THMYCN/p53ER-TAM mice have an increased penetrance of the neuroblastoma phenotype at a younger age. Preliminary results indicate that restoration of p53 function by tamoxifen injection causes the induction of apoptosis in neuroblastoma tumors. This THMYCN/p53ER-TAM may represent an improved model for therapy-resistant, relapsed neuroblastoma and is an ideal platform for testing potential future therapeutic modalities.
Email: gustafsonC@pedi.ucsf.edu

SEL48
Rituximab is a novel neuroblastoma therapy with efficacy against neuroblastoma tumor initiating cells in vitro and in vivo
Angelini, Paola1; Hansford, Loen2; Kaplan, David4; Irwin, Meredith4
1Hospital for Sick Children, Department of Paediatrics and Program in Cell Biology, University of Toronto, Toronto, Canada; 2Hospital for Sick Children, Program in Cell Biology, University of Toronto, Toronto, Canada; 4University of Toronto, Department of Molecular Genetics, Toronto, Canada
Background: Tumour initiating cells (TIC) have been isolated from several solid tumours, and novel therapeutic approaches are under development based on the assumption that these cells are chemoresistant and may be responsible for tumour relapse. Neuroblastoma (NB) TICs isolated from bone marrow metastases express markers consistent with NB and a subset of surface markers characteristic of B-lymphocytes, including CD20 (L. Hansford, ANR 2010). We investigated the sensitivity of NB TICs to Rituximab, a monoclonal chimeric anti-CD20 antibody currently used for the treatment of CD20+ lymphoma.
Method/approach: Immunophenotype of NB TIC (NB12, NB88R, NB122R2) and NB adherent cell lines (SK-N-SH, SK-N-AS, SH-SY5Y) was performed by flow cytometry (FACS). In vitro Rituximab activity was tested by complement-mediated cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC) assays. The effect of Rituximab +/- goat anti-human antibody (to induce hyper-crosslinking) and +/- chemotheraphy was tested by Alamar Blue and FACS (apoptosis and cell cycle analyses). For in vivo studies, tumours established by injection of TICs intradermally in BALB/c nu/nu mice were treated with Rituximab or control human IgG1 20 mg/kg intra-peritoneal days 1 and 4.
Results: NB TIC (but not adherent NB lines, even when grown in TIC conditions) express the B-cell surface molecules CD20, IgM, CD74, CD19, CD22, MHC class II and CD32. Rituximab induced CDC and ADCC in vitro for all tested NB TICs. Treatment with Rituximab alone or hyper-crosslinked reduced TIC viability in vitro, and pre-treatment with chemotherapy was more effective. Rituximab expression is known to be responsible for tumour relapse. Neuroblastoma (NB) TICs isolated from bone marrow metastases express markers consistent with NB and a subset of surface markers characteristic of B-lymphocytes, including CD20 (L. Hansford, ANR 2010). We investigated the sensitivity of NB TICs to Rituximab, a monoclonal chimeric anti-CD20 antibody currently used for the treatment of CD20+ lymphoma.
Conclusion: NB TICs display a unique immunophenotype including B-cell markers and may represent a novel therapeutic target in neuroblastoma.
Email: paola.angelini@sickkids.ca

SEL47
Validation of Survivin as a therapeutic target in neuroblastoma
Lamers, Fieke1; Schild, Linda2; van der Ploeg, Ida3; Elia4; Martil5; Koster, Jan6; Versteeg, Rogier1; Caron, Huib1; Molenaar, Jaap1
1Academic Medical Center, Human Genetics, Amsterdam, Netherlands
The inhibitor of apoptosis protein BIRC5 (Survivin) is a gene located on the SRO of chromosome 17q. Using Affymetrix mRNA expression data, we show that BIRC5 expression is strongly up-regulated in neuroblastoma compared to normal tissue, other adult malignancies and compared to non malignant fetal adrenal neuroblasts. The high BIRC5 expression is correlated to 17q gain indicating that the genetic aberration on 17q is contributing to the overexpression. Finally, the overexpression of BIRC5 strongly correlates to a bad prognosis, independent of 17q gain. To further validate BIRC5 as a potential drug target we used both LNA and tetravalent shRNA to inhibit BIRC5 in neuroblastoma cell lines. Both BIRC5 antisense techniques caused a specific knock down on mRNA and protein level. This resulted in massive apoptosis as indicated by PARP cleavage and an increase of the sub-G1 fraction on FACS analysis. BIRC5 has two functions. One is as an inhibitor of the intrinsic apoptotic pathway, the other is stabilization of the microtubules in the chromosomal passenger complex. We investigated the mechanism by which apoptosis is caused after BIRC5 knockdown. We could not detect interaction of BIRC5 with DIABLO or XIAP, two proteins in the intrinsic apoptosis pathway. However, we could show an interaction between BIRC5 and Aurora Kinase B, a protein in the chromosomal passenger complex. In addition we observed PS3 activation after BIRC5 silencing, and we rescued apoptosis after BIRC5 silencing by CASP2 (caspase-2) inhibition. PS3 and CASP2 are known to be associated as a result of chromosomal instability. Immunofluorescence showed multineucleated cells and aberrantly shaped nuclei after BIRC5 knockdown. This indicates that the cells could not finish the cell cycle properly. We conclude that both BIRC5 LNA and shRNA cause a specific inhibition of BIRC5 which results in a pro-apoptotic effect on neuroblastoma cells via mitotic catastrophe. We are currently determining the IC50 of YM-155, a small molecule BIRC5 inhibitor, in a panel of 25 neuroblastoma cell lines. The aim is to find YM-155 sensitivity markers and to find synergy with other inhibitors based on the results described above.
Email: s.g.lamers@amc.uva.nl
POB1

Identification in vitro and in vivo of tumoral glial precursor cells in neuroblastoma

Garcia, Idoia1; Mayol, Gemma1; de Torres, Carmen1; Mora, Jaume1

Email: jmora@hsjdbcn.org

Conclusions:
GD2+/S100A6+ neuroblasts may represent a tumoral glial morphology, displaying flat and enlarged cytoplasms, distinctive features of I-type NB cells, rare GD2+ neuroblastic cells with concomitant S100A6+ amplification. Interestingly, during in vitro neuronal induced differentiation cells correlating with IF results. For MYCN amplified tumors, all FACS-analyzed showed GD2+/S100A6+ representing less than 10% of the cells, while S100A6 was seen in the stromal-glial bundles and endothelial cells. In NB specimens, GD2 staining was detected in all neuroblastic cells (Acosta S, 2009). The aim of this work was to explore the existence of bipotential tumoral precursor cells in neuroblastoma.

Methods:
Double immunofluorescence (IF) for GD2 and S100A6 was performed in 14 primary NBT and 8 metastatic bone marrow specimens. FACs analysis was performed in 7 enzymatically disaggregated tumor samples. The I-type cell line SK-N-BE2C differentiated with 1µM ATRA was used to model GD2+/S100A6+ cells in vitro.

Results:
In NB specimens, GD2 staining was detected in all neuroblastic cells, while S100A6 was seen in the stromal-glial bundles and endothelial cells but also in dispeased neuroblasts. 12 (85%) of 14 diagnostic samples showed GD2+/S100A6+ neuroblasts, being more abundant (15%) in low-risk than (1%) high-risk tumors. GD2+/S100A6+ cells were also identified in some stromal bundles and blood vessels. All bone marrow specimens analyzed showed GD2+/S100A6+ representing less than 10% of the total. By FACs analysis, double stained cells represented 11-33% of total cells correlating with IF results. For MYCN amplified tumors, all FACS-sorted GD2+/S100A6+不同ially stained subpopulations showed MYCN amplification. Interestingly, during in vitro neuronal induced differentiation of i-type NB cells, rare GD2+ neuroblastic cells with concomitant S100A6+ staining appeared. Subsequently, these GD2+/S100A6+ cells changed morphology, displaying flat and enlarged cyttoplasmatic, distinctive features of the S-type cell phenotype.

Conclusions:
GD2+/S100A6+ neuroblasts may represent a tumoral glial precursor subpopulation in NBT.

Email: jmora@hsjdbcn.org

POB2

Integrated analysis of DNA methylation, copy number and mRNA expression identifies novel candidate tumor suppressor genes in neuroblastoma

Alcock, Leah; Buckley, Patrick; Bryan, Kenneth; Das, Sudipto; Walters, Karen; Stallings, Raymond

The Royal College of Surgeons in Ireland, Cancer Genetics, Dublin, Ireland

Background:
Bi-allelic inactivation of tumor suppressor genes has been previously reported in cancer through the concomitant loss of one gene copy through deletion and hyper-methylation of the remaining allele. The purpose of this study was to identify such concurrent events through the integrated analysis of DNA copy number, methylation and mRNA data sets for neuroblastoma (NB) tumor and cell lines.

Methods:
A set of primary NB was analyzed by high resolution aCGH (n=160), and a subset (n=18) of these tumors was further investigated using methylated DNA immunoprecipitation applied to microarrays to assess genome-wide promoter hypermethylation status. NB cell lines treated with 5-Aza-2’-deoxycytidine (DAC) were profiled using mRNA expression microarrays (NimbleGen).

Results:
Potential bi-allelic inactivation events were identified through an integrated analysis of MYCN amplified (MNA) and 11q- tumor sub-groups profiled for DNA methylation and DNA copy number. In total, 29 genes were both hypermethylated and deleted in a minimum of three mRNA tumor subtypes while 51 genes were similarly identified in a minimum of three tumors from the 11q- subtype. The majority of genes that were both hemizygozus deleted and hypermethylated from the MNA subgroup mapped to either chromosome 1p or 19p, while the majority of such “two hit” genes in the 11q- subtype mapped to either chromosome 11q or 3p. In order to determine if the hypermethylation affecting the hemizygous allele might be functionally significant, we carried out mRNA microarray expression profiling on three different NB cell lines treated with DAC. In total, 37 genes out of the 80 genes (46%) that were concomitantly hypermethylated and deleted in the primary tumors were re-expressed by greater than 1.5 fold in at least one cell line.

Conclusion:
Integrated analyses of high resolution DNA copy number, methylation and mRNA expression data is a powerful approach for candidate gene selection in neuroblastoma. Potential targets identified through this strategy will form the basis of a functional studies.

Email: leahalcock@rcsi.ie

POB3

Ganglioneuroblastoma, nodular subtype and MYCN amplification: the hospital for sick children experience

Angelini, Paola1; Marrano, Paola2; Thner, Paul2; Irwin, Meredith3; Baruchel, Sylvain4

1Hospital for Sick Children, Department of Paediatrics and Program in Cell Biology, University of Toronto, Toronto, Canada; 2Hospital for Sick Children, Department of Paediatric Laboratory Medicine, University of Toronto, Toronto, Canada; 3Hospital for Sick Children, Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada; 4Hospital for Sick Children, New Agent and Innovative Therapy program, Department of Paediatrics, University of Toronto, Toronto, Canada

Background:
Nodular ganglioneuroblastic neuroblastoma (nGNB) represents ~10% of neuroblast tumours. Despite its poor prognosis, only 2% of cases carry MYCN amplification. However, the presence of both stroma-poor and stroma-rich components makes biological studies prone to sampling error. We investigated MYCN copy number in stroma-rich and stroma-poor areas, to clarify MYCN amplification role and potential heterogeneity in nGNB.

Method/approach:
Patients diagnosed with nGNB between 1987-2008 were identified through Health records. MYCN copy number was assessed by chromogenic in situ hybridization (CISH).

Results:
10 patients were identified and represent this study population. The M/F ratio was 2.3. Although 6/10 were older than 18m at diagnosis, only 2 had metastatic disease. VMA was elevated in 7/9 available cases. The 2 cases with stage 4 disease received chemotherapy and all others were treated with surgery only. 3 patients relapsed (1 with stage 4 disease, 1 with stage 1 multifocal, 1 with stage 2B) at a median time 12.5 months (1.4-31). 2 patients are lost to follow-up, 8 are alive, with (N=2 cases) or without (N=4) disease. Tumour tissue adequate to perform CISH was available for 8 cases. At the single neuroblast level, MYCN copy number was heterogeneous within any given sample and among different patients’ samples. Similar results were obtained in ganglion cells (GC). Schwann cells had normal MYCN copy number. In all but one case the increase in MYCN copy number was related to aneuploidy, as determined by FISH using centromere probes for chromosome 1, 2 and 16. One case was defined as MYCN amplification, since the FISH identified a normal diploid number, while MYCN copy number ranged from normal to 5 in neuroblasts, and from normal to 15 in GC. We observed a complete concordance between neuroblasts from nodular areas and GC from stroma-rich areas.

Conclusion:
Extensive investigation of MYCN copy number confirms that MYCN amplification is present only in a minority of cases of nGNB and does not account for the poor prognosis observed in nGNB. A genome wide approach by SNP-array is underway to identify genetic changes associated with this subgroup of neuroblastoma.

Email: paola.angelini@sickkids.ca
Posters

Interleukin-6-mediated activation of the signal transduction and activator of transcription (STAT3) contributes to chemoresistance and tumor progression in neuroblastoma.

Ara, Tasnim1; Nakata, Rie1; Keshelava, Nino2; Seeger, Robert1; DeClerck, Feyer1

1Keck School of Medicine, University of Southern California, San Antonio Research Inst; 2Children's Hosp Los Angeles, Pediatrics, Los Angeles, United States; 3Children's Hosp Los Angeles, Pediatrics, Biochemistry and Molecular Biology, Los Angeles, United States

Background: There is recent evidence that STAT3 activation plays a central pro-tumorigenic role in cancer progression as it is constitutively activated in a number of diverse tumors. Here we tested the hypothesis that STAT3 also contributes to neuroblastoma progression.

Method/Approach: We analyzed a panel of 14 neuroblastoma cell lines including drug resistant and drug sensitive cell lines for STAT3 activation (phosphorylation) and its effect on drug resistance. STAT3 expression was also examined in metastatic bone marrow samples.

Results: We observed that in most neuroblastoma cell lines STAT3 was not constitutively activated. However we documented that it is rapidly activated (15 minutes) when neuroblastoma cells are treated with IL-6 and its soluble agonistic receptor sIL-6R. Twelve of the neuroblastoma cell lines examined expressed the 2 subunits of IL-6 receptor (IL-6Rαβ0 and gp130) but do not make IL-6 and sIL-6R. Activation of STAT3 by IL-6 and IL-6 plus sIL-6R in drug sensitive CHLA255 and SK-N-SH neuroblastoma cells made them resistant to etoposide and methylphenidate. We then documented that treatment of neuroblastoma cells with IL-6 upregulates the expression of several anti-apoptotic proteins, in particular survivin, Bcl-xL, and Bcl-2, and inhibits etoposide-induced cytocyte C released from the mitochondria and activation of the intrinsic apoptotic pathway.

The protective effect of IL-6 on drug-induced apoptosis was abolished when the cells were pretreated with 2.5 µM, a small STAT3 inhibitor, olin by siRNA. We also observed that sIL-6R, which enhances IL-6-mediated STAT3 activation and drug resistance, is produced by monocytes in the presence of neuroblastoma cells. Finally, we found elevated expression of phosphorylated STAT3 and survivin in bone marrow specimens of neuroblastoma patients with bone marrow metastasis.

Conclusion: The data indicate that in neuroblastoma STAT3 activation is primarily mediated by the tumor microenvironment through the production of IL-6 and sIL-6R by stromal cells, and to a point critical for the IL-6/sIL-6R/STAT3 pathway in chemoresistance.

Email: tara@chula.usc.edu

IMMUNOCYTOTOXICITY OF NEUROBLASTOMA - INSIGHTS FROM EXPERIMENTAL MODELS

Ash, Shilpa1; Askensay, Nadir2; Venk, Isaac2

1Schneider Children's Medical Center of Israel, Sackler Faculty of Medicine, Tel Aviv University; 2Sackler Faculty of Medicine, Tel Aviv University

Phox2b-mediated regulation of alkylation expression in neuroblastoma pathogenesis

Bachetti, Tiziana1; Di Paolo, Daniela1; Mirisola, Valentina2; Brignole, Chiara2; Bellotti, Marta2; Caffa, Irene3; Ferraris, Chiara2; Fiore, Michele2; Fornasari, Diego2; Di Lascio, Simondi2; Chiarle, Roberto2; Borghini, Silvia2; Pfeifer, Ursch5; Porzoni, Marco2; Bellolla, Cecchetti5; Persi, Pasqual2; G. Gaslini Children's Hospital, Laboratory of Molecular Genetics, Genoa, Italy; 2G. Gaslini Children's Hospital, Experimental Therapy Unit, Laboratory of Oncology, Genoa, Italy; 3National Cancer Research Institute, Turin, Italy; 4G. Gaslini Children's Hospital, Laboratory of Molecular Genetics, Genoa, Italy; 5University of Milan, Department of Pharmacology, Milan, Italy; 6University of Turin, Dept. Biomedical Sciences and Human Oncology, Turin, Italy

Background and Aims: The immunogenic and complex genetic etiology of neuroblastoma (NB) has been confirmed with the identification of mutations in two genes, encoding for the receptor tyrosine kinase Anaplastic Lymphoma Kinase (ALK) and the transcription factor Paired-like Homebox 2B (PHOX2B), in a limited proportion of NB patients. These two genes have also been found over-expressed in the majority of primary NB samples and cell lines. These observations led us to test the hypothesis of a regulatory or functional relationship between ALK and PHOX2B, lying behind NB pathogenesis.

Methods: The effect of gene expression modulation was studied in vitro in NB cell lines by either siRNA-mediated knock-down or forced-over-expression of each target gene and transcription levels were evaluated by real-time RTPCR and Western blot. PHOX2B binding to ALK promoter was detected by EMISA and is being confirmed by ChIP.

Results: The new finding of a concurrent involvement of ALK and PHOX2B genes in NB pathogenesis provides new insights into molecular biology of NB and opens new perspectives to design novel combined therapeutic approaches.

Email: patriziaperri@ospedale-gaslini.ge.it
POB8
Natural histone deacetylase inhibitor, sulforaphane, inhibits growth and survival of human neuroblastoma
Bayat Mokhtari, Reza1; Yeger, Herman1; Das, Bikul2; Zhang, Libo3; Kumar, Massimo1; Pistoia, Vito1; Rafitghello, Lizzia1; Blaes, Franz1; Hero, Barbara1; G.Gaslini Institute, Laboratory of Oncology, Genoa, Italy; 2Justus-Liebig-University, Giessen, Germany; 3The Hospital for Sick Children, Hematology/Oncology, Toronto, Canada; 4University Children's Hospital, and Center for Molecular Medicine Cologne (CMMC), Department of Paediatric Oncology, Cologne, Germany

Background/aims: Opsonocytosis/myelocytosis syndrome (OMS) is a rare paraneoplastic disorder, often associated in childhood with neuroblastoma (NB). In these patients, NB presents as localized tumor with favourable prognosis. The pathogenesis of OMS is still unknown but the detection of autoantibodies and lymphocytic infiltration in NB samples is suggestive of an ongoing autoimmune response. BCMA (B-cell maturation antigen), and BAFF-R (B-cell activating factor-Receptor) are members of the Tumor Necrosis Factor Receptor Superfamily (TNFRSF) that promote B-cell growth and survival upon interaction with BAFF ligand. Aim of this study was to investigate the role of BAFF, BCMA and BAFF-R in ectopic lymphoid neogenesis of OMS-associated NB.

Methods: Expression of BAFF, BAFF-R and BCMA was evaluated by immunohistochemistry in paraffin embedded specimens of NB patients associated or not with OMS by using Envision System Horse Radish Peroxidase (HRP) (Dako). In vitro expression of BAFF, BAFF-R and BCMA was analyzed by flow cytometry in different NB cell lines before and after treatment with Retinoic Acid or Interferon-γ (IFN-γ). Secretion of BAFF by NB cells was investigated by ELISA kit. In vitro effects of recombinant (r)BAFF were tested by counting the cells with Trypan Blue dye.

Results: BAFF and BCMA were detected both in tumor cells (neuroblasts and ganglion-like cells) and infiltrating B lymphocytes from NB patients with or without OMS. In contrast, BAFF-R was expressed only by lymphoid cells. Similarly, BAFF and BCMA, but not BAFF-R, were expressed in a large panel of NB cell lines. BAFF, BCMA and BAFF-R expression was upregulated by differentiating agents such as RA and IFN-γ. Finally, BAFF was never secreted in NB cells supernatants. Preliminary experiments suggested that BAFF-R did not support proliferation or survival of NB cells. Colony SFN alone or BAFF and their receptors are expressed in tumor tissues from NB patients with or without OMS, and are likely involved in attraction and survival of tumor-infiltrating B cells.

Email: prucci@libero.it

POB9
Promising effects of the PI3K/mTOR inhibitor PI-103 with currently applied chemotherapeutic drugs on neuroblastoma cell lines
Besancon, Odette1; Tytgat, Godelieve1; Leen, René1; Caron, Hubert2; van Kullenburg, André1; Gaslini, Massimo1; Pistoia, Vito1; Raffaghello, Lizzia1; Blaes, Franz1; Hero, Barbara1; G.Gaslini Institute, Laboratory of Oncology, Genoa, Italy; 2Justus-Liebig-University, Giessen, Germany; 3The Hospital for Sick Children, Hematology/Oncology, Toronto, Canada; 4University Children's Hospital, and Center for Molecular Medicine Cologne (CMMC), Department of Paediatric Oncology, Cologne, Germany

Background: Neuroblastoma is a childhood tumor with a poor prognosis and therefore, new therapeutical options are needed. The PI3K/AKT/mTOR pathway is a potent survival-signaling pathway that is aberrantly activated in a variety of human cancers. We investigated the efficacy of the PI3K/mTOR inhibitor PI-103 and determined if inhibition of the PI3K/AKT/mTOR pathway sensitized neuroblastoma cells towards currently applied chemotherapeutic drugs.

Method/approach: Six human neuroblastoma cell lines, three MYCN amplified and three MYCN single copy, were treated with topotecan, gemcitabine, cisplatin, etoposide or doxorubicin alone or combined with 4hr preincubation of PI-103 (80 or 400 nM). Viability in monolayers was measured using the MTS assay and dose-effect curves were generated to perform multiple drug-effect analysis. A combination index (CI) < 1 indicates a synergistic/additive effect of the combination. The effect of PI-103 was also studied in cells growing in spheroids, a three dimensional tumor model and as such a model for micrometastases. Spheroids were treated for 14 days with PI-103 (0 - 600 nM).

Results: In all six neuroblastoma cell lines growing in monolayers a dose- and time-dependent decrease in viability after treatment with PI-103 or currently applied chemotherapeutics was observed. The IC50 values for PI-103 ranged from 130 - 960 nM for the different cell lines. PI-103 showed an inhibiting effect on tumor-spheroids growth (IC50 = 400 nM). A synergistic effect in monolayers was observed for most combinations, with the most pronounced effect for the combination topotecan - PI-103 (CI< 0.65).

Conclusion: Our results showed that the combination of PI-103 with five currently applied chemotherapeutic drugs has a mainly synergistic effect with respect to viability in most neuroblastoma cell lines growing in monolayers, in particular for PI-103 combined with topotecan. Profound growth inhibition of spheroids incubated with PI-103 was observed. Therefore, combinations of PI-103 with currently applied chemotherapeutic drugs might be a promising new strategy in treatment of neuroblastoma.

Email: o.g.besancon@amc.uva.nl
POB11
Role of ATP and myeloid-derived suppressor cells in neuroblastoma

Bianchi, Giovanna1; Prigione, Ignazia1; Emontini, Laura2; Cilli, Michele2; Pellegati, Patrizia3; Di Virgilio, Francesco3; Marigo, Ilaria4; Simonato, Francesca3; Bronte, Vincenzo4; Pistola, Vito4; Raffagallo, Lizzia4; 'G.Gaslini Children’s Hospital, Laboratory Of Oncology, Genoa, Italy; 2National Cancer Institute, Laboratory Of Oncology, Genova, Italy; 3University of Ferrara, Dept of Experimental and Diagnostic Medicine, Section of General Pathology, Interdisciplinary Center for Study of Inflammation, Ferrara, Italy; 'Istituto Oncologico Veneto, Istituto Oncologico Veneto, Padova, Italy

Background/Aim: The biochemical composition of tumor microenvironment is crucial for the modulation of cancer cell growth as well as for the functions of immune cells. Recent findings have shown that solid tumors have an increased concentration of adenosine and extracellular ATP that may exert a pivotal role in the regulation and homeostasis of immunosuppressive cell populations. Aim of this study was to investigate whether:

i) extracellular ATP is a component of neuroblastoma (NB) microenvironment,

ii) myeloid-derived suppressor cells (MDSC) are involved in NB cell growth

Methods: The NXS2 murine NB cell line was stably transfected with plasma membrane lucerase (pmeLUC) probe and inoculated in the tail vein or orthotopically in the adrenal gland of A/J mice. Bioluminescence imaging (BLI) was used to detect extracellular ATP in living animals. MDSC were phenotypically characterized in the blood, bone marrow (BM), spleen and tumor from healthy and NB-bearing mice by cytometric analysis of CD11b, Gr-1, F4/80, IL-4 receptor (r) and CD62L expression. Proliferation of unstimulated and CD3-stimulated splenocytes from healthy and NB-bearing mice was measured by 3H-thymidine incorporation.

Results: Extracellular ATP was specifically detected in the tumor microenvironment of NB bearing mice compared to healthy tissues. The percentage of CD11b+, Gr-1+ cells was found to be higher in the spleen, BM, and blood from NB bearing mice compared to healthy animals. In addition, higher proportions of CD1b+, Gr-1+, IL-4r+ were found in the blood and BM from NB bearing mice vs healthy animals. In contrast, lower percentages of CD11b+, Gr-1+, F4/80+ and CD11b+, Gr-1+, CD62L+ were detected in the blood and BM from NB bearing than control mice. Finally, splenocytes from NB bearing mice proliferated less than those from healthy animals. Preliminary experiments indicate that two MDSC cell lines express the functional purinergic receptor P2X7, through which ATP may be released.

Conclusion: Extracellular ATP and functional MDSC were found to be a relevant component of NB microenvironment and may modulate NB cell growth.

Email: prucci@libero.it

POB12
Non-transcriptional role of MYC and genomic rearrangements in neuroblastoma

Blumrich, Anna1; Muth, Daniel2; Poehler, Christina1; Gade, Stephan2; Simonato, Blumrich, Anne3; Zappata, Marco3; Schwab, Manfred3; Saveljeva, Larissa3; 'German Cancer Research Center (DKFZ), Division of Tumor Genetics, Heidelberg, Germany; 'German Cancer Research Center (DKFZ), Division of Molecular Genetics, Heidelberg, Germany

Background:

Common fragile sites (cFS) represent chromosomal regions that are prone to breakage after partial inhibition of DNA synthesis. They are associated with various forms of DNA instability in cancer cells, and cFS activation is thought to promote tumorigenesis via genomic damage in early stage tumorigenesis. One well-documented genomic alteration departing from cFS is DNA amplification in cancer cells, but its molecular basis remains poorly understood.

Method/approach: The main focus of this study is to map the cFS FRA2C distal of chromosome 2 using six-color FISH and the analysis of its correlation to MYCN amplification in neuroblastoma (NB) cell lines and primary tumors by array CGH.

Results: FRA2C consists of two cFS spanning 747 kb (FRA2Ctel) and 746 kb (FRA2Ccen) at 2p24.3 and 2p24.2, respectively. Both cFS flank a 2.8 Mb non-fragile region containing MYCN. FRA2Ctel and FRA2Ccen are highly enriched in flexibility peak domains compared to non-fragile regions. A perfect 23-23.5 bp (AT/TA) stretch spans the maximum flexibility peak domain in FRA2Ccen. MYCN ampiclon borders and gains clustered in FRA2Ctel or FRA2Ccen in approximately 70% of NB cell lines and primary tumors.

Conclusion: These observations raise the possibility that initiation of MYCN amplification is related to the activation of FRA2Ctel and/or FRA2Ccen.

Email: a.blumrich@dkfz.de

POB13
Expression and clinicla relevance of melanoma-associated antigens in neuroblastoma

Bocca, Paola1; Prigione, Ignazia1; Carlini, Barbara1; Corrias, Maria Valeria1; Ferrone, Sofia2; ‘G.Gaslini Children's Hospital, Laboratory Of Oncology, Genoa, Italy; 2University of Pittsburgh, Hillman Cancer Research Institute, Pittsburgh, United States

Background:

The high molecular weight melanoma-associated antigen (HMW-MAA) and the cytoplasmic melanoma-associated antigen (c-MAA) are expressed by melanoma cells; their serum levels are increased in melanoma patients, and correlate with clinical outcome. The expression of these molecules was evaluated by flow cytometry in Neuroblastoma (NB) cell lines and in short-term cultures of metastatic neuroblastoma (mNB) from NB patients’ bone marrow. c-MAA was strongly expressed in 3 out of 5 NB cell lines and in 3 out of 5 mNB. Its expression was weaker in 2 NB cell lines and undetectable in 2 mNB. HMW-MAA was strongly expressed only in 1 out of 5 NB cell lines and in 2 out of 5 mNB. Its expression was weaker in 2 out of 5 NB cell lines and in 3 out of 5 mNB, and undetectable in 2 NB cell lines. Release of CYT-MAA was evaluated by ELISA. CYT-MAA was released in vitro by 5 NB cell lines and by 3 mNB.

Conclusion: Serum levels of c-MAA were significantly higher in NB patients (median 23.86 ± 2.59 U/ml) than in healthy donors (median 5.733 ± 3.12 U/ml, p=0.0005). The incidence of relapse was significantly higher in patients with serum levels of CYT-MAA >25 U/ml (p=0.0041).

Method/approach: To localize coincidences of MYC binding to DNA, replication origins and double-strand breaks chromatin immunoprecipitation (ChIP) was performed in c-MAA (SH-SYSY, SJ-NB12) and MYCN (Kelly) expressing cell lines. Antibodies against MCM3 and ORC2 (replication origin markers) and MYC and c-MYC as well as pH2AX (double-strand break marker) were used. ChIP enriched DNA fragments were hybridized to a fine tiling array, representing chromosome 2p. The correlation between MYC triggered replication origins and genomic rearrangements on 2p was determined.

Results: For MYCN (Kelly) number of binding peaks on 2p were 2908 and for c-MYC 1799 (SH-SYSY) and 2557 (SJ-NB12). MYCN binding mapped to replication origins at 16 regions (Kelly), c-MYC binding mapped to replication origins at 64 regions (SH-SYSY) and 256 regions (SJ-NB12). Phosphorylated H2AX coincided with replication origins that were bound by MYC at 64 regions (SH-SYSY) and 256 regions (SJ-NB12). The genomic overlap of marker binding for replication origin formation and double strand breaks as well as MYC binding mapped to characteristic genomic rearrangements in NB cell lines, in Kelly (p=0.006), SH-SYSY (p=0.001) and SJ-NB12 (p=0.001). These genomic rearrangements coincide with fragile sites on 2p.

Conclusion: Collectively, number of MYC binding correlated with the genetic background of the cell lines. Correlations analyses suggest that MYC triggered origin formation leads to genomic rearrangements. In consequence, deregulated MYC may contribute to tumorigenesis in a non-transcriptional manner by causing genomic instability in cancer cells.

Email: a.blumrich@dkfz.de

POB14
Expression and clinicla relevance of melanoma-associated antigens in neuroblastoma

Blumrich, Anna1; Muth, Daniel2; Poehler, Christina1; Gade, Stephan2; Simonato, Blumrich, Anne3; Zappata, Marco3; Schwab, Manfred3; Saveljeva, Larissa3; 'German Cancer Research Center (DKFZ), Division of Tumor Genetics, Heidelberg, Germany; 'German Cancer Research Center (DKFZ), Division of Molecular Genetics, Heidelberg, Germany

Common fragile sites (cFS) represent chromosomal regions that are prone to breakage after partial inhibition of DNA synthesis. They are associated with various forms of DNA instability in cancer cells, and cFS activation is thought to promote tumorigenesis via genomic damage in early stage tumorigenesis. One well-documented genomic alteration departing from cFS is DNA amplification in cancer cells, but its molecular basis remains poorly understood.

Method/approach: The main focus of this study is to map the cFS FRA2C distal of chromosome 2 using six-color FISH and the analysis of its correlation to MYCN amplification in neuroblastoma (NB) cell lines and primary tumors by array CGH.

Results: FRA2C consists of two cFS spanning 747 kb (FRA2Ctel) and 746 kb (FRA2Ccen) at 2p24.3 and 2p24.2, respectively. Both cFS flank a 2.8 Mb non-fragile region containing MYCN. FRA2Ctel and FRA2Ccen are highly enriched in flexibility peak domains compared to non-fragile regions. A perfect 23-23.5 bp (AT/TA) stretch spans the maximum flexibility peak domain in FRA2Ccen. MYCN ampiclon borders and gains clustered in FRA2Ctel or FRA2Ccen in approximately 70% of NB cell lines and primary tumors.

Conclusion: These observations raise the possibility that initiation of MYCN amplification is related to the activation of FRA2Ctel and/or FRA2Ccen.

Email: a.blumrich@dkfz.de
POB15
Heterogeneous MYCN amplification - amplicon, genomic background and network analysis

Erichsen, Jennie1; Brunner, Bettina1; Ladenstein, Ruth5; Martinsson, Tommy4; Peters, Peter F1
Children’s Cancer Research Institute, Tumor Biology, Vienna, Austria; 2Medical University of Vienna, Clinical Institute of Pathology, Vienna, Austria; 3Medical University Graz, Department of Pathology, Graz, Austria; 4Göteborg University, Sahlgrenska University Hospital, Department of Clinical Genetics, Institute of Biomedicine, Göteborg, Sweden; 5St. Anna Children's Hospital, Paediatric Oncology, Vienna, Austria

Background: Among 110 MYCN amplified (MNA) neuroblastomas (NB) analyzed at the OCH in the last decade, 26 tumors contained a varying number of tumor areas/without MNA, defined as heterogeneous MYCN amplification (hetMNA) according to INRG biology guidelines. Since the genomic background of hetMNA tumors has not yet been described, we have looked for and characterized segmental and/or numerical chromosome aberrations (SCA and/or NCA) and genetic instability as initiators for amplicon formation.

Methods: Up to 4 tumor pieces of the same hetMNA tumors from 5 patients were cryosectioned. In total, 13 pieces were analyzed by interphase FISH ((i-FISH)) and their DNA was analyzed by MLPA and 250k SNP arrays to investigate the genomic background of hetMNA tumors. MYCN-i-FISH was performed to detect MYCN heterogeneity and combined with DDx probes to assess amplicon composition. H2A.X staining and subsequent MYCN-FISH was performed to correlate DNA double-strand breaks (DSB) occurrence with MNA. Quantification of signals on sections was done with an automatic device.

Results: All analyzed hetMNA tumors showed NCAs, 2 samples from 2 patients each showed only NCAs, 6 pieces from 4 tumors from 2 patients displayed a variety of inconsistent, additional SCAs, and 3 of 4 pieces from a patient had only NCAs. Numerious chromosomes were found to be overrepresented and chromosome 19 was the only disomic one. In four tumor pieces of 4 patients by MLPA/SNP arrays, the fifth showed MNA only in its MYCN-FISH results. One tumor revealed different amplicon compositions in the same piece and another showed two 1p-breakpoints in different pieces. H2A.X staining pattern only partially correlated with MNA cells.

Conclusion: Our data indicates the existence of multiple tumor cell clones and a single hetMNA NB. At the applied resolution, no common SCA is found to be underlying MNA (subtle genomic changes and mutations not tested for). A conclusion on genetic instability as a cause for MNA is premature. Our data strongly supports a detailed approach to hetMNA NB and DNA-based techniques as recommended by the INRG.

Email: dominik.bogen@oczta.onet.pl

POB16
Immunocytochemical study of bone marrow in neuroblastoma patients - Polish experience

Bójek-Marzec, Katarzyna; Wieczorek, Aleksandra; Balwierz, Walenty
Polish-American Institute of Pediatrics, Jagiellonian University, Medical College, Oncology/Hematology, Krakow, Poland

The presence of disseminated neuroblastoma cells in bone marrow is important for clinical staging and risk assessment at diagnosis and for therapy monitoring. Reliable detection of tumor cells in bone marrow may be a factor of great prognostic significance.

In Department of Pediatric Oncology/Hematology in Krakow, from 1 October 2006 to 31 January 2010, frequency of GD2-antigen occurrence were analyzed in 138 bone marrow samples from 41 NB patients. Among all 41 patients evaluated at diagnosis, 23 (56%) had GD2 positive bone marrow immunocytochemistry. In 17 patients, NBL cells were found in classical cytological evaluation. In all cases immunocytochemistry confirmed bone marrow infiltration. In all patients without evidence of NBL cells in immunocytochemistry, there were no blasts found on smears. However, in 6 patients with bone marrow involvement found in immunocytochemistry, classical cytology did not reveal any pathological cells. The number of positive cells in immunocytochemistry ranged between 1 and 180 out of total 1x106 total cell analyzed. The concordance of evidence of bone marrow involvement in classical cytology with bone marrow immunocytochemistry was 84% (44% - negative in both methods, 40% - positive and 16% - discordant between methods), with higher sensitivity of immunocytochemistry.

Despite the fact that cytomorphological screening of bone marrow smears is still the only accepted method for the detection of disseminating neuroblastoma cells, there is a need for checking prognostic value of immunocytochemical assay.

Email: kasibiobek@poczta.onet.pl

POB17
ChiPaway: A tool for visualization and analysis of high-throughput microarray based immunoprecipitation data

Brynn, Kenneth; Buckley, Patrick G.; Murphy, Derek M.; Das, Sudipto; Stallings, Raymond L.
Royal College of Surgeons in Ireland, Department of Cancer Genetics, Dublin, Ireland

Background: The development of high-throughput microarray based techniques have allowed the routine generation of vast amounts of biological data. The current software pipeline for analysing data generated from high-throughput array based immunoprecipitation experiments such as chromatin immunoprecipitation (ChiP) and methylated DNA immunoprecipitation (MeDIP) is generally limited to peak calling and provides limited support for genome-wide visualization and for further downstream analysis such as experimental cross analysis, inter-class comparisons and marker selection. In the course of our research into the involvement of the DNA binding protein MYCN and the impact of DNA methylation in neuroblastoma, we have developed a software application called ChiPaway which aims to fulfil the above needs.

Methods: ChiPaway is a user friendly, cross platform java based, point and click application that allows the user to visualize, interact and compare multiple experimental samples both from the perspective of raw enrichment values and post processed peak data.

Result: As well as basic functionality such as export and import of various formats; genomic visualization and annotation; and co-ordinate and gene name based search methods, ChiPaway also enables rapid assessment of replicates and comparison of peaks generated across alternative sample conditions. Furthermore, ChiPaway also allows comparison between sample classes using machine learning techniques such as cluster analysis and feature selection methods to aid in the discovery of disease markers.

Conclusion: ChiPaway has been developed to support several studies into DNA binding and methylation in neuroblastoma. Ultimately, ChiPaway allows researchers to access genome scale immunoprecipitation experiments simultaneously and rapidly distil these data into usable information to support understanding of disease mechanism and marker selection.

Email: kenneth.bryan@rcsi.ie

POB18
Genome-wide DNA methylation profiling reveals extensive and complex epigenetic alterations in neuroblastomas

Buckley, Patrick G.; Das, Sudipto; Bryan, Kenneth; Stallings, Raymond; Alcock, Leah; Versteeg, Rogier
1The Royal College of Surgeons in Ireland, Cancer Genetics, Dublin, Ireland; 2Academic Medical Center, Human Genetics, Amsterdam, Netherlands

Background: Although a number of studies have reported aberrant methylation and inactivation of selected genes in neuroblastomas (NB), the extent of genome-wide promoter hypermethylation is poorly understood. We have applied methylated DNA immunoprecipitation (MeDIP) to genomic microarrays representing all known promoter and/or CpG islands in the human genome to more fully characterize the epigenome of neuroblastic tumors.

Methods: MeDIP analysis was applied to NB primary tumors (n=18), cell lines (n=7), ganglioneuroblastoma (GNB) (n=4) and ganglioneuroma (GN) (n=6).

Results: The total number of hypermethylated sites per sample ranged from 1,462-5,197. Consistent differences in DNA methylation patterns were identified between cell lines and tumor subtypes, indicating that epigenetic changes play a significant role in adapting cells to in vitro proliferation. Unsupervised hierarchical clustering of methylation data revealed a distinct split between the GN/GNB and NB groups. mRNA microarray expression analyses of cell lines following treatment with 5-aza-2-deoxycytidine allowed us to explore the functional significance of the hypermethylation. The number of genes which were consistently hypermethylated in the GN/GNB group relative to NB was far greater (199 genes) than the opposite comparison (2 genes). Gene ontology analysis carried out on genes hypermethylated in >90% of GN/GNB displayed a statistically significant enrichment for protein kinases, growth factors and mitosis. In total, 70 recurrent large-scale blocks of contiguously hypermethylated promoters/CpG islands were identified, consistent with other studies of breast and colon cancer. The size of these regions ranged from 12.5 kb to 590.6 kb, with a mean length of 96.4 kb, with nearly one-third of the blocks clustering within telomeric regions.

Conclusion: Our results indicate that genome-wide hypermethylation in neuroblastic tumors is highly complex and plays important roles in many cellular processes, including in vitro cell growth and differentiation. We also identify many candidate genes which are potentially silenced through methylation and which will form the basis of functional studies.

Email: pbuckley@rcsi.ie
POB19

Identification of epigenetically regulated genes that predict patient outcome in neuroblastoma
Carén, Helena1; Djøs, Anna2; Nethander, Maria1; Sjögren, Rose-Marie1; Kogner, Per3; Nilsson, Staffan4; Martinsson, Tommy1
1University of Gothenburg, Clinical Genetics, Gothenburg, Sweden; 2University of Gothenburg, Genomics Core Facility, Gothenburg, Sweden; 3Karolinska Institutet, Woman and Child Health, Stockholm, Sweden; 4Chalmers University of Technology, Mathematical Statistics, Gothenburg, Sweden

Background: Epigenetic mechanisms such as DNA methylation and histone deacetylation are important regulators of gene expression and are frequently involved in silencing tumour suppressor genes. The aim of this study is to detect genes abnormally methylated in neuroblastoma, using genome-wide approaches.

Results: We present eight genes (KRT19, PRKCDBP, SCNN1A, POU2F2, TFGB1, COL1A2, DHR53 and DUSP23) that are methylated in neuroblastoma, most of them not previously reported as such, some of which also distinguish between biological subsets of neuroblastoma tumours. A high methylation frequency of SCNN1A, PRKCDBP and KRT19 is significantly associated with adverse outcome in neuroblastoma.

Conclusion: Identification of biomarkers will be important in risk stratification of patients with neuroblastoma in the future and this, methylation status of genes would be a suitable marker. It is essential to make the best prognosis possible to ensure that patients receive the best available treatment, as well as to avoid unnecessary treatment, which can lead to severe side effects in this group of young patients.

Email: helena.caren@gu.se

POB20

Factors affecting the outcome of the p53 mediated DNA damage response in neuroblastoma
Carr-Wilkinson, Jane1; Griffiths, Rebecca2; Elston, Rebecca3; Gamble, Laura, D4; Lunec, John1; Tweddle, Deborah A.5
1Northern Institute for Cancer Research, Newcastle University, Paul O’Gorman Building, Newcastle upon Tyne, United Kingdom; 2Northern Institute for Cancer Research, Newcastle University, Paul O’Gorman Building, Newcastle upon Tyne, United Kingdom

Background: MYCN oncogene amplification occurs in 25-30% of neuroblastoma and is associated with poor prognosis. We previously reported that MYCN amplified (MNA) p53 wild-type neuroblastoma cell lines failed to G1 arrest in response to irradiation, but this could not be attributed to MYCN alone. We hypothesised that a) Genes co-amplified with MYCN and/or b) the predominant cell type (N or S) determines the downstream response to DNA damage in neuroblastoma cell lines.

Method/approach: The MYCN amplicons of five MNA and two non-MNA cell line were mapped using 50K SNP arrays. One MNA (NBL-W) and one non-MNA neuroblastoma cell line (SKNSh) were sub-cloned into N-type and S-type and characterised for expression of BCL-2 and Vimentin, markers for N and S cell types respectively. The response to 4 Gy irradiation was measured using clonogenic survival and FACS analysis for cell cycle arrest and sub-G1 peak for apoptosis. To determine the role of p53 in this process p53 was knocked down using siRNA.

Results: No genes with a potential role in cell cycle regulation were consistently co-amplified in the MNA cell lines excluding a role for co-amplified genes. Mixed N and S type non-MNA SKNSh1 cells underwent a G1 arrest following irradiation and mixed N and S type MNA and high MYCN expressing NBL-W cells failed to G1 arrest following irradiation. Conversely, N-type SHSY5Y and NBL-W cells failed to G1 arrest in response to DNA damage, and showed impaired apoptosis at 4 Gy and MDM2, whereas S-type SHEP and NBL-W cells did undergo a G1 arrest with induction of p21 and MDM2. MYCN was downregulated in NBL-W cells suggesting that the effect of MYCN cannot be excluded. Conversely N type cell lines underwent higher levels of apoptosis than S type cell lines. Following p53 knockdown in SHSY5Y S-type cells there was a decrease in apoptosis and this effect disappeared on the partial depletion of p53.

Conclusion: The downstream response to DNA damage in p53 wild-type neuroblastoma cell lines is p53 dependent, and determined predominantly by the morphological sub-type.

Email: jane.carr-wilkinson@ncl.ac.uk

POB21

Characterization of ALK rearrangements in neuroblastoma
Cazes, Alex1; Boevoi, Valentina2; Agnél2; Barillot, Emmanuel2; Delattre, Olivier1; Janoueix-Lerosey, Isabelle1
1Institut Curie, INSERM U830, Paris, France; 2Institut Curie, INSERM U900, Paris, France; 3Institut Curie, Unité de Génétique Somatique, Paris, France

Background: Neuroblastoma (NB) is an embryonal cancer of the peripheral autonomous nervous system. Recently, activating mutations of the ALK gene, highly expressed in NB, have been identified in sporadic and familial NB cases. The ALK gene encodes a tyrosine kinase receptor that is preferentially expressed in the central and peripheral nervous system during embryonic development. Translocations, leading to fusion proteins containing the tyrosine kinase domain of ALK and involving various partners, have already been shown to be oncogenic in several human cancers, including anaplastic lymphomas, myofibroblastic tumors and a subset of lung carcinomas.

Method/approach: Chromosomal rearrangements were searched by Comparative Genomic Hybridization experiments using an oligonucleotide array covering the entire ALK gene. Inverse PCR experiments were then performed to identify the sequences involved at the breakpoint junctions. In one cell line a NGS (Next Generation Sequencing) strategy was performed to characterize chromosomal rearrangements.

Results: Rearrangements within the ALK gene have been identified in several cell lines and tumors. These rearrangements consist in partial gains or amplifications of ALK, with breakpoints occurring in introns at various positions. Different types of rearrangements have now been characterized, including coamplifications and inverted duplication structures. In one cell line, exhibiting multiple breakpoints within the ALK gene, NGS allowed to pinpoint several of these breakpoints. Further Northern blot experiments will allow to determine whether the rearranged alleles are expressed. Moreover, a protein variant of ALK, identified in a NB cell line, is currently being analyzed by mass spectrometry in order to fully characterize its biological activity.

Conclusion: The complete structural and functional characterization of these rearrangements will allow to determine whether they lead to an oncogenic property of ALK in NB. Such abnormalities of the ALK gene may constitute an alternative mechanism to ALK activation, therefore implicating the ALK receptor in a higher role.

Email: alex.cazes@curie.fr

POB22

Clusterin interacts with HSP60: implications in neuroblastoma development
Chaiwatanasirikul, Korn-Anpong; Sarit, Arturo
Institute of Child Health, UCL, Molecular Haematology and Cancer Biology, London, United Kingdom

Background: Nuclear factor kappa B (NF-κB) is a transcription factor with an established role in the cellular inflammatory immune response and is constitutively activated in different types of human cancers. Previously, a highly conserved heterodimeric sulfated glycoprotein known as Clusterin (CLU) was shown to be a negative regulator of NF-κB activity in neuroblastoma. Suppression of CLU could elicit NF-κB activation and increase markers for epithelial-mesenchymal transition (EMT) process in a MYCN transgenic neuroblastoma model. In our laboratory, we have shown that CLU expression is negatively regulated by the proto-oncogene MYCN, which is widely associated with aggressive types of neuroblastoma tumours. Therefore, we hypothesized that CLU is a tumour and neuroblastoma suppressor protein and aim to investigate the molecular mechanisms by which CLU regulates NF-κB activity in neuroblastoma.

Methods: Luciferase assay was used to investigate which CLU subunits control NF-κB activity. CLU-interacting proteins were identified and confirmed using GSTPull down assay, Mass-Spectrometry and Co-immunoprecipitation.

Results: Only the intracellular form of CLU (cytoplasmic CLU) showed to be a negative regulator of NF-κB activity with the most effective suppression of NF-κB activity within the alpha chain region near the N-terminal. Extracellular CLU (secreted CLU), on the other hand, is not involved in the regulation of NF-κB activity but appeared to be a specific key regulator of AKT activation in the Phosphoinositide-3 Kinase (PI3K) survival pathway. Data from GST-Pull down assay, mass-spectrometry analysis and co-immunoprecipitation demonstrated that a specific chaperone protein named Heat Shock Protein 60 (HSP60) directly interacts with the N-terminal region of intracellular CLU. HSP60 levels correlate poorly with neuroblastoma patient outcome.

Conclusions: This report is the first to describe a novel and direct interaction between intracellular CLU and HSP60, which may play an important role in the regulation of NF-κB activity during neuroblastoma development.

Email: k.chaiwatanasirikul@ich.ucl.ac.uk
POB23

Human mesenchymal stromal cells (hMSCs) enhanced migration and invasion of neuroblastoma cells via SDF-1/CXCR4 and SDF-1/CXCR7 axes

Chen, Godfrey Chi-Fung1; Ma, Ming2

1The University of Hong Kong, Paediatrics and Adolescent Medicine, Hong Kong, Hong Kong; 2The University of Hong Kong, Paediatrics & Adolescent Medicine, Hong Kong, Hong Kong

Background: Neuroblastoma is one of the cancers that commonly invade the bone and bone marrow. The SDF-1/CXCR4 axis has been proposed as an important pathway involved in bone marrow metastasis for various cancers including neuroblastoma. But the role of hMSCs & CXCR7, the other known receptor for SDF-1, in metastatic neuroblastoma is yet to be explored.

Materials and Methods: In this study, we first used a chemokines miniaarray to screen for the chemokines secreted by the hMSCs and confirmed that SDF-1 was one of the secreted factors. W then collected the serum poor conditioned media from hMSC cultures derived from 3 different healthy donors (stored hMSCs previously with IRB approval and written informed consent) and evaluated their SDF-1 levels respectively by EUSA. The expression of CXCR4 and CXCR7 on 5 known neuroblastoma cell lines (BE2C, BE2M17, IMR32, SK-N-LL & SY5Y) with metastatic potential was then determined by flow cytometry. We then performed migration and invasion assay with hMSCs and neuroblastoma co-culture under a transwell system.

Results: SDF-1 can be found in all hMSCs serum poor culture media. Flow cytometry analysis revealed that all of the 5 neuroblastoma cell lines expressed CXCR7, and 4/5 expressed CXCR4. We then found that the migration and invasion of neuroblastoma cells was enhanced by MSCs co-culture or SDF-1 under the transwell migration & invasion assay. The migration efficiency of neuroblastoma in response to hMSCs conditioned media and SDF-1 treatments was considerably higher than that of the control medium (n=3, p<0.01), and such effect could be significantly but not totally blocked by AMD3100, an inhibitor of CXCR4.

Conclusion: Our preliminary data suggested that hMSCs played a significant role in the metastatic potential of neuroblastoma possibly through the release of SDF-1. SDF-1 might act on both CXCR4 and CXCR7 and the blocker of CXCR4 could significantly but not totally block the migration and invasion of neuroblastoma cells.

Email: gcfchan@hkucc.hku.hk

POB24

Alterations of NDK kinase A/ NM23-H1 deregulate c-myc transcription

Chang, Christina; Chan, Kai-Hui; Paris, Larry; Ma, Lin-Jen; Wei, Ren-Shian; Tan, Choon-Yee

National Cheng Kung University, Institute of Molecular Medicine, Tainan, Taiwan

Altered expression and mutations of NDK kinase A (NDPK-A), encoded by nm23-H1, have been detected in patients with metastatic tumors. One of new functions of NDPK-A is to participate in gene regulation. Evidence suggests that the PTPRD gene that encodes PTPs may have diverse influences over NBL cells. For instance, the inhibition of PTPs has been shown to induce either cell cycle arrest or neurotogenesis in NB41 or SH-SYSY NBL cell lines, respectively. In contrast, evidence suggests that the PTPRD gene that encodes PTPs is frequently deleted in NBL, suggesting that some PTPs may potentially harbour tumour-suppressive functions.

We tested the hypothesis that PTP inhibition with sodium orthovanadate (VA) would induce differentiation and suppress the growth of a range of NBL cell lines, while combination treatment with retinoic acid (RA) would increase these effects. We show that treatment with VA decreased proliferation rates, and caused subtle cell-cell specific changes to the cell cycle distribution of 4 NBL cell lines. Treatment with RA, but not VA caused an increase in the percentage of cells in G0, as shown by a reduction in the expression of the proliferative marker Ki67. Furthermore we showed that treatment with VA induced significant increases in neurotogenesis, a cell-structural parameter of differentiation, and that combination treatment with VA and RA greatly enhanced neurotogenesis beyond the effect of either chemical alone. A general inhibitor of PTPs therefore can enhance the effects of RA.

Email: cichang@mail.ncku.edu.tw
POB27
Identification of hypoxia signatures in neuroblastoma cell lines by 11-2 regularization and data filtering
Corriero, Andrea1; Fardin, Paola2; Acquaviva, Massimo3; Barla, Annalisa4; Mosci, Sofia5; Rosasco, Lorenzo6; Veri, Alessandro7; Varesio, Luigi8; Genoa, Italy
Background: Gene expression signatures are clusters of genes discriminating different statuses of the cells and their definition is critical to understand the molecular bases of diseases. The identification of a gene signature is complicated by the high dimensionality of the data and by the heterogeneity of cells response. The 11-2 regularization is an embedded feature selection technique that fulfills all the desirable properties of a variable selection algorithm with the potential to generate a gene signature even in biologically complex settings. We applied this algorithm to detect the signature characterizing the transcriptional response of neuroblastoma tumor cell lines to hypoxia, to a condition of low oxygen tension that occurs in the tumor microenvironment.
Method/approach: We determined the gene expression profile of 9 neuroblastoma cell lines cultured under normoxic and hypoxic conditions. We studied a heterogeneous set of neuroblastoma cell lines to mimic the in vivo situation and to test the validity of the 11-2 regularization. Hierarchical and k-means clustering and supervised approach have been applied to divide the cell lines on the bases of genetic differences. However, the disturbance of this strong transcriptional response masked the detection of the more subtle response to hypoxia. In order to address this issue, data filtering techniques based on prior knowledge have been applied.
Results: The algorithm distinguished the normoxic and hypoxic statuses defining an unbiased output with a leave-one-out error of 17%. This signature is composed by 11 probesets representing 8 genes known to be modulated by hypoxia. We demonstrate that 11-2 regularization outperforms more conventional approaches allowing the identification of a gene expression signature under complex experimental conditions.
Conclusion: The 11-2 regularization, coupled with data filtering, generates an unbiased output with a low classification error. We feel that the application of this algorithm to tumor biology will be instrumental to analyze gene expression signatures hidden in the transcriptome that, like hypoxia, may be major determinant of the disease.
Email: andreacorriero@ospedale-gaslini.ge.it

POB28
Autophagy and its regulation in neuroblastoma
Courroyer, Sonia1; Imbrioglio, Tina V1; Nyalendo, Carine1; Barrelli, Claire1; Tera, Pierre2; Duval, Michel3; Vassal, Gilles3; Sartele, Herve4; 1CHU Sainte-Justine, Centre de recherche, Montreal, Canada; 2CHU Sainte-Justine, Centre de cancérologie Charles-Bruneau, Montreal, Canada; 3Institut de cancérologie Gustave Roussy, Recherche clinique et translationnelle, Villejuif, France
Background/aims: Neuroblastoma (NB) is a frequent paediatric tumour. After combined treatments of chemotherapy, bone marrow transplantation, surgery or radiotherapy, the metastatic tumours still have a low success rate. Finding new therapeutic strategies to increase the survival rate of patients with NB is therefore essential. Autophagy is a self-degradative process that insures a constant protein and organelle turnover. By suppressing altered organelles, autophagy maintains cell survival by adapting to stress, but in some cases can induce cell death. The aim of this study was to determine if autophagy is present and could be regulated in NB.
Methods: Five Tissue Micro Array containing 184 NB diagnosed from 1977 to 2008 were studied. An immunohistochemistry method was used to identify the expression of LC3, a cytosolic protein required for autophagic vacuole (autophagosome) formation, and beclin 1, a positive regulator of autophagy. In addition, two NB cell lines (SK-N-SH and SK-N-DZ) were treated in vitro with six drugs (temozolomide, LY294002, rapamycin, vincristin, doxorubicin and cisplatin). Cell survival was measured by MTT cell proliferation assay. The autophagic vacuoles were labelled with monodansylcadaverine and the result was measured by fluorescence. Autophagosome formation was monitored by immunodetection of LC3 cleavage and beclin 1.
Results: Our study demonstrates that low levels of autophagy are present in a majority of NB. Also, LC3 expression is not correlated with clinical pathological features. By contrast, higher levels of Beclin 1 are expressed in NB of children over one year of age (with poor prognostic). Beclin 1 has a higher expression level in primary tumours than in metastases. In vitro study demonstrates that autophagy could be modulated by chemotherapy that inhibits cell survival. This inhibition was correlated with increase of the autophagic process as shown by the cleavage of LC3, the augmentation of autophagosomes and the expression of beclin 1.
Conclusion: Autophagy is present and could be modulated by chemotherapy in NB. Regulation of autophagy represents a very attractive target to develop new therapeutic strategies for NB.
Email: soniacourroyer@hotmail.com

POB29
All-trans retinoic acid induced differentiation of SK-N-BE cells results in extensive DNA methylation alterations of gene promoter regions
Das, Sudipto1; Buckley, Patrick; Bryan, Kenneth; Watters, Karen; Foley, Niamh; Alcock, Leah; Bray, Isabella; Stallings, Raymond1; Royal College of Surgeons in Ireland and Children’s Research Centre, Our Lady’s Children’s Hospital, Department of Cancer Genetics, Dublin, Ireland
Background: All-trans retinoic acid (ATRA) causes a number of neuronal changes in neuroblastoma (NB) cell lines to undergo differentiation and a similar derivative is included in the final part of the treatment regime for patients with high stage disease neuroblastoma. Here, we examine the impact of ATRA treatment on promoter DNA methylation status in SK-N-BE using a whole genome profiling approach to further understand the molecular mechanism of induced differentiation.
Methods: Methylated DNA immunoprecipitation (MeDIP) was applied to microarrays representing all known promoter and/or CpG island regions of the human genome for pre and post ATRA treated SK-N-BE cells. The transcriptional activity of genes was evaluated using miRNA expression microarrays, and the methylation status of several candidate genes was further validated using bi-sulphite sequencing.
Results: Hybridization of MeDIP samples to microarrays indicated 402 gene promoters were de-methylated 7 days post ATRA treatment, 82 of which were over-expressed greater than 2-fold. One of these genes, nitric oxide synthase 1 (NOS1, re-expressed by 4.8 fold), has been previously shown to promote neuroblastoma cell differentiation (Clari et al J Cell Sci 2004;117:727). Gene ontology analysis of the genes that were de-methylated and re-expressed after ATRA treatment indicated enrichment for signal transduction pathways (p=0.0069). Mechanistically, the widespread demethylation observed following ATRA treatment might be due to the observed decrease in DNMT1 (p = 0.004) and DNMT3B (p = 0.018) gene expression. In contrast, 88 genes became hypermethylated following ATRA treatment, 13 of which were under-expressed by >2 fold. Conclusion: NB SK-N-BE cells induced to undergo differentiation by ATRA were found to have substantial alterations in DNA methylation at numerous gene promoters, potentially as a consequence of altered DNA methylation events triggered by ATRA treatment. These findings are functionally important in neuroblastoma cell differentiation.
Email: sudiptodos@rosci.ie
POB30

Chemokines CXCR5-CXCL13 cross-talk between malignant neuroblastoma cells and schwannian stromal cells suggests a role in the inhibition of metastatic dissemination

Del Grosso, Federica; Coco, Simona; Scaruffi, Paola; Stigliani, Sara; Valdona, Francesca; Benelli, Roberta; Boccardo, Simona; Salvi, Sandra; Truini, Mauro; Croce, Michelle; Ferrini, Silvano; Tonini, Gian Paolo

1National Cancer Research Institute, Translational Paediatric Oncology, Genoa, Italy; 2University of Genoa, Oncology and Genetics (DOBIG), Genoa, Italy; 3National Cancer Research Institute, Oncology and Angiogenesis, Genoa, Italy; 4National Cancer Research Institute, Diagnostic Technologies, Genoa, Italy; 5National Cancer Research Institute, Laboratory of Immunological Therapy, Genoa, Italy

Background: Neuroblastoma stroma-poor (NB-SP) is composed of small undifferentiated Neuroblasts (Nb) and scarce Schwannian Stromal cells (SSc); most of NB-SP onset as metastatic disease. Ganglioneuroblastoma stroma-rich (GBN- SR), 11 NB cell lines and in Nb and SSc isolated by Laser Capture Microdissection. CXCR5 and CXCL13 protein expression was analysed by immunofluorescence, FACS or immunohistochimistry. Cell migration of CXCR5 positive NB cell were performed by chemotaxis assay. The effects of CXCL13 treatments on NB cell lines were investigated by MTT cell proliferation assay.

Results: We found that CXCR5 was more expressed in isolated SSc than in NbN. This finding suggests to us that CXCL13 might have a functional role in the relationship between SSc and NbN and in tumor dissemination.

Method/approach: mRNA expression of CXCL13 and its receptor CXCR5 was evaluated by Real-Time RT-PCR in 14 NB-SP, 14 GBN-SR, 11 NB cell lines and in NbN and SSc isolated by Laser Capture Microdissection. CXCR5 and CXCL13 protein expression was analysed by immunofluorescence, FACS or immunohistochimistry. Cell migration of CXCR5 positive NB cell were performed by chemotaxis assay. The effects of CXCL13 treatments on NB cell lines were investigated by MTT cell proliferation assay.

Conclusion: Our findings suggest that CXCR5-CXCL13 axis could mediate a cross talk between NbN and SSc by creating a tumor environment in which malignant neuroblasts are entrapped and inhibited to grow under the influence of CXCL13 released by schwannian stromal cells. This mechanism could affect the ability of NbN to migrate and give distal metastasis explaining why GBN-SR disease do not show tumor dissemination.

Email: gianpaolo.tonini@istge.it

POB31

N-glycosylation of ALK as a potential target for disruption of prosurvival signaling pathways in neuroblastoma cell lines

Del Grosso, Federica; De Mariano, Marilena; Passoni, Lorenza; Paleari, Laura; Tonini, Gian Paolo; Longo, Lucio

1National Cancer Research Institute (IST), Translational Pediatric Oncology, Genoa, Italy; 2University of Genoa and National Cancer Research Institute (IST), Translational Pediatric Oncology, Genoa, Italy; 3Istituto Nazionale Tumori (INT), Pediatric Oncology, Milano, Italy; 4National Cancer Research Institute (IST), Functional Genomics, Genova, Italy; 5Italian Neuroblastoma Foundation and National Cancer Research Institute (IST), Translational Pediatric Oncology, Genoa, Italy

Background: The Anaplastic Lymphoma Kinase (ALK) is a 200-220 kDa N-glycosylated tyrosine kinase (TK) receptor, whose expression is restricted to the developing nervous system. The catalytic domain of ALK was originally identified in the 1(2;5) translocation that produces the oncogenic fusion protein NPM-ALK and occurs in most of the Anaplastic Large Cell Lymphomas (ALCL). Recently, ALK was identified as the main predisposition gene to both familial and sporadic neuroblastoma (NB) by discovering of activating missense mutations in the TK domain. We examined the effects of tunicamycin, a specific inhibitor of N-glycosylation, on NB cell lines characterized by different ALK features.

Methods: We employed the following NB and ALCL cell lines: SH-SY5Y and LAN-5 (ALK mutation F1174L), NB1 (ALK amplified) and, as controls, LA1-SS (no ALK expression) and SU-DHL1 (NPM-ALK). Cells were treated with 500 nM tunicamycin in time course experiments for up to 60h. Total protein lysates were immunoblotted using primary antibodies for ALK, AKT, STAT3, ERK1/2 and matching phospho-proteins. Cell growth inhibition was assessed with the xCELLigence instrument, which can quantify adherent cell proliferation and viability in real-time. Cell death was performed by FACScan flow cytometry after incubation with propidium iodide and annexin-V.

Results: Tunicamycin treatment of NB cells expressing ALK resulted in the accumulation of a hypoglycosylated ALK band (180 kDa) and decreased amounts of the full size receptor (200-220 kDa). Interestingly, ALK phosphorylation was markedly decreased in the ALK driven NB cell lines (SH-SY5Y, LAN-5, NB1) after 12h and over 60h. The phosphorylation of downstream ALK effectors (AKT, STAT3, ERK1/2) was also considerably decreased. On the contrary, tunicamycin had no effects on phosphorylation of ALK effectors in LA1-SS and SU-DHL1 cells. Moreover, cell viability of ALK driven NB cells was impaired by N-glycosylation inhibition but was minimally affected in control cell lines.

Conclusion: Our results are suggestive that enzymatic steps regulating N-glycosylation are potential targets that may be exploited for novel therapeutic approaches in NB.

Email: luca.longo@istge.it
POB33

The calcium-sensing receptor gene is inactivated by genetic and epigenetic mechanisms in neuroblastomas and its overexpression reduces neuroblastoma proliferation in vitro and in vivo

de Torres, Carmen1; Casalà, Carla1; Ordoñez, José Luis2; Miguel, Solange3; Muneci, Francisca2; Galván, Patricia1; Rodríguez, Eva1; Mayol, Gemma2; García, Idola1; Martí, Elisa1; de Alava, Enrique1; Lavarrino, Cinzia1; Mora, Jaume1
1Hospital Sant Joan de Déu, Developmental Tumor Biology Laboratory, Espugues de Llobregat, Barcelona, Spain; 2Céntrio de Investigaciones del Cánecer, Molecular Pathology Laboratory, Salamanca, Spain; 3Hospital Vall d’Hebron, Research Unit, Barcelona, Spain; 4Consejo Superior de Investigaciones Científicas, Instituto de Biología Molecular, Barcelona, Spain

Background: We have previously reported that the calcium-sensing receptor (CaR) is expressed in differentiated, favorable neuroblastic tumors (NT) and it is up-regulated upon differentiation induction. In both in vitro and in second-hand neuroblastomas (NB), however, CaR expression is undetectable in undifferentiated NB. The transcription of CaR is under the control of two promoters and promoter 2 (P2) is GC rich. Aim: To analyze the genetic and epigenetic mechanisms responsible for the CaR gene inactivation in NT. To evaluate the in vitro and in vivo effects of CaR overexpression on NB cell lines proliferation and metastatic capacities.

Methods: CaR mRNA was analyzed by qRT-PCR in NB cell lines treated with 5-aza-2’-deoxycytidine (DAC) and/or trichostatin A (TSA). Methylation status of P2 was evaluated by bisulite specific PCR and sequencing. A specific probe for the CaR locus was generated to perform fluorescence in situ hybridization (FISH). SK-N-LP and SK-N-D cell lines were stably transfected with pCMV-GFP or pCMV-CaR-GFP. The proliferation rate of independent clones was assessed in vitro and in nude mice. Also, their metastatic capacity was evaluated in a chick embryo model.

Results: CaR mRNA was undetectable in 7/9 cell lines. Increased CaR mRNA levels were seen in LAN1, IMR32, LA1-55n, BE(2)-C, SK-N-D, SK-N-LP, LA1-55s cell lines following DAC+TSA, but not in SH-SY5Y and SK-N-AS cells. Percentage of methylated cytosines in P2 was 8% in control cells, SH-SY5Y and SK-N-AS cells, but it was 21-56% in the other cell lines. Hypermethylation of P2 was found in NT correlated with undifferentiated histology (P = 0.002), age at diagnosis >12mo (P = 0.026), high clinical risk (P = 0.009), MYCN amplification (P = 0.04) and absence of CaR-expressing cells (P = 0.02). Allelic losses of the entire chromosome 3 were seen among NB, GB and GR. Stably transfected pCMV-CaR-GFP clones displayed statistically significant decreased in vitro and in vivo proliferation rates. However, the metastatic pattern of pCMV-CaR and pCMV-GFP clones was similar.

Conclusion: The CaR gene is silenced by genetic and epigenetic mechanisms and it exhibits tumor suppressor properties in NT.

Email: cdtorres@hsjdbcn.org

POB34

3D miRNA mutation screening in neuroblastoma

De Wilde, Bram1; Leefler, Steve1; Mesldagh, Pieter1; Vanderstraeten, Nathalie1; De Schrijver, Joachim1; Pattyn, Filip1; De Preter, Katleen1; Van Peer, Gert2; Stallings, Ray2; Gemma1; García, Idoia1; Martí, Elisa3; de Alava, Enrique3; Lavarino, Cinzia3; Mora, Jaume1
1Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium; 2Hospital Sant Joan de Déu, Developmental Tumor Biology Laboratory, Espugues de Llobregat, Barcelona, Spain; 3Centro de Investigaciones del Cánecer, Molecular Pathology Laboratory, Salamanca, Spain; 4Hospital Vall d’Hebron, Research Unit, Barcelona, Spain;

Background: We have previously reported that the calcium-sensing receptor (CaR) is expressed in differentiated, favorable neuroblastic tumors (NT) and it is up-regulated upon differentiation induction. In both in vitro and in second-hand neuroblastomas (NB), however, CaR expression is undetectable in undifferentiated NB. The transcription of CaR is under the control of two promoters and promoter 2 (P2) is GC rich. Aim: To analyze the genetic and epigenetic mechanisms responsible for the CaR gene inactivation in NT. To evaluate the in vitro and in vivo effects of CaR overexpression on NB cell lines proliferation and metastatic capacities.

Methods: CaR mRNA was analyzed by qRT-PCR in NB cell lines treated with 5-aza-2’-deoxycytidine (DAC) and/or trichostatin A (TSA). Methylation status of P2 was evaluated by bisulite specific PCR and sequencing. A specific probe for the CaR locus was generated to perform fluorescence in situ hybridization (FISH). SK-N-LP and SK-N-D cell lines were stably transfected with pCMV-GFP or pCMV-CaR-GFP. The proliferation rate of independent clones was assessed in vitro and in nude mice. Also, their metastatic capacity was evaluated in a chick embryo model.

Results: CaR mRNA was undetectable in 7/9 cell lines. Increased CaR mRNA levels were seen in LAN1, IMR32, LA1-55n, BE(2)-C, SK-N-D, SK-N-LP, LA1-55s cell lines following DAC+TSA, but not in SH-SY5Y and SK-N-AS cells. Percentage of methylated cytosines in P2 was 8% in control cells, SH-SY5Y and SK-N-AS cells, but it was 21-56% in the other cell lines. Hypermethylation of P2 was found in NT correlated with undifferentiated histology (P = 0.002), age at diagnosis >12mo (P = 0.026), high clinical risk (P = 0.009), MYCN amplification (P = 0.04) and absence of CaR-expressing cells (P = 0.02). Allelic losses of the entire chromosome 3 were seen among NB, GB and GR. Stably transfected pCMV-CaR-GFP clones displayed statistically significant decreased in vitro and in vivo proliferation rates. However, the metastatic pattern of pCMV-CaR and pCMV-GFP clones was similar.

Conclusion: The CaR gene is silenced by genetic and epigenetic mechanisms and it exhibits tumor suppressor properties in NT.

Email: cdtorres@hsjdbcn.org

POB35

Bmi-1 promotes neuroblastoma cell proliferation by regulation of cyclin levels

Ding, Jane1; Mao, Ling2; Ding, Han-Fei3; Medical College of Georgia, Cancer Center, Augusta, United States

Background: Bmi-1 is a member of the Polycomb Group family of transcription repressors and has a critical role in maintaining the proliferative and tumorigenic potential of neuroblastoma cells. The molecular mechanism for the oncogenic activity of Bmi-1 in neuroblastoma pathogenesis remains poorly defined.

Methods: We have generated human neuroblastoma BE(2)-C cells with inducible knockdown of Bmi-1 expression. With this system, we examined, in both the molecular and cellular levels, the effect of Bmi-1 knockdown on neuroblastoma cell proliferation.

Results: We found that knockdown of Bmi-1 arrested BE(2)-C cells in the S phase. This action of Bmi-1 is independent of p14ARF and p16INK4a, as downregulation of Bmi-1 had no effect on p16INK4a expression and overexpression of p14ARF showed no significant impact on the survival or proliferation of neuroblastoma cells. By contrast, Bmi-1 knockdown resulted in a marked decrease in the protein levels of cyclins, which complex with cyclin-dependent kinases to drive cell cycle progression. Microarray analysis of gene expression profiles and quantitative real-time PCR revealed no changes in mRNA levels of the cyclins between control and Bmi-1-knockdown BE(2)-C cells. Interestingly, Bmi-1 knockdown is associated with upregulation of several F-box proteins that function as substrates for the Skp1-Cul1-F-box protein-Rbx1 (SCF) ubiquitin ligase complexes and are responsible for the specific ubiquitination of many cell cycle regulators including cyclins.

Conclusion: Our findings suggest a critical role of Bmi-1 in the control of cyclin degradation in neuroblastoma cells by regulating expression of F-box proteins.

Email: hding@mcg.edu

POB36

Prickle1: Possible tumour suppressive role in neuroblastoma

Dyberg, Cecilia1; Papachristou, Panagiotis2; Ringsedt, Thomas2; Kogner, Per1; Johnsen, John Inge1; 1Karolinska Institutet, Neonatal unit, Department of Woman and Child Health, Stockholm, Sweden; 2Centro de Investigaciones, Instituto de Biología Molecular, Barcelona, Spain

Background: Wts are a family of intercellular signalling factors that regulate a wide range of functions during embryonic development including cell proliferation, cell fate determination, differentiation, cell polarity and cell migration. They bind to Frazzled receptors and signaling thereafter proceeds via Dishevelled (Dvl) proteins to several intracellular pathways known as the canonical and non-canonical signalling pathways. Activation of the canonical pathway results in build up of beta-catenin levels and targets gene transcription. Increased Wnt/ beta-catenin signalling is associated with many forms of cancer, including neuroblastoma. For long it has been suspected that the non-canonical Wnt signaling pathways could interfere with canonical signalling. This is now genetically confirmed, although the mechanism is still unclear. In this study we investigated the function of Prickle1, a planar polarity (PCP) protein, in regulation of the cytoskeleton and Wnts/ beta-catenin activity in neuroblastoma.

Method/approach: The expression of human Prickle1 was examined in SH-SY5Y neuroblastoma cells and the interactions with other PCP proteins were evaluated using immunoprecipitation. Prickle1 and Prickle1 mutants were overexpressed through transfection in SH-SY5Y and the effects on Rac1 and Actin were analyzed by immunocytochemistry. Prickle1 and beta-catenin levels were quantified by Western blotting.

Results: We found that Prickle1 expression driven both in SH-SY5Y cells. Preliminary, increased levels of Prickle1 seem to affect the cytoskeleton of SH-SY5Y cells, as assayed by Rac1 and actin immunoreactivity. Immunoprecipitation demonstrated that Prickle1 is present in complex with Vang2, another planar cell polarity (PCP) protein known to interfere with the actin cytoskeleton.

Conclusion: The planar cell polarity protein Prickle1 is expressed in neuroblastoma with a potential tumour suppressive role with significance for tumour cell behavior by affecting the cytoskeleton. However, the exact mode of interaction between Prickle1 and Vang2 remains to be elucidated.

Email: cecilia.dyberg@ki.se
Focal amplifications and deletions at miRNA loci in neuroblastoma

Background: Recent studies have advanced insights into the genetic basis of neuroblastoma (NB) but more in depth knowledge is required in order to define new molecular targets for therapeutic agents. As part of our ongoing investigation for the role of miRNAs involved in NB, we performed a genome wide screening for focal copy number alterations at miRNA loci.

Method/approach: Array CGH data were obtained from 188 NB samples and 33 cell lines using a 44K/60K custom oligonucleotide array platform, specifically enriched with probes for miRNAs, transcribed ultra conserved regions (T-UCRs) and genes or genomic regions recurrently implicated in NB. Circular binary segmentation values were wave corrected and extracted from our in-house developed database arrayCGHbase. The R environment was used to identify genes residing in focal (defined as smaller than 1 Mb) aberrations.

Results: Array CGH screening detected focal copy number alterations affecting 64 different miRNA loci. Of particular interest was a high level of gain of the miR-17-92 locus in cell line NLF, a miRNA cluster known to be directly regulated by MYCN and shown to be amplified in other tumor types. Focal deletions were detected at 33 miRNA loci, 16 of which were implicated in at least two tumors. Several of these miRNAs are known or presumed to be involved in neuronal development or tumorigenesis. For example, we provide for the first time evidence that miR-15a/16, a known tumor suppressor miRNA, might be implicated in NB as it is deleted in several cases. In addition to these findings, our screening also revealed new information regarding protein coding genes such as a rare amplification of two tyrosine kinase receptors and the telomerase reverse transcriptase hTERT gene as well as a focal deletion of TCF4 and a new recurrent distal 1q deletion.

Conclusion: This study revealed focal copy number alterations targeting miRNAs as well as rare amplifications of a role of miRNAs in NB pathogenesis. Further functional analyses are ongoing in order to determine their contribution to the NB phenotype. Previously unmapped copy number alterations were also detected for important protein coding genes, such as hTERT and TCF4.

Email: annelies.fieuw@ugent.be

POB39

Involvement of delta-like 1 homolog (drosophila) in the development of chemoresistance in neuroblastoma cell lines

Background: As in other cancers, the development of chemoresistance represents a major obstacle in the successful treatment of high grade neuroblastoma (NB). In addressing the mechanisms underlying the chemoresistant phenotype of NB, we previously reported overexpression and activation of FZD1, and hence the association of pathological activation of the wnt/j-catenin pathway to the resistant phenotype of NB.

Method/approach: In this study, a closer analysis of the gene expression profile of doxorubicin-resistant cells (LAN-1-R) was performed.

Results: This analysis allowed us to identify Delta-like 1 homolog (drosophila) or DLK1 as another, moderately but significantly, overexpressed gene in the resistant variants. DLK1, a member of the Notch/delta/serrate family of proteins, is expressed in several embryonic tissues and in adult adrenal glands. DLK1 is also highly expressed in neuroendocrine tumors such as NB, suggesting a possible involvement in the development of the disease. We confirmed the increase in DLK1 expression by real-time quantitative PCR in LAN-1-R vs the non resistant LAN-1 cells with a 5.2-fold stimulation. Higher amounts of DLK1 protein were detectable on the cell membrane of the LAN-1-R by Western blot, as well as released in resistant LAN-1-R cells culture fluid as compared to non resistant LAN-1 cells. To further explore the contribution of DLK1 to the multidrug resistant and malignant phenotype of NB cells, DLK1 was overexpressed in different NB cell lines with variable endogenous DLK1 expression, or silenced by lentiviral-mediated micro-adapter shRNA, in LAN-1-R or NB6 cell lines. The resulting resistance to drugs, tumorigenic and tumor-initiating properties are presented.

Conclusion: Our data which fully support a recent report, convincingly implicating DLK1 in enhanced tumorigenic and tumor-initiating properties are presented. These observations which associate DLK1 to multiple mechanisms leading to the particularly malignant behavior of NB, deserve further investigation in clinical settings.

Email: Marjorie.Flath@chuv.ch
POB41
NF-kB and IRF1, but not MYCN, control the expression of MHC class I and endoplasmic reticulum aminopeptidases in human neuroblastoma cells
Forloni, Matteo1; Albini, Sonia1; Lorenzi, Silvia1; Citoldi, Loredana2; Boldrini, Renata2; Giannini, Giuseppe3; Natali, Pier Giorgio4; Giacomini, Patrizio4; Fruci, Doriana1
1Ospedale Pediatrico Bambino Gesù1, Giovanni Paolo II, Rome, Italy; 2Ospedale Pediatrico Bambino Gesù2, Division of Pathology, Rome, Italy; 3Sapienza University, Experimental Medicine, Rome, Italy; 4Regina Elena Cancer Institute, Laboratory of Immunology, Rome, Italy
Amplification and overexpression of the MYCN oncogene characterize the most aggressive forms of neuroblastoma and are believed to severely down-regulate MHC class I expression by transcriptional inhibition of the p50 NF-kB subunit. We found that in human neuroblastoma cell lines, high MYCN expression is not responsible for low MHC class I expression, since neither transfection-mediated overexpression nor siRNA suppression of MYCN affects MHC class I and NF-kB p50 protein levels. Furthermore, we identified NF-kB as the main regulator of MHC class I, since the p56 NF-kB subunit binds MHC class I promoter in chromatin immunoprecipitation experiments, and MHC class I expression is enhanced by p56 transfection and reduced by inhibition of p56 activity. Interestingly, IRF1 is also involved in the regulation of MHC class I expression, since its overexpression particularly when combined with the overexpression of NF-kB p56, reverses the MHC class I-low phenotype in the most aggressive neuroblastoma cell lines. Like MHC class I, the endoplasmic reticulum aminopeptidases ERAP1 and ERAP2, known to generate MHC class I binding peptides, are regulated by NF-kB and IRF1 and contain functional NF-kB and IRF1-binding elements in their promoters. Consistent with these findings, nuclear p56 and IRF1 could only be detected in the maturating neuroblastic cells that express higher levels of MHC class I molecules in tumor specimens. These findings provide molecular insight into defective MHC class I expression in neuroblastoma, and indicate that activating NF-kB and IRF1 in MHC class I-low, aggressive neuroblastoma cells could be instrumental for successful application of T cell-based immunotherapy.
Email: fruci@qopbg.net

POB42
Differential expression of PI3K-Akt pathway genes in neuroblastoma.
Fransson, Susanne1; Abel, Frida1; Eriksson, Helena1; Martinsson, Tommy1; Ejeskär, Katarina1
University of Gothenburg, Dep. Clinical genetics, Göteborg, Sweden
Background: The phosphatidylinositol 3-kinase (PI3K)-Akt pathway transmits intracellular signals that regulate cell growth, proliferation and survival and is frequently affected in human cancer. Neuroblastoma of different stages are known to be biologically diverse and in this study we investigated whether expression of PI3K-Akt pathway genes differ between favourable and aggressive neuroblastomas.
Methods: The level of mRNA expression of 88 PI3K pathway genes in 24 primary neuroblastoma of different stages was investigated by whole genome GeneChips (HUI33A and HUI33plus2.0, Affymetrix). The 12 genes that showed significant difference of expression between either INRG localized versus metastasizing tumors, or between INSS Stage 1-2 versus Stage 4 neuroblastomas in the microarray study were confirmed by real-time PCR with TaqMan in a larger set of tumors (52 samples). In another set of 24 neuroblastomas the protein expression was examined by Western Blot.
Results: The PI3K pathway genes PDGFRα and IGF1R were significantly up-regulated in MYCN+ and PIK3CD, BAD, PRKCB1, PRKCCZ and FOXO3 were significantly down-regulated in aggressive compared to favourable neuroblastoma tumours in the microarray analysis. Three of the down-regulated genes (CDC42, PRK3CC, PRKCCZ) are located in the 13q36 chromosomal region, and could partly be due to 1p-deletion in the aggressive neuroblastomas.
Conclusion: Some of these genes are also involved in other cell signalling pathways, like MAPK signalling (PDGFRα, BAD, PRKCB1). Taken this into account, our conclusion is that the PI3K-Akt pathway is up-regulated in favourable neuroblastoma tumors, and other pathways, like the MAPK signalling pathway, are more important in the aggressive tumours.
Email: susanne.fransson@cngen.gu.se

POB43
The impact of MYCN on the response to MDM2-p53 antagonists in neuroblastoma
Gabriel, Laura D1; Tweddle, Deborah A.2; Luneç, John2
1Northern Institute for Cancer Research, Newcastle University, Paul O’Gorman Building, Newcastle upon Tyne, United Kingdom; 2Northern Institute for Cancer Research, Newcastle University, Paul O’Gorman Building, Newcastle upon Tyne, United Kingdom
Background: MYCN amplification is a major negative prognostic marker occurring in 25-30% of neuroblastomas. MYC proteins are transcription factors with roles in both cell proliferation and apoptosis. We have recently published evidence showing that p53 is a direct transcriptional target of MYCN in neuroblastoma and p53 mediated apoptosis is an important mechanism of MYCN dependent apoptosis (Chen et al 2010, Cancer Res). Co-amplification of MDM2 with MYCN provides a contributory mechanism by which MYCN amplified neuroblastoma evades p53 mediated apoptosis. MDM2-p53 antagonists activate wild-type p53 that is suppressed by MDM2 and have been found to have antitumour activity in neuroblastoma preclinical models. Hypothesis: The activity of MDM2-p53 antagonists is increased in the presence of MYCN.
Method/approach: Growth inhibition assays for the MDM2-p53 antagonists Nutlin-3 and MI-63 were carried out in the tetracycline regulatable MYCN SHEP Tet21N cell line. Knockdown of MYCN was achieved using siRNA in two MYCN and MDM2 co-amplified neuroblastoma cell lines (NGP and LS) followed by treatment with Nutlin-3 and MI-63. Induction of p53 responsive genes and apoptotic markers were observed using Western blot and changes in cell cycle by flow cytometry. Apoptosis was determined by caspase activity and annexin V staining.
Results: The GI50s for Nutlin-3 and MI-63 increased 4 fold in MYCN- compared to MYCN+ Tet21N cells. Following treatment with MDM2-p53 antagonists alone, an increased p53 response was observed with subsequent G arrest in NGP cells and apoptosis in both NGP and LS cells. After MYCN knockdown and treatment with Nutlin-3 and MI-63, no further effect was seen on the proportion of cells in G0. However, a reduction in levels of apoptotic markers was observed by Western blot in both cell lines and a significant reduction in caspase activity was observed in NGP cells (50% reduction compared to control after MYCN knockdown, p<0.0001).
Conclusion: The reduction of MYCN expression decreases the apoptotic response to MDM2-p53 antagonists. MDM2-p53 antagonists may therefore be particularly effective in high risk MYCN amplified neuroblastoma.
Email: l.d.gamble@ncl.ac.uk

POB44
The role of MDMX on the response to MDM2-p53 antagonists in neuroblastoma
Gabriel, Laura D1; Tweddle, Deborah A2; Luneç, John
Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, United Kingdom
Background: The MYCN protein plays a dual role in driving proliferation and sensitising cells to apoptosis. MDM2 is the major negative regulator of the p53 tumour suppressor and in neuroblastoma cell lines it is often co-amplified with MYCN and in these circumstances may be a mechanism by which MYCN amplified neuroblastoma circumvent MYCN mediated apoptosis. Most neuroblastomas have wildtype p53 and MDM2-p53 antagonists are being investigated to reactivate p53 through inhibition of MDM2. MDMX is also a negative regulator of p53 and is similar in structure to MDM2, but whereas MDM2 regulates p53 stability and activity, MDMX regulates activity only. MDMX removal or inhibition may be necessary to fully activate the p53 response.
Hypothesis: Knockdown of MDMX results in an enhanced response of MYCN and MDM2 co-amplified neuroblastoma cell lines to MDM2-p53 antagonists.
Method/approach: Knockdown of MDMX was achieved using siRNA in two MYCN and MDM2 co-amplified neuroblastoma cell lines (NGP and LS), followed by treatment with the MDM2-p53 antagonists, Nutlin-3 and MI-63. Induction of p53 responsive genes and apoptotic markers were observed using Western blot and changes in cell cycle by flow cytometry. Apoptosis was assessed by caspase activity and annexin V staining.
Results: An increased p53 response was observed following MDM2-p53 antagonist treatment alone, shown by a G arrest in NGP cells and induction of apoptosis in both cell lines. Following MDMX knockdown alone, an increase in p21WAF1 was observed indicating that the p53 response is activated. However, upon addition of MDM2-p53 antagonists following MDMX knockdown the NGP cells still arrested in G1 but in both cell lines the proportion of cells undergoing apoptosis decreased.
Conclusion: This data indicates that cells are more resistant to MDM2-p53 antagonists following MDMX knockdown, suggesting that MDMX expression may be important in determining the response to MDM2-p53 antagonists in neuroblastoma.
Email: l.d.gamble@ncl.ac.uk
POB45 Distinctive expression patterns of MicroRNA in neuroblastoma tumors of poor and favorable outcomes

Gatellier, Charles-Henry; Meurice, Guillaume; Bauer, Matthieu; Nageotte, Damien; Schmit, Sébastien; Bérasain, Christophe; Gattellier, Charles-Henry; Meurice, Guillaume; Bauer, Matthieu; Nageotte, Damien; Schmit, Sébastien; Bérasain, Christophe

Abstract Book 159

Background: We therefore analyzed a neuroblastic tumor series for expression of microRNAs (miRNAs) and their function in neuroblastoma cell lines. The aim was to develop new therapeutic concepts for relapsed tumors. Protein analyses showed different clinical features in NB, offering new insights into PA pathways and PA metabolism.

Methods: We measured the expression of a representative set of miRNAs (all p < 0.005). Interestingly, high miRNA expression also showed significant correlation with poor survival prognosis in Kaplan-Meier analyses stratified for patients without MYCN amplification, suggesting an additional role for miRNA independent of MYCN. Conversely, high OAZ2 mRNA expression correlated with increased survival and with several favorable clinical NB characteristics (all p < 0.003). In addition, we provide first evidence for MYCN-associated transcription factors M2D and M2D7 in OAZ2 expression. In NB cell cultures, ectopic over-expression of MYCN altered ODC, but not OAZ2 mRNA levels. Conclusion: Our data suggest that elevated ODC and low OAZ2 mRNA expression levels correlate with several unfavorable genetic and clinical features in NB, offering new insights into PA pathways and PA metabolism-targeting therapies may be useful.

POB46 Neuroblastoma differentiation signaling pathways

Geerts, Dirk; van Nes, Johan; Revel, Ingrid; Huizinga, Gerda; Selderink, Nathalie; van Sluis, Peter; Koster, Jan; Versteeg, Rogier

Academic Medical Center - University of Amsterdam, Human Genetics, POB45

Background/Aims: The differentiation potential of neuroblastoma, including the (trans-) differentiation of neuroblastoma into ganglioglia onoma or glioma is poorly understood. Elucidation of the signal transduction pathways involved in this phenomenon can identify basic aspects of neuroblastoma pathogenesis. Here we therefore studied a neuroblastoma tumor series for expression of the transcription factor genes involved in nerve crest development and tested their function in neuroblastoma cell lines.

Methods: Genome-wide miRNA profiles (Affymetrix Hu133 Plus 2.0) of 110 neuroblastic tumors and 24 neuroblastoma cell lines were generated. In addition, we manipulated the expression of the transcription factor genes MEIS1, MSX1, MYCN, NOTCH3, and PHOX2B in neuroblastoma cell lines by shRNA-mediated knockdown and inducible over-expression. Time-course experiments after expression manipulation were profiled to identify the downstream pathways of these genes.

Results: Expression profiling showed that MEIS1, MYCN, and PHOX2B were highly expressed in most neuroblastomas, but only weakly in ganglioglia onoma. MSX1 showed the opposite expression pattern. Indeed, a mutational negative regulation between MSX1 and PHOX2B was identified in IMR32 and SHNB cell lines with inducible PHOX2B and MSX1 expression. In agreement with a role in differentiation, MSX1 induced cell cycle arrest in neuroblastoma cell lines. MSX1 activated central genes in neural differentiation routes, like DLK1, HEY1, and NOTCH3 in the Delta-Notch, and DKK1-3 and SFRP1 in the Wnt pathway. These target genes showed the same expression patterns in neuroblastoma versus ganglioglia onoma tumors as MSX1. Together, these data suggest that MSX1 is a key transcription factor activating neural pathway genes in neuroblastoma, while MEIS1, MYCN, and PHOX2B repress these signal transduction routes.

Conclusion: We have identified core signaling networks governing neural differentiation in neuroblastoma. PHOX2B, mutated in $3% of neuroblastomas, and MYCN are part of this network. We propose that this network is instrumental in the balance between differentiation and progressive disease in neuroblastoma.

Email: D. Geerts@amc.uva.nl

POB47 The polyamine metabolism genes ornithine decarboxylase and antizyme 2 predict aggressive behavior in neuroblastomas with and without MYCN amplification

Geerts, Dirk; Koster, Jan; Albert, David; Kuomaa, Dana-Lynn; Feith, David; Pegg, Anthony; Volkman, Richard; Caron, Hubil; Versteeg, Roger; Bachmann, Andrea; Schwab, Manfred; Westermann, Frank

Institut Gustave Roussy, CNRS-UMR 8126, Villejuif, France; Institut Gustave Roussy, Paediatric Department, Villejuif, France; Institut Gustave Roussy, Bio-Pathology, Villejuif, France

Background: High risk neuroblastoma (HRNB) includes i) tumors with MYCN amplification (MNA or type C) independent of stage and ii) non-MYCN (NNM) tumors of stage 4 from patients over 18 months of age. Among these patients, a very small group (6%) exhibits an unexpected long survival rate. Among these patients, a very small group (6%) exhibits an unexpected long survival rate. Signatures of high risk neuroblastoma in these patients have been shown.

Methods: We analyzed the expression of ODC and ODC-activity regulating genes antagonizes 1-3 (OAZ1-3) and AZ inhibitors 1-2 (AZ-IN1-2) in human neuroblastoma (NB) tumors and correlated these with genetic and clinical features of NB. Since ODC is a target gene of MYCN, the correlation between ODC and MYCN was of special interest.

Results: ODC mRNA expression in NB tumors was significantly predictive of decreased overall survival probability and correlated with several unfavorable clinical NB characteristics (all P < 0.005). Interestingly, high ODC mRNA expression also showed significant correlation with poor survival prognosis in Kaplan-Meier analyses stratified for patients without MYCN amplification, suggesting an additional role for ODC independent of MYCN. Conversely, high OAZ2 mRNA expression correlated with increased survival and with several favorable clinical NB characteristics (all P < 0.003). In addition, we provide first evidence for MYCN-associated transcription factors M2D2 and M2D7 in ODC regulation. In NB cell cultures, ectopic over-expression of MYCN altered ODC, but not OAZ2 mRNA levels.

Conclusion: Our data suggest that elevated ODC and low OAZ2 mRNA expression levels correlate with several unfavorable genetic and clinical features in NB, offering new insights into PA pathways and PA metabolism-targeting therapies may be useful.

Email: s.gogolin@dkfz.de
Neuroblastoma (NB) is the most common and deadly tumor of childhood. Amplification of the MyCN oncogene characterizes the subset of aggressive NBs resistant to various therapeutics. However, the data on the role and significance of MyCN amplification for cell survival is controversial. The involvement of mitochondria in the regulation of cell death pathways makes targeting of these organelles a promising strategy for tumor cell elimination. A redox-silent analogue of vitamin E, α-tocopherol succinate (α-TOS), was shown to cause apoptosis via production of reactive oxygen species (ROS) and Bax-mediated release of cytochrome c from mitochondria.

Methods: MycN expression in T21N cells was regulated by doxycyclin. Analysis of ROS production was performed using mitochondria-incorporated sensor. Calcium fluxes were monitored by confocal microscopy.

Results: α-TOS facilitates induction of mitochondrial permeability transition (MPT) in NB T21N cells, Jurkat T-lymphocytes, and isolated rat liver mitochondria. α-TOS stimulates a rapid entry of Calcium into the cell with subsequent accumulation of these ions in mitochondria - a prerequisite step for MPT induction. ROS produced by α-TOS can contribute to MPT via oxidation of thiol groups in the adenine nucleotide translocase and alteration of redox state of glutathione or pyridine nucleotides. α-TOS also facilitates MPT induction via moderate uncoupling of mitochondria, since MPT is stimulated by low Calcium accumulation in mitochondria and prior inhibition of mitochondrial Calcium uptake significantly mitigated apoptotic response. Interestingly, downregulation of Mycn markedly decreased the level of Calcium accumulation in mitochondria. As a result, the level of Ca2+ in cytosol remained elevated that caused processing and release of another pro-apoptotic mitochondrial protein, AIF.

Conclusions: Downregulation of Mycn made cells resistant towards cisplatin, α-TOS. Thus, different mechanisms are involved in cell death induced by these drugs, and involvement of MyCN has a pathway-specific character.

Email: Boris.Zhitovitsky@ki.se

POB52
Expression QTL analysis of tumor susceptibility in a mouse model of neuroblastoma

Hackett, Christopher1; Kwok, Pui2; Young, Song3; Khan, Javed4; Allan, Balmain5; Weiss, William A.6
1University of California, Biomedical Sciences Graduate Program, San Francisco, United States; 2University of California, Dermatology, San Francisco, United States; 3University of California, Pediatrics Oncology Branch, Gaithersburg, United States; 4University of California, Helen Diller Family Comprehensive Cancer Center, San Francisco, United States; 5University of California, Neurology, Pediatrics, Neurosurgery, and Helen Diller Family Comprehensive Cancer Center, San Francisco, United States

We created a transgenic mouse model for neuroblastoma by targeting expression of the MYCN oncogene to the neural crest under the control of the tyrosine hydroxylase promoter. Tumor penetrance in this model is highly dependent on mouse strain genetic background, providing the opportunity to explore the molecular development of tumors using gene perturbations. Transgenic mice on a FBV1N background are almost completely penetrant. We generated a backcross population of (TH-MYCN-FBV1N x 129/SVJ) x 129/SVJ mice that displayed a 33% tumor incidence. We genotyped 200 backcross mice with 348 markers and identified a locus on chromosome 10 linked to tumor susceptibility with a lod score of 4.5. Using a QTL model, we found several other significant interacting loci. To identify candidate genes at these loci, we analyzed RNA from 46 tumors and 116 superior cervical ganglia from backcross mice using Affymetrix mouse exon arrays, and tested for genetic control of gene expression (expression QTLs). We have identified several dozen highly-significant expression QTLs, many mapping to tumor susceptibility loci and sharing common molecular signaling and metabolic pathways. We have also identified several more QTLs at an exon-level resolution, suggesting possible differential splicing of transcripts between strains that may play a role in tumor development. Further analysis of gene correlation networks and expression QTLs should illuminate novel molecular pathways and potential drug targets in neuroblastoma.

Email: weiss@cgf.ucsf.edu
POB53
Age-dependent genotypes in aggressive neuroblastoma: MYCN amplification facilitates a few-hit/early age process
Hedborg, Fredrik1; Celinka, Chan1; Martinsson, Tommy1; Kogner, Per2; Dunsmuir, Jan1; Trägård, Catarina1; Diaz de Ståhl, Teresita1
1Uppsala University, Dept of Genetics and Pathology and Dept of Women's Sexual Health, Uppsala University; Dept of Genetics and Pathology, Uppsala, Sweden; 2Gothenburg University, Dept of Clinical Genetics, Sahlgrenska Academy, Gothenburg, Sweden; 3Karolinska Institutet, Dept of Women and Child Health, Childhood Cancer Research Unit, Stockholm, Sweden; 4Karolinska Institutet and Uppsala University, Dept of Women's and Children's Health, Stockholm and Uppsala, Sweden

Background: Presence of MYCN amplification (MNA) or of 11q loss represents two major tumor genotypes in high-risk neuroblastoma (HR-NB). Most HR-NBs are diagnosed after 18 months of age but can occur in younger children in association with MNA. The aim of the present investigation was to further explore the age-dependence of the tumor genetics of aggressive neuroblastoma.

Method/approach: A consecutive series of Swedish HR-poor outcome NB, comprising 29 HR and 4 Intermediate Risk tumors, were analyzed for DNA copy number alterations, assessed on a 32K clone-based array platform. Affymetrix 250K and Illumina 610Q SNP array chips were used for validation. The age-dependence of MNA status was compared to data from 111 HR-NBs annotated in the Swedish NB registry over a 25-year period.

Results: MNA was associated with young age at diagnosis, mean age 30.6 months (n=15) - found in 1011 tumors of the youngest (range 4-30 months) but absent in 10/11 tumors of the oldest children (range 57-169 months) of our series. On the opposite, mean age at diagnosis of patients with non-MNA tumors was significantly higher, 65.6 months (n=18), and 12 of these tumors displayed 11q loss (mean age at diagnosis 69.5 months). Furthermore, we observed that while MNA tumors harbored few additional genetic aberrations (mean 0.6), in tumors with 11q loss these were more numerous (mean=11.5) and showed increasing tendency by age at diagnosis (p<0.037). The age-dependence of MNA status in HR-NB was verified in the Swedish NB registry, with mean ages at diagnosis of 29.4 and 54.8 months for MNA (n=25) and non-MNA (n=46) tumors, respectively.

Conclusion: The data suggests two major pathways in the genesis of HR-NB. Most HR-NBs are diagnosed after 18 months of age but can occur in younger children in association with MNA. The aim of the present investigation was to further explore the age-dependence of the tumor genetics of aggressive neuroblastoma.

Email: fredrik.hedborg@genpat.uu.se

POB54
Modeling the neuroblastoma tumor initiating cell microenvironment in 3D culture
Herland, Anna1; Wigerup, Caroline2; Johansson, Sofie2; Påhlman, Sven2; Hedborg, Fredrik1; Cetinkaya, Cihan2; Martinsson, Tommy3; Kogner, Per4; Kamager, Fredrik1; Cetinkaya, Cihan2; Martinsson, Tommy3; Kogner, Per4
1Karolinska Institutet, Department of Cell and Molecular Biology, Herland, Anna1; Wigerup, Caroline2; Johansson, Sofie2; Påhlman, Sven2; Hedborg, Fredrik1; Cetinkaya, Cihan2; Martinsson, Tommy3; Kogner, Per4

Background: Neuroblastoma (NB) is one of the most frequent and deadly solid tumors in children. However, further elucidation of the NB tumor development and its progression is poorly understood. MicroRNA (miRNA) deregulation was recently identified as a major contributor to cancer initiation and progression. As it has been shown that abnormal DNA methylation at miRNA CpG islands contributes to tumor formation, we aimed to investigate the potential role of epigenetically silenced miRNAs in NB pathogenesis.

Method/approach: Using quantitative PCR preceded by stem-loop megaplex reverse transcription, we measured the expression level of 384 miRNAs in a panel of 7 NB cell lines before and after treatment with a demethylating agent (5-aza-2'-deoxycytidine, DAC), a histone deacetylase inhibitor (trichostatine A, TSA), or a combination of both. miRNA profiles were also determined for 100 primary NB tumors, and normal NB progenitor cells that have been isolated by laser capture microdissection from fetal adrenal glands.

Results: Based upon the association with a CpG island and the absence of expression in a subset of NB tumors, miR-544b and miR-544c were identified as strong candidate suppressor miRNAs. Those miRNAs belong to the same cluster located on 11q23.1, a region critically deleted in a subset of aggressive NB. Reconstitution of expression of the down regulated miRNAs resulted in decreased cell proliferation, miR-449a, a member of the same family of conserved miRNAs, was reactivated by epigenetic treatment in all investigated cell lines, thus also pointing at a putative tumor suppressor activity. Using miR-449a mimics forced overexpression indeed resulted in cellular differentiation and cell cycle arrest and demonstrated significant cell viability reduction in wild type T332 NB cells. Identification of critical target genes is ongoing.

Conclusion: miRNA profiling succeeded in identifying specific NB miRNAs silenced by epigenetic mechanisms in NB. Hence, this analysis may enable the identification of epigenetically regulated miRNAs that contribute to NB pathogenesis and which represent new targets for therapy.

Email: Jasmin.Hoebeck@UGent.be

POB55
Epigenetically silenced microRNAs contribute to neuroblastoma pathogenesis
Hoebeck, Jasmin1; Westdijk, Pieter1; Pattyn, Filip2; De Preter, Katleen2; Rihani, Ali2; Vermeulen, Joliete1; Van Maeleken, Tom1; Lefever, Steve1; Nuyten, Justine1; Vult, Nutter1; De Paepe, Anne1; Spelmans, Frank1; Van den Bergh, Jo1
1 Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium; 2KU Leuven, Institute for Molecular Biotechnology, Leuven, Belgium

Background: Neuroblastoma (NB) is one of the most frequent and deadly solid tumors in children. However, further elucidation of the NB tumor development and its progression is poorly understood. MicroRNA (miRNA) deregulation was recently identified as a major contributor to cancer initiation and progression. As it has been shown that abnormal DNA methylation at miRNA CpG islands contributes to tumor formation, we aimed to investigate the potential role of epigenetically silenced miRNAs in NB pathogenesis.

Method/approach: Using quantitative PCR preceded by stem-loop megaplex reverse transcription, we measured the expression level of 384 miRNAs in a panel of 7 NB cell lines before and after treatment with a demethylating agent (5-aza-2'-deoxycytidine, DAC), a histone deacetylase inhibitor (trichostatine A, TSA), or a combination of both. miRNA profiles were also determined for 100 primary NB tumors, and normal NB progenitor cells that have been isolated by laser capture microdissection from fetal adrenal glands.

Results: Based upon the association with a CpG island and the absence of expression in a subset of NB tumors, miR-544b and miR-544c were identified as strong candidate suppressor miRNAs. Those miRNAs belong to the same cluster located on 11q23.1, a region critically deleted in a subset of aggressive NB. Reconstitution of expression of the down regulated miRNAs resulted in decreased cell proliferation, miR-449a, a member of the same family of conserved miRNAs, was reactivated by epigenetic treatment in all investigated cell lines, thus also pointing at a putative tumor suppressor activity. Using miR-449a mimics forced overexpression indeed resulted in cellular differentiation and cell cycle arrest and demonstrated significant cell viability reduction in wild type T332 NB cells. Identification of critical target genes is ongoing.

Conclusion: miRNA profiling succeeded in identifying specific NB miRNAs silenced by epigenetic mechanisms in NB. Hence, this analysis may enable the identification of epigenetically regulated miRNAs that contribute to NB pathogenesis and which represent new targets for therapy.

Email: Jasmin.Hoebeck@UGent.be

POB56
The interaction between GRP75 and retinoic acid receptor-α retinoid X receptor-α is essential for retinoic acid-induced neuronal differentiation of neuroblastoma cells
Hsu, Wen-Ming1; Shih, Yu-Yin2; Juan, Hsueh-Fen3; Tsay, Yeu-Guang1; Liao, Yung-Feng2
1National Taiwan University Hospital, Department of Surgery, Taipei, Taiwan; 2Academia Sinica, Institute of Cellular and Organismic Biology, Taipei, Taiwan; 3National Taiwan University Hospital, Department of Life Science, Taipei, Taiwan; 4National Yang-Ming University, Graduate Institute of Biochemistry and Molecular Biology, Taipei, Taiwan

Background/Aims: The tumorigenesis of neuroblastoma (NB), the most common extracranial solid tumor in children, is thought to be caused by defects in the normal development of neuroblasts, resulting in aberrant cell-cycle exit and cell differentiation. Retinoic acid (RA) has been used in clinic practice as a differentiation therapy for NB. Given that GRP75 is a favorable prognostic factor and is essential for RA-induced neuronal differentiation of NB cells. The aim of this study is to determine the molecular mechanism underlying GRP75-mediated regulation of RA-elicited neuronal differentiation.

Methods: Co-immunoprecipitation was employed to determine whether GRP75 can interact with RA receptors, retinoic acid receptor (RAR)α and retinoid X receptor (RXR)α. The functional role of GRP75-bound RARα/RXRα heterodimers in gene expression was determined by real-time PCR and chromatin immunoprecipitation.

Results: We demonstrated that GRP75 can be translocated into nucleus and physically interact with RARα and RXRα and that the nuclear GRP75-bound RARα/RXRα complexes can augment RA-elicited regulation of gene expression. Furthermore, GRP75 can govern the recruitment of RA-bound RARα/RXRα complexes to RARE in the regulatory regions of RA target genes, resulting in the transcriptional modulation downstream of RA signaling. Our data also showed that GRP75 is essential for stabilizing RARα/RXRα complexes in the presence of RA by reducing the proteasome-mediated degradation of RARα and RXRα.

Conclusions: Our present findings delineate a novel mechanism underlying the GRP75-dependent regulation of RARα/RXRα-mediated gene expression in the RA-induced neuronal differentiation, providing the basis for the development of novel therapeutics for NB.

Email: billwenshu@gmail.com
POB57

Neurot1 is involved in the development of neuroblastoma especially at INK4a/ARF domain.

HuanQ. Peng1; Kishida, Satoshi1; Ichikawa, Hitoshi1; Kadomatsu, Kenji1
1Nagoya University, Molecular Biology, Nagoya, Japan; 2Nagoya University Medical Faculty, Pediatric Hematology/Oncology, Nagoya, Japan

Background: MYCN transgenic (Tg) mouse is a model for neuroblastoma(NB). Because the celiac ganglion is the tumor origin in most cases, and the period of tumor onset is restricted (around 2-week old in homozygous mice), these mice are preferred to use for gene expression profiles in the precancerous and initial stages of NB. We seek for candidate molecule targets for NB therapy through the analysis of these expression profiles.

Method/approach: To examine gene expression profiles, we dissected following tissues: 1) normal celiac ganglion (wild-type mice, 2-week old), 2) celiac ganglion at hyperplasia stage (homozygous mice, 2-week old), 3) NB at initial stage (homozygous mice, 3-week old), 4) NB at initial stage (homozygous mice, 9-10-week old). CDNs were synthesized and hybridized to MG 430 2.0 Array (Affymetrix). From the interested candidates we pick out neurot1 as our first priority to focus on. We develop autograft tumor from MYCN hemizygous mice. We also developed tumor sphere formation to manipulate neurot1 expression in vitro. For knocking down neurot1 expression we use shRNA against neurot1. We inoculated autograft tumor sphere treated or not by neurot1 shRNA to nude mice to check tumorigenesis.

Results: 626 genes were upregulated in hyperplasia ganglia comparing to normal ones. Among them, 15 genes, containing 6 transcription factors, were upregulated more than 10 times. Among them neurot1 was an important transcription factor which had already been reported to be essential for neurogenesis and maturation of adult-born neurons. We used human NB cell lines to evaluate its role in tumor growth. Data showed that neurot1 could enhance NB cell survival and increase the proportion of S and G2/M phase in the cell cycle. Compared to control group autograft tumor sphere whose neurot1 expression knocked down by shRNA showed lower growth speed and sphere formation speed. Data from in vivo tumorigenesis experiment also shows neurot1 is essential for tumor development.

Conclusion: Neurot1 is involved in the tumorigenesis of neuroblastoma at an initial stage. Targeting neurot1’s expression may be helpful to cure this disease.

Email: phuang@med.nagoya-u.ac.jp

POB58

An observation of chromosomal abnormalities and MYCN and AURKA gene changes in neuroblastoma patients

Inandiklioglu, Nilah1; Yilmaz, Sema2; Demirhan, Osman1; Bayram, Ibrahim1; Erbey, Fatih2; Tanyeli, Atilla2
1Cukurova University Medical Faculty, Pediatric Hematology/Oncology, Adana, Turkey; 2Cukurova University Medical Faculty, Medical Biology, Adana, Turkey

Background: Neuroblastoma is an embryonal tumor of the sympathetic nervous system. Numerous gene abnormalities and the MYCN gene are involved in the etiology of neuroblastoma. Paraffine tissue was studied in hot regions where oncogenes and protooncogenes are present and are identified in 85.7% of stage IV patients. The percentages obtained in the present study were found to be related with literatures.

Results: The MYCN and AURKA gene analysis in paraffine tissue of 9 (36%) patients were determined through the FISH technique. The chromosomal abnormalities in both patients and the control group were investigated in blood samples through the utilization of standard cytogenetic procedures. Of 21 (%84) patients had chromosomal abnormalities. 18.4% of the cells of the patient group and 2.6% of the control group showed chromosomal defects. The difference between the patient and the control group was considered to be statistically significant (p<0.0001). It was reported that 72% of these abnormalities were structural while that of 28% were quantitative. Of these abnormalities, 1q21, 1q32, 2p24, 2q21, 2q31, 4q31, 9q11, 9q22, 13q14, 14q11, 14q24 and 15q22 were identified to be critical regions in the formation of NB. These areas were also reported to be the hot regions where oncogenes and protooncogenes are present and are involved in the etiology of neuroblastoma. Paraffine tissue was studied in 6 (66.7%) of the 9 patients, MYCN and AURKA gene amplifications were identified in 85.7% of stage IV patients. The percentages obtained in the present study were found to be related with literatures.

Conclusion: 1q21, 1q32, 2p24, 2q21, 2q31, 4q31, 9q11, 9q22, 13q14, 14q11, 14q24 and 15q22 were the most frequent chromosomes abnormalities. Many recurrent chromosome abnormalities associated with poor clinical outcomes have been identified in neuroblastoma. Several genetic abnormalities have been reported as prognostic markers.

Email: semayilmaz@hotmail.com

POB59

Chromosomal instabilities in a neuroblastoma patient

Inandiklioglu, Nilah1; Yilmaz, Sema2; Demirhan, Osman1; Bayram, Ibrahim1; Acipayam, Can2; Erbey, Fatih2; Tanyeli, Atilla2
1Cukurova University Medical Faculty, Medical Biology, Adana, Turkey; 2Cukurova University Medical Faculty, Pediatric Hematology/Oncology, Adana, Turkey

Aim: To investigate chromosomal aberrations in blood samples of 2 years-old boy diagnosed with grade IV neuroblastoma.

Introduction: Neuroblastoma is an embryonal tumor and originates in progenitor cells of the sympathetic neural tissue, accounting for eight percent of all neoplasia in childhood. The biology underlying the clinical heterogeneity of neuroblastoma is directed to histopathologic and genetic abnormalities. Many recurrent chromosome abnormalities associated with poor clinical outcomes have been identified in neuroblastoma. Several genetic abnormalities have been reported as prognostic markers.

Method: GTG-banding in blood samples was performed.

Results: Hidden recurrent deletions and translocations in various chromosomes were detected. All deletions were unbalanced. The most prevalent recurrent unbalanced deletions resulted in del(1)(p32-qter) (1/50), del(3)(cen-qter) (1/50), del(2)(q31-tet) (1/50), del(7)(q11-tet) (1/50), del(7)(q11.1-qter) (1/50), del(9)(q11.1-qter) (2/50)X2, del(9)(q3-tet) (1/50), del(10)(q24-qter) (1/50), del(12)(p11-qter) (1/50), 46,X,del(13)(q14.3-p13.1) (1/50), del(15)(q25-qter) (1/50), del(18)(q21-tet) (1/50), del(19)(q13.3-qter) (1/50). Translocations [46,X,der(3)(3;12)q26-q15-tet] (1/50), 46,X,der(11) (11;11)X18(2q5-q21-tet) (1/50), and marker [47,X,Y,ace,del(2)(q31-tet) (1/50)] were found the most frequent chromosomal abnormalities. Furthermore, sex chromosome aneuploidies were the other significant (47,X,XY,6 translocation). Recurrent deletions and translocations in various chromosomes should be taken into account among poor prognostic factors in neuroblastoma patients.

Reference:

Email: semayilmaz@hotmail.com

POB60

Constitutive activated Hen2 expression in neural crest cells could be a trigger of neuroblastoma development

Isogai, Eriko1; Ohira, Miki1; Haraguchi, Seiki2; Nakagawara, Akira1
1Chiba Cancer Center Research Institute, Lab. of Cancer Genomics, Chiba, Japan; 2Chiba Cancer Center Research Institute, Lab. of Embryonic, Genetic Engineering, Chiba, Japan; 3Chiba Cancer Center Research Institute, Div. Biochem., Innovative Cancer Therap., Chiba, Japan

Background: Mash1 is one of the cell fate determination genes of the sympathoadrenal lineage. Its expression is abnormally upregulated especially in aggressive neuroblastomas. However, the molecular mechanism remains unclear. We have reported that LIM-only protein, LMO3 and basic helix-loop-helix protein, HEN2 act as oncogenes by upregulating Mash1 expression in neuroblastoma cells by suppressing the inhibitory function of HES1 against Mash1 transcription (Hen2 2006). To confirm these previous results in vivo, we made transgenic mice of Hen2 and Lmo3 and examined the phenotypes.

Methods: Transgenes mouse Hen2 and mouse Lmo3 were expressed under the control of mouse Wnt1 promoter. Neural crest cells (NCCs) were prepared from intestine of transgenic mice and the littmate control embryos because the preparation and culture are easier than that from neural tube. Differentiation of NCCs was induced by culturing in low mitogen and growth factor culture medium. Differentiation, proliferation and cell death were examined by immunostaining, BrDU incorporation and TUNEL assay.

Results: When differentiation was induced, proliferation and survival rates of NCCs prepared from transgenic mice embryos were increased as compared with those prepared from littermate controls. Differentiation rates of these transgenic NCCs were decreased and maturity differentiated less than wild-type cells. The Mash1-positive cells were slightly increased in transgenic NCCs. Because continued expression of Mash1 is recently reported to be involved in the development of aggressive neuroblastomas, constitutive expression of Hen2 could be a cause of promoted proliferation of NCCs. Since hydrophychal was developed in Hen2 and Lmo3 transgenic mice, similar effects of Lmo3 on proliferation, survival and differentiation of NCCs were expected.

Conclusions: We previously indicated Hen2 upregulates expression of Mash1 by suppressing the inhibitory function of HES1 transcription. Mash1 transcription in neuroblastoma cell lines. The present study also suggested that this activity of mouse Hen2 may exist in NCCs and that Hen2 could act as a trigger of neuroblastoma in NCCs.

Email: eisogai@chiba-crc.jp
**POB61**
A novel orphan receptor, NLRR3, induces neuronal differentiation and is negatively regulated by MYCN in neuroblastoma

**Kishida, Satoshi**1; Cao, Dong Liang1; Ichikawa, Hitoshi2; Kadomatsu, Kenji1

**Abstract**
Tumor-initiating cell formation in MYCN Tg mice

**Background:**
The human NLRF family genes were originally identified from the cDNA project of neuroblastoma (NB) we generated as the genes whose expression is differential between favorable and unfavorable NBs. The NLRF family genes encode orphan receptors with unknown function. Our preliminary data showed that expression of NLRF1 is high in unfavorable NBs, whereas that of NLRF3 is high in favorable NBs. However, their precise molecular mechanisms remain elusive.

**Method/approach:**
Luciferase reporter, transcriptional profiling, immunoprecipitation and chromatin immunoprecipitation (ChIP) assays were performed to investigate the transcriptional regulation of NLRF3 by MYCN. Quantitative real time PCR was applied to examine the mRNA expression.

**Results:**
Expression of NLRF3 mRNA was significantly high in favorable NBs as compared to unfavorable ones (n=36 vs 50, p<0.001), whereas that of NLRF1 was high in unfavorable NBs (p=0.028). The multivariate analysis showed that expression of NLRF3 was an independent prognostic factor. The immunohistochemical study showed that NLRF3 is strongly positive in favorable NB cells, though it mainly localized in the cell nuclei. NLRF3 is up-regulated during retinoic acid-induced differentiation of RTBM1 NB cells accompanying with down-regulation of endogenous MYCN, whereas it was decreased after induction of MYCN expression in SH-SYSY NB cells. In addition, expression of NLRF3 was down-regulation or repression of MYCN was up-regulated by knockdown of MYCN at both mRNA and protein levels. Furthermore, like NLRF3, Mx2-1, which is a co-repressor of MYCN, is highly expressed in favorable NBs (p=0.0004), suggesting that MYCN-repression may be regulated by MYCN and Mx2-1. Indeed, MYCN repressed transcription of NLRF3 in cooperation with Max and Miz-1. The chromatin immunoprecipitation (ChIP) analysis also demonstrated that MYCN, Max and Miz-1 recruited on to the promoter regions of NLRF3 gene and act as a transcriptional repressor complex for NLRF3 expression.

**Conclusion:**
NLRF3 regulates neuronal differentiation and survival and its expression is negatively regulated by MYCN in association with Mx-2 in aggressive NBs.

**Email:** jesmindreams2003@yahoo.com

**POB62**
Comprehensive screen for genes involved in tumorigenesis and tumor-initiating cell formation in MYCN Tg mice

**Kishida, Satoshi**1; Cao, Dong Liang1; Ichikawa, Hitoshi1; Kadomatsu, Kenji1

**Abstract**
Comprehensive screen for genes involved in tumorigenesis and tumor-initiating cell formation in MYCN Tg mice

**Background:**
MYCN transgenic (Tg) mouse is a model for neuroblastoma. These mice spontaneously develop neuroblastoma whose onset is preceded by mesenteric gangliogliomas. The analysis of the gene expression and chromosomal aberration patterns in MYCN Tg mice, we are trying to identify the genes involved in tumorigenesis and tumor-initiating cell (TIC) formation. Those genes must be potential targets for molecular therapy and prognosis prediction.

**Method/approach:**
In order to examine the chromosomal aberration, we carried out CGH array analysis (Agilent, 44K) with 12 tumors from MYCN Tg mice. Spleen from each mouse was supplied for the comparative genomic hybridization analysis. On the other hand, we prepared tumors from MYCN Tg mice, and tumor spheres cultured from those tumors. Tumor sphere contains putative TICs. We carried out gene expression array analysis (Affymetrix, MG430 2.0 Array) with those samples to identify the TIC-specific genes.

**Results:**
CGH array analysis showed that the gene C was deleted in all 12 tumors. Although there is no report about the relationship between the C and neuroblastoma, its function is implicative enough. C is essential for the normal differentiation of neural crest-derived cells. We also picked up 43 genes and 48 genes down-regulated. ALK expression was increased in normal and in some samples. This may be related to tumorigenesis. In agreement with this, we have shown that endogenous NPY stimulates proliferation of NB cells and tumor angiogenesis via its Y2 receptors (Rs), while blocking Y2Rs inhibits tumor growth. The goal of this study was to determine the interactions of the NPY system with another known growth-promoting factor in NB - BDNF.

**Conclusion:**
BDNF expression was measured by real-time RT-PCR, cell viability by MTS assay and apoptosis by caspase 3/7 activity.

**Results:**
The study was performed on NB cells derived from primary tumors. CHLA-15, SMS-KAN and post-therapy tumors (SK-N-BE2, CHLA-20, SMS-KAN) expressing the functional BDNF receptor, TrkB, as determined by RT-PCR and MAPK activation upon BDNF treatment. We have shown that BDNF up-regulates the expression of NPY and its Y2Rs in NB cells and, more dramatically, induces expression of Y5Rs, which are not detectable in most NB cell lines under basal conditions. In agreement with this, expression of Y5Rs correlated with TrkB expression in human NB samples. Since BDNF is a known survival factor for NB cells, implicated in its resistance to chemotherapy, we sought to determine if pro-survival activity of BDNF is mediated by NPY, Y2 antagonist, and even more dramatically Y5 antagonist, reduced the anti-apoptotic effect of BDNF in chemotherapy-treated NB cells, while NPY mimicked the effect of BDNF. Moreover, treatment with chemotherapy up-regulated the expression of both systems - BDNF and TrkB, as well as NPY and its Rs. This was further supported by elevated expression of NPY and Y5Rs in cells derived from chemotherapy-treated patient (CHLA-20) as compared to cells derived from primary tumors of the same patient (CHLA-15).

**Conclusion:**
While Y2Rs are the main NPY Rs constitutively expressed in NBs and responsible for its proliferative effect, expression of Y5Rs is induced by BDNF and chemotheraphy and enhances the pro-survival functions of the peptide. These anti-apoptotic actions of NPY can additionally augment the known direct survival effects of BDNF.

**Email:** kshida@med.nagoya-u.ac.jp

**POB63**
Common pathways in neuroblastoma and early-onset breast cancer

**Kompanje, Kenneth**1; Madan, Katrina2; Hoon, Mary3; John4; Witte, John1

**Abstract**
Common pathways in neuroblastoma and early-onset breast cancer

**Background:**
Recent experience of families with cases of early-onset breast cancer (BRC) and neuroblastoma (NBL) has suggested that mutations or copy number changes in the same loci may predispose both diseases. While it is likely that interactions between higher frequency alleles factor into the development of NBL and BRC, it is difficult to predict such interactions without the use of novel methods. To test the hypothesis that BRC and NBL carry mutations in the same pathways, we have begun a systematic reanalysis of genome wide association study (GWAS) results from NBL and BRC.

**Method/approach:**
We combined p-values from both GWAS using Fisher’s method and used empirical distribution-free statistical testing to search for enriched Gene Ontology categories in each GWAS. Over 2,000 Gene Ontology categories were tested for enrichment of significant associations, and enrichment testing results were adjusted for false discovery rate (FDR).

**Results:**
While there appeared to be relatively little overlap of significant associations between the two studies at the individual SNP level, gene ontology analysis indicated a strong over-representation of highly significant SNPs near genes encoding proteins required for cell adhesion, ion transport, and transmembrane receptor tyrosine kinase signaling in both BRC and NBL (p<0.01).

**Conclusion:**
Gene Ontology analysis of GWAS results from BRC and NBL suggests that the activation of different genes in many of the same pathways affects cancer progression similarly in both diseases. While it is likely that interactions between higher frequency mutations may lead to common outcomes, identification of the interactions within members of these pathways could provide a molecular basis for shared predisposition.

**Email:** kenkompass@gmail.com

**POB64**
Neuropeptide Y in neuroblastoma - Interactions with BDNF and effect on cell survival

**Kuan-Celanier, Anna**1; Lu, Congyi1; Everhart, Lindsay2; Toreisky, Jeremy1; Kittilska, Joanna1

**Abstract**
Neuropeptide Y in neuroblastoma - Interactions with BDNF and effect on cell survival

**Background:**
Neuropeptide Y (NPY) and brain-derived neurotrophic factor (BDNF) are neurotrophic factors that play important roles in neurodevelopment and neuroregeneration. NPY and its receptors (Y receptors) are expressed in multiple tissues, including the central and peripheral nervous systems. NPY is involved in the regulation of neuronal survival, growth, and differentiation.

**Results:**
We observed a significant up-regulation of NPY and its receptor, Y2Rs, in NB cells derived from primary tumors of the same patient (CHLA-15). The up-regulation of NPY and its Y2Rs was accompanied by increased expression of BDNF and TrkB, which are key receptors for neurotrophins. The study was performed on NB cells derived from primary tumors (CHLA-15, SMS-KAN) and post-therapy tumors (SK-N-BE2, CHLA-20, SMS-KAN) expressing the functional BDNF receptor, TrkB, as determined by RT-PCR and MAPK activation upon BDNF treatment. We have shown that BDNF up-regulates the expression of NPY and its Y2Rs in NB cells and, more dramatically, induces expression of Y5Rs, which are not detectable in most NB cell lines under basal conditions. In agreement with this, expression of Y5Rs correlated with TrkB expression in human NB samples. Since BDNF is a known survival factor for NB cells, implicated in its resistance to chemotherapy, we sought to determine if pro-survival activity of BDNF is mediated by NPY, Y2 antagonist, and even more dramatically Y5 antagonist, reduced the anti-apoptotic effect of BDNF in chemotherapy-treated NB cells, while NPY mimicked the effect of BDNF. Moreover, treatment with chemotherapy up-regulated the expression of both systems - BDNF and TrkB, as well as NPY and its Rs. This was further supported by elevated expression of NPY and Y5Rs in cells derived from chemotherapy-treated patient (CHLA-20) as compared to cells derived from primary tumors of the same patient (CHLA-15).

**Conclusion:**
While Y2Rs are the main NPY Rs constitutively expressed in NBs and responsible for its proliferative effect, expression of Y5Rs is induced by BDNF and chemotheraphy and enhances the pro-survival functions of the peptide. These anti-apoptotic actions of NPY can additionally augment the known direct survival effects of BDNF.

**Email:** jbk4@georgetown.edu
Involvement of the CXCL12/CXCR4/CXCR7 axis in the malignant progression of human neuroblastoma

Liberman, Julie1; Flahaut, Marjorie1; Mühlthaler-Mottet, Annick1; Coulon, Aurélie1; Joseph, Jean-Marc2; Gross, Nicole1
1University Hospital of Lausanne, CHUV, Pediatric Oncology Research Unit, Lausanne, Switzerland; 2University Hospital of Lausanne, CHUV, Pediatric Surgery Unit, Lausanne, Switzerland

Background: Chemokines and their receptors, particularly the CXCR4/CXCL12 axis, have been involved in tumor progression in several cancers, including neuroblastoma. We previously reported a tumor-type-specific and microenvironment-related growth-promoting role for the CXCR4 receptor. Growth-promoting effects were highly significant only when NBs were orthotopically implanted in adrenal glands of nude mice, thus highlighting the impact of the tumor microenvironment on the CXCR4-mediated NB growth. We further explored the participation of tumor microenvironment in the behaviour of CXCR4-expressing cells, by 2D co-culture strategy. We also addressed the role of CXCR7, the recently identified second CXCL12 receptor. Although reported to confer atypical properties to cancer cells, the role of CXCR7 in the cross-talk with the microenvironment is still unknown.

Method/approach: A 2D co-culture of CXCR4 expressing NB cells and fibroblasts was developed. CXCR7 participation in the CXCR4/CXCL12/CXCR7 axis was analysed by gain of function strategies and in vitro differentiation assays.

Results: When co-cultured on a layer of fibroblasts prepared as a human immortalized and irradiated cell line, CXCR4 overexpressing cells displayed a significantly faster but ligand-independent growth as compared with controls. In parallel, a screening of NB tumor samples revealed a CXCR7 staining pattern as specifically associated to differentiated and/or mature cells of the tumor. NB cell lines showed a specific CXCR7 expression, which increased upon exposition of cells to differentiation agents. Moreover, overexpression of CXCR7 was shown to be supportive in vitro survival and growth of NB cells. In contrast, when co-expressed with CXCR4, CXCR7 led to a decrease of NB growth which was only observed in presence of CXCL12.

Conclusion: This study confirms the microenvironment-related growth-promoting role for CXCR4. Our prelinogy, Retinoid acid and epigenetic modulators induce CASZ1 expression during NB cell differentiation. CASZ1 has two major protein isoforms, hcasz5 (CASZ1b) and hcasz11 (CASZ1a), which contain 5 and 11 zinc fingers, respectively. Retinoic Acid, Myc, or hcasz11 in NB cells enhanced cell adhesion, inhibited migration and suppressed tumor growth in vitro soft agar colony formation assays and in vivo tumor growth in mouse xenografts. Expression profiling revealed that >95% of hcasz5 target genes overlap with hcasz11 targets and are involved in cell proliferation and developmental processes. Consistent with their function at suppressing tumor growth, both hcasz5 and hcasz11 induced NB tumor suppressor genes NGFR (~15-fold), TrkA (~2-fold) and clusterin (~10-fold). To test the impact of loss of CASZ1 isoforms on neural differentiation, we utilized in vitro Embryonic Stem (ES) cell differentiation models. Wild type ES cells (CASZ1+/+) formed Embryoid Bodies (EBs) that can be induced to differentiate to neuronal cells that express beta III tubulin. However, ES cells hemizygous for CASZ1 (CASZ1-/-) expressed 50% less CASZ1 mRNA and formed ~½ the EBs compared to wild-type ES cells. Moreover, CASZ1-/- ES cells failed to differentiate into neuronal cells. CASZ1-/- and CASZ1+/+ ES cells had similar alkaline phosphatase activity (a marker of ES cell self-renewal) and the loss of CASZ1 did not affect expression of critical neural genes such as Neurog1, Pax6, Oct3 and Nanog. Thus, CASZ1+/+ ES cells have self-renewal properties similar to wild-type cells but have a block in their ability to form EBs and differentiate into neuronal cells (our study demonstrates that CASZ1 levels are critical for neural differentiation and supports the hypothesis that haploinsufficiency of CASZ1 contributes to neuroblastoma tumorigenesis).

Email: izuizhu@mai.tlh.gov

Screening of ALK mutations and abnormalities in neuroblastoma cell lines and Italian neuroblastoma cases

Longo, Luca; De Mariano, Manuela; Del Grosso, Federica; Passoni, Lorenza; Deferrari, Raffaella; Mazazzza, Mariangela; Girardi, Luigi; Lukesch, Roberto; Garaventa, Alberto; Tonin, Gian Paolo
1Italian Neuroblastoma Foundation and National Cancer Research Institute (IST), Translational Pediatric Oncology, Genoa, Italy; 2University of Genoa and National Cancer Research Institute (IST), Translational Pediatric Oncology, Genoa, Italy; 3Istituto Nazionale Tumori (INT), Pediatric Oncology, Milano, Italy; 4Bambino Gesù Children’s Hospital, Pediatric Oncology, Roma, Italy; 5G. Gaslini Children’s Hospital, Pediatric Hematology-Oncology, Genoa, Italy

Background: The Anaplastic Lymphoma Kinase (ALK) has emerged as a genetic driver in at least a subset of neuroblastoma cases (NB). We screened for ALK tyrosine kinase (TK) mutations additional 71 Italian sporadic NB cases to those already reported [1]. Moreover, we screened individuals with infant Italian familial NB, SKH. We also performed an SILAC analysis using the Vysis LSI ALK Dual Color, Break Apart Rearrangement Probe. Immunoafflits for pALK and ALK were performed on total protein lysates. ALK and MYCN gain was investigated by Multiplex Ligation-dependent Probe Amplification (MLPA). ALK rearrangements were studied by FISH analysis using the Vysis LSI ALK Dual Color, Break Apart Rearrangement Probe.

Results: Overall, we detected 6/114 (5.3%) ALK mutations in Italian NB population and 5 of these alterations occur at amino acids F1174 and R1275, which are the two most frequently impaired. We also found a novel missense mutation, 3509T>G (I1170S), which we already reported [1]. Moreover, we detected the R1192P ALK mutation in a recently collected NB cell line. Furthermore, we found 6 ALK-positive NB cases in our study dataset and 2/120 cases in a panel of 30 NB cell lines, which were also partially investigated for alk expression. Moreover, we sought for other mechanisms of ALK activation such as gain/amplification and translocations in sporadic NB cases and cell lines.

Methods: Direct sequencing for ALK exons 20-28 was performed by BigDye Terminator v1.1 kit on the ABI-Prism 3130 genetic analyzer. Immunoafflits for pALK and ALK were performed on total protein lysates. ALK and MYCN gain was investigated by Multiplex Ligation-dependent Probe Amplification (MLPA). ALK rearrangements were studied by FISH analysis using the Vysis LSI ALK Dual Color, Break Apart Rearrangement Probe.

Conclusion: To our knowledge, we identified 11 ALK mutations in 113 NB cases without MYCN gain revealed no ALK gain too. On the contrary, 31 out of 33 (93.9%) MYCN gain NB cases showed also ALK gain. Finally, FISH analysis performed on 8 congenital and familial NB revealed no ALK translocation. However, the increased number of cells were confirmed to carry a deletion of the ALK TK telomeric region.

Conclusion: In summary, our study adds information about the involvement of ALK in NB, although further efforts should be made to define all the possible mechanisms that impair ALK activity.


Email: luca.longo@itge.it

doi:10.1002/ajp2.11012010
TRIM16 acts as a tumour suppressor via inhibitory effects on cytoplasmic vimentin and nuclear E2F1 in neuroblastoma cells

Methods: NB TICs have been cultured in vitro and the expression of TRIM16 was analyzed by quantitative PCR. A novel assay is being developed that allows for systematic and accurate analysis of the role of TRIM16 in NB TIC biology, based on the formation of microislands of NB TICs surrounded by substrate immobilized epithelial ligands and Eph receptors.

Results: We have mapped the expression of epithelial ligands and Eph receptors in NB TICs by quantitative PCR, which shows significant overlap with the previously reported expression profile of epithelial and nuclear E2F1 in neuroblastoma cells. Furthermore, we have implemented a method to immobilize patterns and gradients of proteins. This is the cornerstone for the development of an assay to systematically investigate NB TIC responses to Ephrin/Eph presentation.

Conclusion: The expression profile of cytoplasmic vimentin and nuclear E2F1 in neuroblastoma cells.

POB70

TRIM16 acts as a tumour suppressor via inhibitory effects on cytoplasmic vimentin and nuclear E2F1 in neuroblastoma cells

Background: Cell-cell communication via the Ephrin/Eph pathway plays a central role in development, mediating guidance and stabilizing tissue borders. Ephrin ligands and Eph receptors are misspliced in most cancers. In neuroblastoma, expression levels of EphB6, EphrinB2, and EphrinB3 predict a favorable outcome. Tumor initiating cells (TICs) are highly tumorigenic cells that have the ability to self-renew and differentiate and are thought to be involved in treatment resistance and disease relapse. TICs have been isolated from neuroblastoma bone marrow metastasis. Our guiding hypothesis is that Ephrin/Eph signaling modulates the neuroblastoma TIC state. The knowledge gained has the potential to aid in the development of strategies that target ephrin/Eph in the treatment of neuroblastoma. We aim to investigate the role of ephrin/Eph signaling, differential on the proliferation and migration of neuroblastoma tumor initiating cells (NB TICs) as well as the cross talk between growth factors and ephrin/Eph signaling in NB TICs.

Methods: NB TICs have been cultured in vitro and the ephrin/Eph expression was analyzed by quantitative PCR. A novel assay is being developed that allows for systematic and accurate analysis of the role of ephrin/Eph in NB TIC biology, based on the formation of microislands of NB TICs surrounded by substrate immobilized epithelial ligands and Eph receptors.

Results: We have mapped the expression of epithelial ligands and Eph receptors in NB TICs by quantitative PCR, which shows significant overlap with the previously reported expression profile of ephrin/Eph of neural crest stem cells. Furthermore, we have implemented a method to immobilize patterns and gradients of proteins. This is the cornerstone for the development of an assay to systematically investigate NB TIC responses to Ephrin/Eph presentation.

Conclusion: The expression profile of cytoplasmic vimentin and nuclear E2F1 in neuroblastoma cells.

POB71

High-risk neuroblastoma without MYCN amplification - 11q-deletion tumors reveal a poor prognostic chromosome instability phenotype with later onset

Analysis of chromosomal aberrations is used to predict clinical prognosis of children with neuroblastoma and to stratify risk-based therapy. MYCN amplification (MNA) alone is incomplete as a poor prognostic factor and 11q status has recently been included in risk classification. We analyzed 170 neuroblastomas using high-density SNP microarrays and describe and compare the high-risk groups defined by MNA (n=37) and 11q-deletion (n=21). Median age at diagnosis was 21 months for the MNA group and 42 months for 11q-deleted, while median survival from diagnosis was 16 months for MNA and 40 months for 11q-deletion. Overall survival was similarly poor, 35% at eight years for both groups.

Conclusion: The high frequency of chromosomal breaks in the 11q-deleted group is suggestive of a chromosomal instability phenotype gene located in 11q, and one such gene, H2AFX, is located in 11q23.3 (within the 11q-deleted region in all tumors). Furthermore, in the groups with segmental aberrations without MNA or 11q-deletion, children with tumors with 17q gain had worse prognosis than those with segmental aberrations without 17q gain, who had a favorable outcome in our material. This study has implications for understanding the role of neuroblastoma tumor genetics, prognostic assessment, and choice of therapy for different risk groups and stresses the use of genome-wide microarray analyses in clinical management to evaluate patient diagnosis, risk and treatment.

Email: tommy.martinsson@gu.se

POB72

Appearance of the novel activating F1174S ALK mutation in neuroblastoma correlates with disease progression: aggressive tumour behaviour and unreponsiveness to therapy

Background: Mutations in the kinase domain of Anaplastic Lymphoma Kinase (ALK) have recently emerged as important players in neuroblastoma biology, both in familial and sporadic subsets. Here we report the appearance of a novel ALK mutation in neuroblastoma, correlating with disease progression and aggressive tumour behaviour.

Methods: A sporadic MYCN-nonamplified infant INSS stage 4 neuroblastoma assessed as INRG intermediate risk was treated with chemotherapy according to SIOPEN infant protocol 99.3. At disease progression 9 months from diagnosis resistant disease emerged and the tumour genetics was reevaluated.

Results: Analyses of genomic DNA from biopsy samples initially showed ALK sequence to be wild-type (homozygous 3521T/3521T i.e. F2274/F1174). However, during disease progression mutation of amino acid F1174 to a serine within the ALK kinase domain was observed, which correlated with metastatic progression and resistance to chemotherapy. Thus, the DNA from tumor 9 months after diagnosis was homozygous for mutation 3521T-C, i.e. F1174S/F1174S. We show here that mutation of F1174 to serine generates a potent gain-of-function mutant, as observed in two independent systems.

Firstly, PC12 cell lines expressing ALKF1174S displayed ligand independent activation of ALK and further downstream signaling activation. Secondly, analysis of the ALKF1174S in Drosophila models confirms that this mutation meditates a strong rough eye phenotype upon expression in the developing eye.

Conclusion: We report a novel neuroblastoma ALKF1174S mutation, which displays ligand independent activity in vivo, correlating with aggressive clinical disease progression resistant to further therapy and oncogenic gain of function in different models.

Email: tommy.martinsson@gu.se
POB73
An integrative genomics screen uncovers ncRNA T-UCR functions in neuroblastoma tumours
Mestdagh, Pieter1; Fredlund, Erik1; Pattyn, Filip1; Rihani, Ali1; Van Maerken, Tom1; Vermeulen, Joëlle1; Kamps, Candy1; Menten, Bjorn1; De Preter, Katleen1; Schramm, Alexander1; Schulte, Johannes1; Noguera, Rosa1; Schleiermacher, Gudrun2; Janoeux-Lerosey, Isabelle2; Laerum, Geneviève2; Powell, Rob1; Nitter, David1; Marine, Jean-Christophe2; Ringnér, Markus2; Speleman, Frank1; Vandesompole, Jo1; Van Peer, Gert1; Medaglia, Chiara5; Schlierf, Stefanie6; Schulte, Johannes6; Mestdagh, Pieter1; Boström, Anna-Karin2; Impens, Francis3; Fredlund, Erik4; De Antonellis, Pasqualino5; von Stedingk, Kris2; Ghesquiere, Bart3; Skapek, Stephen X.1; Volchenboum, Samuel L.2; 1Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium; 2Lund University, Department of Oncology, Lund, Sweden; 3University Hospital, Essen, Pediatric Hematology and Oncology, Essen, Germany; 4University Medical Centre, Gothenburg, Sweden; 5Institut Curie, Department of Paediatric Oncology, Paris, France; 6Ghent University Hospital, Department of Pediatric Oncology, Ghent, Belgium; 7Primerdesign, Primerdesign, Southampton, United Kingdom; 8Ghent University, Laboratory for Molecular Cancer Biology, Ghent, Belgium

Background: Different classes of non-coding RNAs, including T-UCRs, have recently been implicated in the process of tumourigenesis. Our results define a T-UCR expression landscape in neuroblastoma and suggest widespread T-UCR involvement in diverse cellular processes that are deregulated in the process of tumourigenesis.

Method/approach: In this study, we designed an RT-qPCR based T-UCR profiling platform to examine the expression and putative function of a novel class of non-coding RNAs known as transcribed ultrasonically expressed regions (T-UCRs) in neuroblastoma.

Results: Genome wide expression profiling of 481 T-UCRs revealed correlation between specific T-UCR expression levels and important clinicogenetic parameters such as MYCN amplification status. A functional genomics approach based on the integration of multi-level transcriptome data was adapted to gain insights into T-UCR functions. Assignments of T-UCRs to cellular processes such as TP53 response, differentiation and proliferation were verified using various cellular model systems.

Conclusion: For the first time, our results define a T-UCR expression landscape in neuroblastoma and suggest widespread T-UCR involvement in diverse cellular processes that are deregulated in the process of tumourigenesis.

Email: pieter.mestdagh@ugent.be

POB74
Mir-17-92 is a master regulator of TGFβ-pathway activity in neuroblastoma
Mestdagh, Pieter1; Boström, Anna-Karin1; Impens, Francis1; Fredlund, Erik1; De Antonellis, Pasqualino1; von Stedingk, Kris2; Ghesquiere, Bart3; Van Peer, Gert1; Medaglia, Chiara5; Schlierf, Stefanie6; Schulte, Johannes6; Schramm, Alexander1; Zollo, Massimo1; Govaert, Kris1; Avetion, Hakart1; Speleman, Frank1; Vandesompole, Jo1; Van Peer, Gert1; Medaglia, Chiara5; Schlierf, Stefanie6; Schulte, Johannes6; Schramm, Alexander1; Zollo, Massimo1; Govaert, Kris1; Avetion, Hakart1; Speleman, Frank1; Vandesompole, Jo1; 1Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium; 2Lund University, Department of Laboratory Medicine; Lund, Sweden; 3University Hospital, Essen, Pediatric Hematology and Oncology, Essen, Germany

Background: The miR-17-92 gene cluster is often activated in cancer cells, including neuroblastoma (Mestdagh et al., Oncogene, 2010). Thus far, the role of this known miR-17-92 coding gene target, constitutes somewhat small and efforts to identify the downstream effectors have been restricted to the study of one or two miRNAs from this cluster.

Method/approach: Here, we examined the effects of entire cluster miR-17-92 activation on global protein expression in neuroblastoma. Using quantitative mass spectrometry, we analyzed the response of thousands of proteins upon miR-17-92 activation.

Results: Analysis of the responsive proteins revealed cooperation between individual miR-17-92 mRNA and implicates miR-17-92 in multiple hallmarks of the tumorigenic program including proliferation and cell adhesion. In addition, we show that miR-17-92 is a potent inhibitor of TGFβ-signaling. By acting both upstream and downstream of pSMAD2, miR-17-92 dampens TGFβ-response in a multifaceted way.

Conclusion: Our results associate impaired TGFβ-signaling to poor outcome of neuroblastoma patients and further elucidate the miR-17-92 – TGFβ connection. In vivo assessment of miR-17-92 activation in mouse xenografts is currently ongoing, and results will be presented.

Email: pieter.mestdagh@ugent.be

POB75
Phosphoprotein and expression analyses of a MYCN-amplified neuroblastoma cell line
Mikan, Kelly1; Kristiansdottr, Kollaf2; Chlenski, Alexandre3; Regan, Kelly1; Skapek, Stephen X.1; Volchenboum, Samuel L.2; 1University of Chicago, Department of Pediatric Oncology, Chicago, United States; 2University of Chicago, Ludwig Center for Metastasis Research, Chicago, United States; 3University of Chicago, Department of Pediatric Oncology, Computation Institute, Chicago, United States

Background: When faced with oncogenic signals, cells activate tumor suppressive responses including irreversible cell cycle arrest or apoptosis. The ARF-MDM2-p53 signaling pathway is crucial for this response is subverted in essentially all human cancers. Surprisingly, neuroblastomas rarely demonstrate ARF or p53 inactivation or MDM2 amplification at diagnosis. We are using genome-wide and proteomic approaches to probe how MYCN-amplified neuroblastoma cells tolerate oncogene expression.

Methods: neuroblastoma cell line, with MYCN constitutively over-expressed but silenced under tetracycline control. After incorporation of stable isotope labeled amino acids for quantitation, cells were harvested, phosphoproteins isolated and GeLC-MS/MS performed. Gene expression analysis was performed at 4, 24, or 48 hours using the Hu1.0ST Affymetrix gene array. Results: proteins, and a subset was analyzed by Western blotting to validate increased amounts of nucleophosmin (NPM1), matrin-3 (MATR3), and SET protein (SET) and decreased amounts of aldehyde dehydrogenase (ALDH1A1). Gene expression analysis of revealed a unique profile for MYCN-amplified cells, including increased TAF4B and decreased MGP and RARRES3. There was no correlation between gene expression and phosphoproteome hits, suggesting post-transcriptional regulatory mechanisms. As NPM1 is known to interact with the ARF/p53 pathway, we are exploring studies of NPM induction. siRNA-NPM knockdown in MYCN-amplified SK-N-BE2 cells revealed no differences in proliferation or viability, indicating that suppression of NPM expression does not restore p53-independent functions.

Conclusion: Our proteomic and gene expression analyses suggest several other interesting targets for study, which are being validated by further proteomic analyses as well as in tumor samples obtained from COG. Modeling of cellular pathways important in MYCN-mediated tumorigenesis may identify interesting proteins for further signaling pathway analysis as well as potential targets for therapeutic development.

Email: slv@uchicago.edu

POB76
Analysis of cellular mediators of oncogenic signaling originating from activated ALK in neuroblastoma
Miyake, Izumi1; Kamata, Reiko2; Futami, Hitoyasu2; Sakai, Ryuichi2; 1Kitasato University School of Medicine, Department of Pediatrics, Sagamihara, Tokyo, Japan; 2National Cancer Center Research Institute, Growth Factor Division, Tokyo, Japan

We had previously demonstrated that a unique signaling pathway is activated in a subset of neuroblastoma cells under the effect of constitutive activation of ALK (anaplastic lymphoma kinase) due to gene amplification the ALK gene (Miyake et al., 2002). ALK is a receptor tyrosine kinase (RTK) originally found as an oncogenic fusion protein in anaplastic large cell lymphomas and is subsequently shown to be essential for the survival of the neuroblastoma cells with the amplified ALK gene (Oasajima-Hakomori et al., 2005). Recently, several reports indicated that activating mutations of ALK alleles are critical oncogenic factors in advanced neuroblastoma, therefore ALK is highlighted as a new target of the therapy of this disease. To investigate the identity of oncogenic signals induced by activated ALK in neuroblastoma cells, we have been analyzed the modification of signaling molecules under the control of the activated ALK were examined. We have previously showed that activating ALK associated with PTB domain of ShcC, followed by the constitutive activation of downstream signals. In this study, it was revealed that ALK formed complex with P130Cas via SH2 domain of ShcC and regulated the phosphorylation of P130Cas in ALK-activating neuroblastoma cell lines. P130Cas is known to be a docking protein contributed tumorigenicity as a potential substrate of oncogenic Src tyrosine kinase. Here it was confirmed that downregulation of P130Cas suppressed the potential of anchorage independent growth and motility of neuroblastoma cells. Interestingly, ALK harboring activating mutation of Y1174L and K1062M, appeared to form more rigid complex with p130Cas comparing to the wild type of ALK, suggesting that this enhanced potential to associate with p130Cas might have some relationship with oncogenic activation of ALK.

Email: rsakai@ncc.go.jp
POB78
Nucleotide excision repair and in vivo neuroblastoma chemoresistance to irinotecan

Munier, Fabienne1; Regaigner, Marie2; Philippe, Cathy3; Legendt, Marion3; Lazan, Vauclair4; Vassal, Gilles1
1Institut Gustave Roussy, UMR 8203 and Pediatrics, 39 rue Camille Desmoulins, Villejuif, France; 2Institut Gustave Roussy, UOF, 39 rue Camille Desmoulins, Villejuif, France

Background: Acquired drug resistance is a major obstacle to successful treatment of neuroblastoma (NB) by chemotherapy. To study the mechanisms of resistance to irinotecan in a therapeutic setting, we have established a resistant NB xenograft model in vivo. Resistance was induced by the topoisomerase I inhibitor irinotecan through sequential and repeated administration in tumor-bearing mice. Resistance was reversible after irinotecan treatment was stopped. The classical mechanisms of topoisomerase I inhibitors resistance were not involved and lack of cross-resistance with alkylating and platinating agents suggested that cell death mechanisms downstream DNA damage remained functional (Calvet et al. Br J Cancer. 2004; 91, 1205-1212).

Methods: We investigated events leading to cell death after DNA damaging treatment. DNA damage after irinotecan exposure and irradiation were compared in resistant IGR-NBBR and sensitive parental IGR-NB xenografts by following the induction of γ-H2AX (a DNA double-strand break (DSB) marker). The early cell fate decision after irinotecan injection at 15, 30 and 60 minutes was investigated at RNA level, using gene expression profiling by Agilent 44K array.

Results: In general, gene expression profiling revealed an implication of JNK survival pathway and an activation of several DNA repair mechanisms, primarily the nucleotide excision repair (NER).

Conclusion: In vivo resistance to irinotecan is due to very efficient DNA repair that could be initiated very early through the JNK pathway. To our knowledge, this is the first report showing that NER is implicated in the in vivo resistance to topoisomerase I inhibitors. Interestingly, effectors of NER are known to participate in DSB repair and could also remove DNA-topoisomerase I cleavage complexes. Our findings provide a strong rationale for investigation of NER and JNK pathway inhibition to reverse chemoresistance in our model.

This work was supported by Region l’Ile de France

Email: fabienne.munier@igr.fr

POB79
A role of human Sgo1 on the growth of human neuroblastoma cells

Murakami-Tonami, Hiko; Kishida, Satoshi; Kadomatsu, Kenji
Nagoya University, Graduate School of Medicine, Nagoya, Japan

Background/Aims: Shugoshin (Sgo) protein was first identified in fission yeast as a protector of centromeric cohesion and is required for accurate chromosome segregation. Like fission yeast protein, human Sgo proteins (hSgo1 and hSgo2) are essential to accurate chromosome segregation during both mitosis and meiosis. However, little is known whether hSgo1 is involved in cancer. The aim of this study is to reveal the role of hSgo1 in neuroblastoma cells. MYCN is known to be amplified in many neuroblastoma cells.

Methods and results: We have compared the gene expression profiling using microarray among the tissue specimen from ganglion, hyperplasia and tumor respectively. According to the microarray results, we found that hSgo1 was expressed greater than fold in hyperplasia and tumor samples than in normal ganglion. Next we checked the mRNA level of hSgo1 in human neuroblastoma cell lines. We found that hSgo1 expression was higher in MYCN amplified cell lines than MYCN single copy cell lines. To assess the effects of hSgo1 expression on neuroblastoma cell lines, hSgo1 knockdown was performed by transfecting SK-N-AS MYCN single copy cell line and IMR32 MYCN amplified cell line with a short hairpin RNA expression vector. hSgo1 knockdown IMR 32 cells proliferated slowly, but hSgo1 knockdown SK-N-AS cells grew at the same level as control cells.

Conclusions: These findings suggested that MYCN regulates hSgo1 expression and that hSgo1, in cooperation with MYCN, has an important role in growth of human neuroblastoma cells.

Email: ybuna@med.nagoya-u.ac.jp

POB80
NF-κB signaling in neuroblastoma

Nowakowska, Natalija; Visserleij, Roesje; Geerts, Dirk
Academic Medical Center - University of Amsterdam, Human Genetics, Amsterdam, Netherlands

Background/Aim: The NF-κB is a complex signaling pathway controlling inflammation and immune response, and is involved in the balance between pro- and anti-apoptotic gene expressions. Recently, a major role for NF-κB in tumor proliferation and apoptosis evasion was determined. We were therefore interested whether NF-κB governs these processes in neuroblastoma. It has previously been described that chemo-resistance of neuroblastoma cell lines can be overcome by inhibition of the NF-κB pathway, but it remains to be determined whether the classical (NFκB1/ RELA) and - (IκB) and/or the alternative (NFκB2/RELB) routes are involved, and which target genes play a role. Affymetrix expression profiles of 88 neuroblastomas generated in our laboratory showed correlations between the mRNA expression of several important NF-κB pathway genes with patient survival and tumor metastasis. These data suggested a role for the NF-κB pathway in neuroblastoma pathogenesis and drug responsiveness.

Methods: Several NF-κB reporter neuroblastoma cell lines were generated. The expression of the five key NF-κB transcription factors (NFκB1, NFκB2, RELA, RELB, and c-REL) was manipulated with shRNA silencing. Gene knock-downs were confirmed on protein and transcriptional activity level. The effects of NF-κB ligand stimulation and TRAIL treatment were examined.

Results: We found that the NF-κB pathway can indeed be constitutively active in neuroblastoma cell lines. However, these cell lines also showed an additional increase in NF-κB activity upon stimulation with IL1B, TNFα, or TRAIL. No activation was detected with the alternative NF-κB pathway ligand BAFF. Silencing of RELA gave strong growth inhibition and abolished TNFα resistance. Silencing IKKa and NFκB2 did not change NF-κB activity, again excluding a role for the alternative NF-κB pathway in neuroblastoma.

Conclusion: We found a role for the classical NF-κB pathway in neuroblastoma cell growth. Gene expression profiling analysis on stimulated cell lines, as well as on cell lines with altered NF-κB transcription factor activity will be used to identify the NF-κB target genes involved in neuroblastoma growth and chemo-resistance.

Email: N.E.Nowakowska@amc.uva.nl

Abstract Book 167

Posters
**POB81**

Activation of the transcription factor FOXO3/FKHL1 by dororubicin and etoposide induces autophagy but not programmed cell death in neuroblastoma cells

Obexer, Petra1; Hagenbuchner, Judith1; Hermann, Martin1; Kuznetsov, Andrey2; Ausserlechner, Michael3

1Medical University Innsbruck, Department of Pediatrics IV, Innsbruck, Austria; 2Tyrolean Cancer Research Institute, Pediatric Oncology Laboratory, Innsbruck, Austria; 3University Medical Center Innsbruck, Department of General and Trauma Surgery, Innsbruck, Austria

**Methods:** To investigate the relevance of FOXO3 activation in neuroblastoma cells, we knocked down the endogenous FOXO3 expression with two siRNAs targeting different regions of the respective mRNA. FOXO3 knockdown reduced both, population and clonogenic growth and increased the amount of dead cells. Furthermore, knockdown of FOXO3 expression induced cytosolic vacuolation and the accumulation of autophagosomes and autolysosomes in BE(2)-C cells as evidenced by electron microscopy, LC-3 conjugation and acridine orange staining. Bafilomycin A1, an inhibitor of autophagy, efficiently blocked this phenotype. Enforced expression of FOXO3 reduced autolysosome formation, whereas a catalytically impaired mutant did not. This indicates that FOXO3 plays an important role in regulating autophagy in neuroblastoma cells.

**Results:** Etoposide and doxorubicin treatment activates FOXO3, induces ROS and ROS-sensitive protein kinases through its activation. Furthermore, FOXO3 knockdown reduced autolysosome formation, whereas a catalytically impaired mutant did not. This indicates that FOXO3 plays an important role in regulating autophagy in neuroblastoma cells.

**Conclusions:** FOXO3 knockdown reduces cell viability and cell cycle progression, and stress resistance in neuronal cells and downstream targets of hyperactive PKB in neuroblastoma (NB). FOXO3/FKHL1 was shown to protect against reactive oxygen species (ROS) by regulating detoxifying enzymes. We investigated the relationship between FOXO3 activation and its involvement in FOXO3-induced apoptosis of human NB cells.

**Methods:** For studying subcellular shuttling of FOXO3 by live cell fluorescence imaging an ECFP-FOXO3-allele was retrovirally expressed in NB cells. Generation of ROS was assessed by fluorescence staining using the ROS-sensitive dye reduced MitoTracker Red CM-H2XROS. NB cell lines for constitutive or conditional expression of Birn, BcIXL, or shRNA directed against Birn and Sestrin3 were generated by retroviral gene transfer.

**Results:** Etoposide and doxorubicin treatment activates FOXO3, induces ROS and elevates the expression of the proapoptotic proteins Noxa and Bim in NB cells. Conditional activation of FOXO3 induced two sequential waves of ROS and death, the latter one being associated with a reduction in Noxa. Knockdown of BcIXL or retroviral overexpression of the prosurvival BcIXL both prevented ROS production and delayed apoptosis which implies that FOXO3-induced ROS is downstream of Birn proteins. The decline after the first ROS wave correlated with increased expression of the peroxiredoxin Sestrin3. Knockdown of Sestrin3 prevented the ROS decline and accelerated cell death in NB cells.

**Conclusions:** The combined data suggest that programmed cell death by FOXO3 involves ROS production downstream of BcIXL and FOXO3 in parallel activates ROS-protection by Sestrin3. Prolonged FOXO3 activation overcomes Sestrin3 protection, induced secondary ROS burst and eventually leads to cell death in human NB cells.

Email: petra.obexer@i-med.ac.at

---

**POB82**

Histone deacetylase 10 contributes to the regulation of autophagy in neuroblastoma

Obenhoff, Ing1; Linke, Jan-Peter1; Böck, Barbara C1; Milde, Tilo1; Lodrini, Marco1; Fischer, Matthias1; Roth, Wolfried1; Kaden, Sylvia1; Gröne, Hermann-Josef1; Deubzer, Hedwig E1; Witt, Olfra1

1German Cancer Research Center (DKFZ), CCU Pediatric Oncology, Heidelberg, Germany; 2German Cancer Research Center (DKFZ), Division of Vascular Oncology and Metastasis, Heidelberg, Germany; 3University Children’s Hospital of Cologne, Department of Pediatric Hematology, Oncology and Stem Cell Therapy, Cologne, Germany

**Results:** Histone deacetylase 10 (HDAC10) was found up-regulated in a large series of neuroblastoma tumors associated with good prognosis, suggesting that autophagy may play a role in neuroblastoma tumor biology in vivo. Our data show that knockdown of HDAC10 induces neuroblastoma cell death and that HDAC10 participates in the regulation of autophagic processes in neuroblastoma cells.

Email: loehme@dkfz.de

---

**POB83**

Aberrant activation of ALK kinase by a short form ALK protein in neuroblastoma

Okubo, Jun1; Takita, Junko2; Nishimura, Masashi3; Kiuchi, Akira1; Igarashi, Takashi1; Hayashi, Yasuhide1; Ogawa, Seoro1; Seishi3

1Univ of Tokyo, Pediatrics, Pediatri; 2Okita; 3Kato, Motohiro; Chen, Yuyan; Sainada, Masashi; Kiuchi, Akira; Igarashi, Takashi; Hayashi, Yasuhide; Ogawa, Seoro; Seishi

**Background:** Anaplastic lymphoma kinase (ALK) was originally identified from a rare subtype of non-Hodgkin’s lymphomas carrying (t2;5) translocation, where ALK was constitutively activated as a result of a fusion with nucleoschinin (NPM). Aberrant ALK fusion proteins were also generated in inflammatory fibrosarcoma and a subset of non-small cell lung cancers, implicated in their pathogenesis. More recently, we and others reported that ALK is also activated constitutively by gene mutations and/or an alternative splicing of its receptor-like kinase in sporadic as well as familial cases of neuroblastoma. Here we show another mechanism of ALK activation in neuroblastoma.

**Methods:** To examine the ALK status in neuroblastoma cells, we performed expression and re-sequences analyses of ALK in a total of 30 neuroblastoma-derived cell lines. Functional analyses of ALK protein were also performed using immunoprecipitation, in vitro kinase assay, colony formation assay, and RNA interference (RNAi)-mediated ALK knockdown.

**Results:** Of the 30 samples, we found a cell line showing an aberrant ALK protein with lower molecular weight. Re-sequencing of the ALK cDNA from the sample revealed a deletion of exons 2 and 3. The short form of ALK protein was autophosphorylated and exhibited a stronger in vitro kinase activity compared to the wild-type kinase. In addition, the short form of ALK protein was more resistant to a pan-ALK inhibitor, ponatinib, and exhibited a higher colony forming capacity in soft agar and tumorigenicity in nude mice. Furthermore, we demonstrated that RNAi-mediated knock down of ALK in the cell line expressing the short form of ALK resulted in suppression of cell growth, indicating its role in the development of neuroblastoma.

**Conclusions:** Our current findings indicate that amino-acid deletion is a novel oncogenic mechanism of ALK in neuroblastoma.

Email: j-okubo@umin.ac.jp

---

**POB84**

MYCN sensitizes human neuroblastoma cells to apoptosis by HIPK2 activation through a DNA damage response

Petroni, Mariangela1; Veselli, Veronica1; Paci, Andrea1; Rinaldo, Cinzia1; Massimi, Isabella1; Carbonari, Maurizio1; Dominici, Carlo1; McDowell, Heather1; Rinaldi, Christian1; Scarpiniti, Isabella1; Frati, Luigi1; Bartolazzi, Armando1; Cifulo, Alberto1; Soddu, Silvia2; Giannini, Giuseppe1; Giannini, Giuseppe1

1Sapienza University of Rome, Experimental Medicine, Rome, Italy; 2Regina Elena Cancer Institute, Experimental Oncology, Rome, Italy; 3Sapienza University of Rome, Clinical Medicine, Rome, Italy; 4Sapienza University of Rome, Pediatrics, Rome, Italy; 5Alder Hey Children’s NHS Foundation Trust, Oncology, Liverpool, United Kingdom; 6S. Andrea Hospital, Pathology, Rome, Italy

**Methods:** Of the 30 samples, we found a cell line showing an aberrant ALK protein with lower molecular weight. Re-sequencing of the ALK cDNA from the sample revealed a deletion of exons 2 and 3. The short form of ALK protein was autophosphorylated and exhibited a stronger in vitro kinase activity compared to the wild-type kinase. In addition, the short form of ALK protein was more resistant to a pan-ALK inhibitor, ponatinib, and exhibited a higher colony forming capacity in soft agar and tumorigenicity in nude mice. Furthermore, we demonstrated that RNAi-mediated knock down of ALK in the cell line expressing the short form of ALK resulted in suppression of cell growth, indicating its role in the development of neuroblastoma.

**Conclusions:** Our current findings indicate that amino-acid deletion is a novel oncogenic mechanism of ALK in neuroblastoma.

Email: j-okubo@umin.ac.jp
POB85
Expression of TWEAK/Fn14 in neuroblastoma; implications in apoptotic resistance and survival

Pettersen, Ingvik1; Baryawno, Ninib1; Rasmussen, Agnes2; Bakkelund, Wenche1; Zykov, Svetlana1; Winberg, Jan-Olof3; Moens, Ug3; Kogner, Per1; Johnsen, John Inge1; Svendsen2; Baldur
1University of Tromsø, Department of Cell Biology and Histology, Tromsø, Norway; 2Karolinska Institutet, Childhood Cancer Research Unit, Stockholm, Sweden; 3University of Tromsø, Department of Cell Biology, Tromsø, Norway; 4University of Tromsø, Department of Medical Biochemistry, Tromsø, Norway; 5University of Tromsø, Department of Microbiology and Virology, Tromsø, Norway

Background: Tumor necrosis factor-like weak inducer of apoptosis (TWEAK), a member of the tumor necrosis factor (TNF) family of cytokines, acts on responsive cells via binding to a cell surface receptor called Fn14. TWEAK binding to a Fn14 receptor or constitutive Fn14 overexpression has been shown to activate nuclear factor kappaB (NF-kB) signaling, which is important in oncogenesis and cancer therapy resistance.

Methods: Expression of TWEAK/Fn14 was analysed in neuroblastoma (NB) primary tumors and cell lines by RT-PCR, western blot, ELISA and immunohistochemistry. The effect of recombinant TWEAK on NB cells was assessed by analysis of NF-kB, Survivin, Bcl2, MMP-9 and gene silencing of TWEAK/Fn14.

Results: The treatment of NB cells with recombinant TWEAK in vitro causes increased survival and apoptotic resistance. This effect is partly due to the activation of the NF-kB signaling and increased expression of anti-apoptotic proteins. TWEAK induced cell survival was significantly reduced by silencing the function of TWEAK/Fn14 by siRNA. TWEAK also induced release of MMP-9 in NB cells.

Conclusions: In conclusion, TWEAK/Fn14 in NB suggests that TWEAK functions as an important regulator of NB growth, invasion, and survival and that therapeutic intervention of the TWEAK/Fn14 pathway may be an important clinical strategy in helping to regulate neuroblastoma carcinogenesis.

Email: balsve@iki.se

POB86
Green Tea Catechins inhibit neuroblastoma growth in vitro and in vivo

Piotrowska, Izabela1; Chayka, Olesya; Cantilena, Sandra; Sala, Arturo
1University of Tromsø, Department of Cell Biology and Histology, Tromsø, Norway

Background: Green Tea Catechins (GTC) are natural compounds with known anti-cancer activity. In recent years many reports have shown that GTC inhibit proliferation and induce apoptosis of various types of cancer. Capsaicin is a natural compound extracted from red peppers. It has been shown that capsaicin can inhibit the growth of xenotransplanted human prostate cancer cells in mice. In this study we investigated whether GTC and capsaicin could suppress the growth and survival of neuroblastoma (NB) primary tumors and cell lines by RT -PCR, western blot, ELISA and immunohistochemistry.

Methods: The effect of GTC on NB cells was evaluated by comparing cell lines with different MYCN copy numbers (IMR32, NLF, CHP212, SK-N-AS, and SY5Y; 100, 25, 2, 2, 2 copies/cell, respectively). Second, the impact of Trk receptor overexpression has been shown to activate nuclear factor kappaB (NF-kB) signaling, which is important in oncogenesis and cancer therapy resistance.

Results: We found that both GTC and capsaicin are potent inducers of tumour cell death and simultaneous treatment of NB cells with combination of the drugs resulted in induction of cell death at a level comparable with that of doxorubicin in vitro. Kaplan Meyers survival analysis shows that treatment of MYCN transgenic mice with GTC significantly prolonged their life span in comparison to control group. In xenograft model, GTC administration delayed tumour growth and reduced tumour size. Affymetrix chip analysis allowed the identification of genes differentially expressed as a result of GTC treatment in both xenograft and MYCN model.

Conclusions: The data presented in this study suggests that GTC are effective inducers of NB cell death and tumour regression in vivo and in vitro. Our data indicate that GTC could be used for chemoprevention of tumour relapse in children with neuroblastoma. Furthermore, the identification of molecular pathways modulated by GTC in vivo should result in the discovery of new targets for therapeutic approaches in neuroblastoma.

Email: i.piotrowska@hit.uct.ac.uk

POB87
N-myc gene expression: Impact on leukocyte infiltration in 3D neuroblastoma spheroids

Puppato, Maur1; Battaglia, Florian1; Gambini, Claudio2; Gregorio, Andrea1; Fardin, Paolo1; Varesio, Luigi1
1G.Gaslini Institute, Laboratory of Molecular Biology, Genoa, Italy; 2G.Gaslini Institute, Laboratory of Pathological Anatomy, Genoa, Italy

High N-myc expression is associated with advanced neuroblastoma stage and poor prognosis, but the relationship between N-myc and immunity has remained obscure. Multicellular neuroblastoma spheroids are a 3D in vitro model system that can reflect the pathophysiological in vivo situation of avascular neuroblastoma microregions and micrometastatic sites; in particular the core region of spheroids well mimics hypoxic conditions of neuroblastoma.

To investigate whether N-myc gene expression together with hypoxia affects leukocyte infiltration we used 72 hours cocultures of peripheral blood mononuclear cells (PBMCs) and preformed 4 days old SHEP21N spheroids. Both the starting spheroids culture and the following spheroids-PBMCs coculture was grown with (N-myc-; bare SHEP21N N-myc expression) or without (N-myc+; high SHEP21N N-myc expression) tetracycline. The distribution of hypoxia and leukocyte infiltration was determined from 5-um-thick paraffin-embedded spheroids sections using respectively monoclonal antibodies to pimonidazole (Hypoxprobe kit) together with antibodies to hypoxics markers (e.g. HIF1α, HIF2α, VEGF, CaX) and appropriate leukocyte-specific antibody (CD3, CD20 or CD68).

Moreover overusing Affymetrix GeneChip we studied the differences in gene expression profile of N-myc+ and N-myc-SHEP21N spheroids. The gene expression data were analyzed using GeneSpring GX 7.3 software. We found that distribution of hypoxic regions and hypoxics markers was similar both in N-myc+ and N-myc- SHEP21N spheroids while infiltration of leukocytes, especially macrophages, was detectable only into N-myc- SHEP21N spheroids. Regarding gene expression experiments we found that some chemokines (e.g. CXCL12 and CXCL14) were upregulated in SHEP21N spheroids that barely express N-myc gene relative to SHEP21N spheroids with high N-myc expression.

Our data suggest a negative correlation between overexpression of a prognostically relevant oncogene, N-myc, and leukocyte infiltration into neuroblastoma tumors. Therefore N-myc mediated tumorigenesis may be coupled with mechanisms of immune escape that are dependent on N-myc involvement in the regulation of immunologically relevant genes.

Email: maurapuppato@ospedale-gaslini.ge.it

POB88
Use of a microgravity culture system to assess biological behavior in neuroblastoma cell lines

Redden, Robert1; Iyer, Radhika; Urbanski, Laura; Minturn, Jane; Brodeur, Garrett2; Doolin, Edward1
1The Children's Hospital of Philadelphia, Department of General Surgery, Philadelphia, United States; 2The Children's Hospital of Philadelphia, Department of Pediatrics, Philadelphia, United States

Background: Clinical and biological features are used to predict outcome in NB patients. However, these assessments are inconsistent. We evaluated a microgravity culture system (rotary bioreactor), which allows NB cells to develop 3D structures (organoids), to predict biological and clinical behavior in these tumors.

Methods: We studied two characteristics of NB that impact malignant growth, cohesion, and morphology can be measured in a reproducible way. This would allow rapid evaluation of different biological perturbations that are dependent on immune escape that are dependent on NB patients.

Results: Cells aggregated to form tumor-like organoids. Organoid size and shape correlated with malignant potential. MYCN-amplified cell lines expressing subclones was also investigated. Single cell suspensions of MYCN transgenic mice were used to predict outcome in NB patients. However, these assessments are inconsistent. We evaluated a microgravity culture system (rotary bioreactor), which allows NB cells to develop 3D structures (organoids), to predict biological and clinical behavior in these tumors.

Conclusions: This 3D culture system provides the assessment of biological and potentially clinical behavior of NB in a more refined way than traditional cell culture. Specific biological characteristics such as growth, cohesion, and morphology can be measured in a reproducible way. This would allow rapid evaluation of different biological perturbations on cellular behavior.

Email: redden@email.chop.edu
POB89

A biological link between p53 and MYCN/MYC expression in neuroblastoma

Regan, Paul Lucas1; Edo, Robby1; Iekagi, Naoko1; Rappaport, Erin2; Tang, Xiao1

1University of Illinois at Chicago, Anatomy and Cell Biology, Chicago, United States; 2Children’s Hospital of Philadelphia, NAP Core, Philadelphia, United States

Background: We previously showed that TSA (an HDAC inhibitor) and Epoxomycin (a proteasome inhibitor) as single agents and in combination significantly suppressed growth of MYCN-amplified neuroblastoma (NB) cells. However, these compounds had contrasting effects on MYCN expression. TSA down-regulated MYCN expression, but Epoxomycin and the TSA/Epoxomycin combination led to MYCN hyper-expression (defined as markedly increased expression beyond that observed in the untreated cells). The expression of p53 was also increased in MYCN-amplified cells treated with Epoxomycin or the TSA/Epoxomycin combination. We examined (i) the pattern of gene expression induced by MYCN hyper-expression in MYCN-amplified cells, and (ii) a potential functional relationship between p53 and MYCN/MYC in NB.

Methods: Transient transfection of MYCN and TP53 into NB cells was done by electroporation. Gene expression profiling, TaqMan real-time PCR, and Western blot assays were used to detect expression patterns of genes and proteins.

Results: We confirmed that ectopic MYCN expression in MYCN-amplified IMR5 cells resulted in growth suppression. Gene expression profiling analysis revealed that the hyper-expression of MYCN in the MYCN-transfected IMR5 cells led to an increased expression of genes involved in growth suppression and apoptosis (EGR1, EPH2A, KLF2, PERP, SEL1L). The expression of PERP and EPHA2 was confirmed by TaqMan real-time PCR and Western blot assay, respectively. Co-transfection of TP53 and MYCN in IMR5 cells led to high p53 expression but a reduction in MYCN expression (below the levels of endogenous MYCN). Transfection of TP53 into IMR5, SYSY, and SK-NAS reduced endogenous MYCN and MYC expression in these cells. Consistent with these observations, treatment of IMR5 and SYSY cells with Doxorubicin, CocCl2, or Roscovitine resulted in an increased p53 expression and a reduction of MYCN and MYC expression.

Conclusions: Although high MYCN expression sustains growth of MYCN-amplified NB, the hyper-expression of MYCN is deleterious to survival of these cells. Transfection of p53 expression has a suppressive effect on MYCN/MYC expression in NB cells.

Email: xaotang@uic.edu

POB90

Mitochondria-related destabilization of MYC family proteins in neuroblastoma cells by OSU-03012, FCCP and Salinomycin

Regan, Paul Lucas1; Fox, Autumn1; Torres, Jaime1; Jacobs, Joshua1; Horuchi, Makoto1; Ishi, Takayuki1; Tang, Xiao1; Iekagi, Naoko1; Ito, Takeshi1; Tsuji, Takehito1; Keita1; Sato, Yoshihara1; Kouchi, Katsunori3; Shirasawa, Hiroshi1; Yoshida, Hideo1

1Graduate School of Medicine, Chiba University, Pediatric Surgery, Chiba City, Japan; 2Graduate School of Medicine, Chiba University, Virology, Chiba City, Japan; 3Tokyo Women Medical University Yachiyo Medical Center, Pediatric Surgery, Yachiyo City, Japan

Background: Previously, we reported that Sindbis virus (SIN) AR339 strain has a possibility as a novel agent for human cervical and ovarian cancer therapy. In this study, we examined whether the human neuroblastoma cells are also susceptible to SIN.

Methods: We evaluated the oncolytic effects of SIN on human neuroblastoma cell lines and its therapeutic efficacy in vivo against human NB cells transplanted into nude mice. And to determine the major components of SIN involved in the oncolysis, we constructed a number of SIN expressing plasmids encoding the structural proteins, capsid, E2, and E1, which were transfected into neuroblastoma cells.

Results: SIN infection induced remarkable oncolytic effects on 14 human NB cell lines; IMR32, NB69, SK-N-SH, SH-SY5Y, NB-1, CHP134, IMR32, GOTO, NBMB, NLF, NGP, SMS-KAN, SMS-CKN, LAN-5, and RT-BM-1, but not on normal human keratinocytes in vitro. In nude mice, i.t. and i.v. inoculation of SIN resulted in significant regression of NB xenograft tumors. In addition, we found the oncolytic effect of UV-inactivated, replication-defective SIN against NB cell lines; IMR32, NB69, NGP, and SK-N-SH, and showed that it was induced by apoptosis. This implies that sfructural proteins of SIN play a major role in this oncolysis. Therefore, we constructed expression plasmids encoding each structural protein, capsid, E2, and E1, which were transfected into neuroblastoma cells. Transfection of the plasmid expressing E1 showed the highest cytotoxicity. The expression plasmid of E2 also showed cytotoxic effect, but the plasmid for Capsid and the vector displayed almost no cytotoxicity.

Conclusions: These results imply that the E1 protein plays an important role in the oncolysis of NB cells by UV-inactivated SIN. As the E1 protein has been considered to contribute to the viral fusion by changing the membrane permeability, it was suggested that the E1 protein of SIN causes the oncolysis of NB cells by the apoptotic signal transmitted during the fusion of membranes.

Email: eyahata@graduate.chiba-u.jp

POB91

Nutlin-3 induced mRNA expression changes in neuroblastoma cells

Rihani, Ali1; Van Maerken, Tom1; Yigit, Nurten1; Nuyten, Justine1; Mestdagh, Pieter1; Speleman, Frank1

Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium

Background: Less than 2% of neuroblastomas tumors harbor a p53 mutation at diagnosis. The inhibition of p53 by its antagonist MDM2 makes p53 an attractive target for molecular therapy, whereby its release from MDM2 leads to its activation and consequently to cell cycle arrest and apoptosis. A recently discovered antagonist of MDM2, nutlin-3, can specifically target MDM2 and modulate p53 activity.

Methods: We assayed for the deregulated miRNAs, cellular effects as cell differentiation and cell viability assays were evaluated.

Results: Deregulated miRNAs after activation of p53 include mir-223, mir-449a, mir-15a, mir-15b, mir-16, mir-323, mir-424, mir-20b, and mir-133a. Functional validation of a first candidate shows that forced mir-133a overexpression induces differentiation in NGS cells. Some predicted targets of this miRNA include EGFR, NDRG1, BCL2L1, and CSF2; these genes have been reported to be involved in tumorigenesis of several types of cancer.

Conclusion: Further experiments are ongoing to functionally validate the importance of the deregulated miRNAs in the p53-mediated cellular responses. We believe that dissecting the p53 pathway and unravelling miRNAs involved in various p53-mediated cellular responses will help us to understand the pathogenesis of neuroblastoma and to delineate new targets for therapeutic intervention.

Email: alrihati@ugent.be
POB93
A novel whole genome amplification approach is useful to perform aCGH in microdissected human ganglioneuroblastoma and neuroblastic components of ganglioneuroblastomas
Scarfelli, Paola1; Coaco, Simona1; Valdora, Francesca2; Stigliani, Sara1; Zhang, Yan1; Chen, Pengchun1; Smutko, John1; Tonini, Gian Paolo2; National Research Institute (ISGiBiO), Genoa, Italy; University of Genova, Department of Oncology and Genetics (DOBIO), Genoa, Italy; NuGEN Technologies, Inc., NuGEN, San Carlos, CA, United States
Background: Tools for subtle characterization of genomic DNA (gDNA) from small clinical samples require linear whole genome amplification (WGA). We show results of Early Access Program (EAP) for Single Primer Immortalization (SPIA) and the technology to analyze minute samples (i.e., cells isolated by LCM of heterogeneous tumors).
Results: The EAP provided a high consistent performance in beta test among the EAPs. Array quality metrics were highly correlated between unamplified and amplified DNAs, indicating that SPIA from as little as 10 ng of starting gDNA generates robust array-CGH results. In Nb and SS cells it successfully identified 1p36-pter imbalance detected by FISH in 40% of neuroblastoma.
Conclusion: aCGH can be employed in the Agilent-CGH workflow without any deviation from recommended protocol; b) SPIA method needs lesser amounts of starting gDNA in comparison to Sigma WGA; c) amplified DNA produces high-quality chromosomal karyotypes that perfectly match results from unamplified gDNA; d) Such approach provides a means to decrease the amount of required DNA for aCGH, which could open the way for the technology to analyze minute samples (i.e., cells isolated by LCM of heterogeneous tumors).
Funds: Fondazione Italiana per la Lotta al Neuroblastoma, MIUR. Participation of P.S. to ARNO2010 is supported by NuGEN
Email: paola.scaruffi@istge.it

POB94
Analysis of expression and inhibition of the Sonic Hedgehog signaling pathway in neuroblastoma
Schlaepfer, Paola1; Laczko, Paula1; Martinsson, Tommy2; Johnsen, John Inger1; Kogner, Per3; Castrellas, Javier S1; University of Navarra School of Sciences, Brain Tumor Biology Unit, Pamplona, Spain; 2University of Navarra, Department of Health Sciences, Pamplona, Spain; 3University of Gothenburg, Department of Clinical Genetics, Gothenburg, Sweden; 4Karolinska Institutet, Childhood Cancer Research Unit, Department of Women’s and Children’s Health, Stockholm, Sweden
Background/aims: The Hedgehog (Hh) signaling pathway has been implicated in the development of several types of solid tumors, including embryonic tumors such as medulloblastoma, although its role in neuroblastoma has not been fully investigated yet. The aim of this study was to analyze the role of the Hh signaling pathway, if any, in NB.
Methods: The expression of SMO, PTCH1 and GLI1 marker genes was analyzed by qRT-PCR and western blot in 12 neuroblastoma cell lines and 28 NB tumor samples. Treatment with the SMO inhibitor cyclopamine was carried out in 12 NB cell lines. Inhibition of the pathway was determined by the fold change decrease of GLI1 at RNA level. Following cycloamine treatment we analyzed changes in cell proliferation, apoptosis and CD133, BCL2 and BAX1 gene expression.
Results: High expression levels of the pathway components in most neuroblastoma cell lines as well as in 25% of the NB tumor samples was detected, suggesting a persistent activation of the Hh pathway in a subset of NB. Genomic amplification of the GLI1 gene was detected in one tumor expressing very high levels of GLI1. Inhibition of the pathway using the SMO antagonist cyclopamine, induced a dramatic decrease in the proliferation capacity of the NB cell lines treated. An increase in the presence of miRNA fragments together with a decrease in the BCL2/BAX1 ratio was found. Moreover, cycloamine reduced the levels of GLI1 and CD133 expression in NB cell lines.
Conclusion: Components of the Hh pathway are highly expressed in neuroblastoma and could be an important feature of neuroblastoma development.
Email: pschlaepfer@alumni.umue.es

POB95
Anaplastic Lymphoma Kinase (ALK) activates the small GTPase Rap1 via the Rho-GeF C3G in neuroblastoma
Schonherr, Christina1; Yang, Hai-Ling2; Vigny, Marc3; H. Palmer, Ruthi1; Hallberg, Bengt1; Umeå University, Department of Molecular Biology, Umeå, Sweden; 2Beijing Forestry University, College of Biological Sciences and Biotechnology, Beijing, China; 3UB39 INSERM/UPMC IFM, Paris, France
Background: The oncogene ALK RTK was detected as the translocation product PFM-ALK in a subset of Anaplastic Large Cell Lymphomas, but more fusion proteins have been found recently. While many studies investigated the mechanisms of action employed by oncogenic ALK fusion proteins, the physiological function of ALK and its downstream targets in mammals is still unclear, but ALK (<10% of NB) is suggested to be involved in the normal development and function of the nervous system. In D. melanogaster ALK can be activated by the natural ligand Jelly Belly (Jeb), promoting formation of the visceral musculature of the gut and guidance cues for axons in the optic lobe. No Jeb exists in mammals. Neuroblastoma, which is derived from neural crest cells and can occur in the entire peripheral sympathetic nervous system, accounts for approximately 15% of all pediatric cancer. Activation of the ALK locus as well as recently reported activating ALK point mutations have been observed in neuroblastoma cell lines and patient samples. The small GTPase Rap1 is involved in the regulation of many cellular processes, amongst others neurite outgrowth and neuronal polarization, which are mediated by sustained MAPK-pathway activation. Rap1 activity is controlled by activating GEFs and inhibitory GAPs. To date a role for Rap1 in the development of neuroblastoma has not been investigated.
Method/approach: We have developed a tetracycline inducible cell system in order to characterize downstream ALK signalling by examining whether ALK is able to activate Rap1 and contribute to differentiation and proliferatiion processes.
Results: Indeed, ALK activates Rap1 via C3G. The activation of the C3G-Rap1-pathway results in neurite outgrowth of PC12 cells, which is inhibited by siRNA-mediated knockdown of Rap1 or C3G. Significantly, this pathway also appears to function in the regulation of proliferation of neuroblastoma cell lines such as SK-N-SH and SH-SY5Y, after abrogation of Rap1 activity reduces cell growth.
Conclusion: These results suggest that ALK activation of Rap1 may contribute to cell proliferation and oncogenesis of neuroblastoma driven by gain-of-function mutant ALK receptors.
Email: christina.schonherr@mobiot.umu.se

POB96
Deep sequencing of the small-RNA transcriptome reveals differential expression of microRNAs in high-risk vs low-risk neuroblastoma
Schulte, Johannes1; Marschall, Tobias1; Martin, Marcel2; Rosenstiel, Philipp2; Messdagh, Pieter; Schlierf, Stefanie1; Thor, Theresa1; Vandesompole, Jo1; Egger, Angelika3; Schreiber, Stefan1; Rahmann, Sven1; Schramm, Alexander1
1University Clinic Essen, Childrens Hospital, Essen, Germany; 2TU Dortmund, LS Informatik 11, Dortmund, Germany; 3University Hospital Schleswig-Holstein, Inst of Clinical Molecular Biology, Kiel, Germany; 4Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium
Background: Small non-coding RNAs, in particular microRNAs, regulate fine-tuning of gene expression in general, and can act as oncogenes or tumor suppressor genes. Differential miRNA expression has been reported to be of functional relevance for the biology of neuroblastoma (NB) and other tumors.
Method/approach: Using next-generation sequencing (NGS), the unbiased and absolute quantification of the small-RNA transcriptome is now feasible. We here analyzed the small-RNA transcriptome in 10 NB with a maximally divergent clinical course (five favorable stage 1 versus five unfavorable MYCN-amended stage 4) using a SOLiD NGS approach generating a total of 188,000,000 reads.
Results: MiRNA expression profiles obtained by deep sequencing correlated well with real-time PCR data. Favorable and unfavorable NB could easily be separated using cluster analysis and significant differences between the miRNA transcriptomes of favorable and unfavorable NB were retrieved. Oncogenic miRNAs of the miR-17 cluster and miR-181 family were overexpressed in unfavorable NB. In contrast, the putative tumor suppressive miRNAs, miR-542-5p and miR-628, were present in favorable NB and virtually absent in unfavorable NB. High expression of various miR star (miR*) species was detected, and correlation of miRNA expression to the expression of the respective miRNA* varied markedly. In-depth sequence analysis also revealed extensive post-transcriptional miRNA editing. Finally, 13 putative novel miRNAs were identified using miRDeep. These of these novel miRNAs were further analyzed, and expression could be confirmed in a cohort of 70 primary neuroblastomas.
Conclusion: NGS is a viable tool to explore the small-RNA transcriptome and to identify novel miRNAs. Our results provide absolute miRNA expression counts and novel insights into the correlation of miR/miR* expression. Furthermore, we addressed the phenomenon of miRNA editing resulting in a variety of iso miRs. The functional implication of the latter mechanism in tumor biology warrants further analysis.
Email: alexander.schramm@uni-due.de
POB97

Prognostic significance of NKp30 spliceoforms in neuroblastoma.

Valteau-Couanet, Dominique

2Keck Sch of Medicine, Univ Southern Calif, Saban Research Inst,
1Keck Sch of Medicine, Univ Southern Calif, Saban Research Inst,
Silverman, Ayaka1; Fukaya, Yasushi1; Shimada, Hiroyuki2; Groshen, Susan3; Seeger, Robert1; DeClerck, Yves4

Evaluation of MCPIP expression patterns and their impact on the survival of neuroblastoma cell lines

Simpson, Anisha M.; Light, Jennifer E.; Minturn, Jane E.; Ho, Ruth; Iyer, Radhika; Varela, Carly R.; Mangino, Jennifer L.; Zhao, Huaqing; Kolla, Venkatakrishnan; Brodeur, Garrett M.

The Children’s Hospital of Philadelphia, Division of Oncology, Philadelphia, United States

POB98

Galecitin-3 binding protein/90 kDa Mac-2 binding protein stimulates interleukin-6 expression in the neuroblastoma microenvironment

Silverman, Ayaka1; Fukaya, Yasushi1; Shimada, Hiroyuki2; Groshen, Susan3; Seeger, Robert1; DeClerck, Yves4

Aim: To evaluate NKp30 spliceoforms in NB patients and their influence on disease dissemination and prognosis.

Methods: NKp30 spliceoforms were analyzed by Real-time reverse transcription-PCR in peripheral mononuclear blood cells from 94 NB patients treated in Gustave Roussy Inst from 1964 to 2010. Unsupervised hierarchical clustering was applied to data obtained and correlated with patient’s clinical data.

Results: Unsupervised hierarchical clustering classified patients in 3 subgroups. Forty-four out of 84 patients (47%) exhibited the predominant NKp30b isoform, 28 (30%) the predominant NKp30c isoform and 22 (23%) the NKp30a isoform. Among the 39 metastatic NB, 27 (69%) exhibited the predominant NKp30b (P=0.001). Finally, among the 49 localized NB with a follow-up more than 2 years, 1/12 (8%) with predominant NKp30a isoform relapsed comparing with 4/28 (14%) with predominant NKp30c or c isoforms.

Conclusion: This approach appears to correlate with dissemination of NB. Prospective studies are in progress to confirm the impact of NKp30 spliceoforms on NB prognosis. New drugs that modulate alternative splicing of NKp30 receptor may therefore represent a new therapeutic approach in NB.

POB99

TrkAIII isoform expression is associated with aggressive behavior in human neuroblastoma.

Simpson, Anisha M.; Light, Jennifer E.; Minturn, Jane E.; Ho, Ruth; Iyer, Radhika; Varela, Carly R.; Mangino, Jennifer L.; Zhao, Huaqing; Kolla, Venkatakrishnan; Brodeur, Garrett M.

The Children’s Hospital of Philadelphia, Division of Oncology, Philadelphia, United States

Background: TrkA is the receptor for nerve growth factor (NGF). High TrkA expression typically is associated with favorable clinical outcome in NB. Conversely, TrkAIII isoform expression in NB is associated with a poor clinical outcome.

Methods: To evaluate TrkAIII isoform expression, we examined a large number of NB cell lines and evaluated the prevalence of TrkAIII isoform expression in NB tumors. The NKp30 isoform is an important receptor for NGF in NB. We investigated the effect of TrkAIII expression on cell survival in vitro.

Results: A total of 404 NB cell lines were examined for the presence of TrkAIII isoform expression. Among the 404 cell lines, 26 (6.4%) expressed the TrkAIII isoform. In a large number of NB tumors, TrkAIII isoform expression was associated with poor clinical outcome.

Conclusion: TrkAIII isoform expression is associated with aggressive behavior in human neuroblastoma.

Acknowledgements: This study was supported by the European Commission’s project MKTD-CT-2006-042586 and by the Polish Ministry of Science 63/6 2007/2007.

Email: hanna.rioka@uj.edu.pl
POB101

NR1L1, a direct target gene of MYCN, modulates aggressive growth of neuroblastoma by selectively enhancing EGFR and IGF signals through the components of lipid rafts

Takatori, Atsushi; Hossain, MD. Shamim; Akter, Jesmin; Ogura, Atsushi; Nakamura, Yohei; Nakagawa, Akira
Chiba Cancer Center Research Institute, Division of Biochemistry and Innovative Cancer Therapeutics, Chiba, Japan

Background: Human NR1L1 (neuronal leucine-rich repeat protein 1) gene was originally identified from our neuroblastoma (NB) cDNA project. We previously reported that high level of NR1L1 mRNA expression is significantly associated with poor prognosis of NB patients. Indeed, the up-regulation of NR1L1 in both NB and non-NB cells enhances cell proliferation. However, the molecular mechanisms remain unclear.

Method: To analyze the effects of NR1L1 on downstream signals of growth factors, Western blotting, in vitro EGFR binding assay, immunoprecipitation (IP) and gradient fractionation were performed. To examine the role of NR1L1 in development, NR1L1 knockout mice were generated by conventional targeting strategy.

Results: MYCN directly bound to the promoter region of NR1L1 gene and activated its transcription. Overexpression of NR1L1 caused more phosphorylation of EGFR and IGF-IR, but not FGFR and TrkA, as well as ERK upon their ligands treatment in NB and other cell lines. Scatchard plot in EGFR binding assay showed a higher Bmax value in NR1L1-overexpressing cells than control cells and the slope of the Hill plot was 0.829 in NR1L1-overexpressing cells, suggesting that NR1L1 makes the binding sites more heterogeneous and increases EGF binding. However, IPA assay showed no obvious interaction between NR1L1 and EGFR or IGF-IR. Gradient fractionation revealed that NR1L1 proteins co-localized with EGFR and IGF-IR in a relatively high density fraction, the so-called SHB/Bip-like treatment of mGIPD, which disrupts lipid rafts, attenuated the NR1L1 function to accelerate EGF signaling. In mouse embryo, NR1L1 was expressed mainly in nervous system, myocyte and branchial arch which is derived from neural crest. A1 staining, NR1L1 knockout mouse showed less body weight than wildtype mice, suggesting that NR1L1 expression is essential for normal development and growth.

Conclusion: NR1L1 may sensitize EGFR and IGF signaling to enhance the cell growth by altering their membrane localization. The development of therapeutic agents against NR1L1 could be of benefit to the cure of high-risk NB.

Email: atakatori@chiba-cc.jp

POB102

Aurora A kinase is a possible target of OSU-03012 to destabilize MYC family proteins

Tamura, Yosuke; Taniguchi, Naoko
1 Chiba University, Graduate School of Medicine, Bioinformatics, Chiba, Japan; 2University of Illinois at Chicago, Anatomy and Cell Biology, Chicago, United States

Background: The 3-phosphoinositide-dependent kinase-1 (PDK1) inhibitor, OSU-03012, rapidly destabilizes MYCN and MYC proteins in neuroblastoma (NB) cells. However, OSU-03012 does not affect the phosphorylation status of AKT, suggesting that PDK1 is not the prime target of OSU-03012. Collectively, these data from our group indicate that OSU-03012 uses mechanisms that are PDK1-independent to destabilize MYC/MYCN and suppress growth of NB cells. In this study, we explore one of these mechanisms. It has been reported that Aurora kinase phosphorylates GS3Kδ, leading to its inactivation, and prevents MYCN/MYCN destabilization. Accordingly, Aurora A knockdown results in reduced efficacy of OSU-03012 and our observations, we hypothesize that one of the targets of OSU-03012 is Aurora A kinase.

Method: In silico molecular docking analysis was used to investigate whether OSU-03012 has any likelihood of binding to Aurora kinase.

Results: The 3D structure of Aurora A (PDB ID: 3DAJ) was obtained from the Brookhaven Protein Databank. The structures of OSU-03012 and FGX were constructed by using MOE (version 2007, CCG, Montreal, Canada). FGX is an Aurora kinase inhibitor, a derivative of Compound 6 that was discovered through site-specific dynamic combinatorial chemistry by Cancilla et al. (Bioorg Med Chem Lett 2008;18: 3977-81). Docking simulations and interaction energy calculations were performed by MOE Dock of MOE, based on the coordinate co-crystalization of FGX and Aurora A (PDB ID: 3DAJ). The resulting most stable docking structures between Aurora kinase A and FGX or OSU-03012 were displayed. Our analysis showed that the calculated interaction energy between Aurora kinase A and FGX was -89.273 kcal/mol, whereas that between Aurora kinase A and OSU-03012 was -109.901 kcal/mol. Thus, OSU-03012 has a higher likelihood of binding to Aurora A kinase.

Conclusion: These results suggest that OSU-03012 affects multiple cellular targets including Aurora A kinase to exhibit its growth suppressive, cell death-inducing, and MYCN destabilizing effects on neuroblastoma cells. Biochemical verification is currently pending.

Email: ikegaki@uic.edu

POB103

Expression and function of RET in neuroblastoma cell lines

Zeh, Laura H; Ho, Ruth; Simpson, Anisha M; Iyer, Radhika; Minturn, Jane E; Brodeur, Garrett M
The Children's Hospital of Philadelphia, Department of Pediatrics, Philadelphia, United States

Background: NB is the most common extracranial tumor in children and is associated with a high mortality rate. Receptor tyrosine kinases (RTKs) have been suggested as important molecules in the development of NB. RET plays an important role in the development of normal sympathetic neurons, and it has been implicated in NB pathogenesis. RET has four ligands (GDNF, NRTN, ARTN, or PSPN) and four co-receptors (GFRα1-4), which bind to RET and begin the signaling cascade. RET is expressed in many NBs, but little is known about the expression of RET and its co-receptors in NB cell lines and tumors, or the activation of RET in response to specific ligand exposure. We examined the presence of RET and GFRα1-3 in six NB cell lines and the phosphorylation of RET in response to ligand.

Methods: Methods: IMR5 were grown. The mRNA expression of RET, and GFRα1-3 was quantified by TaqMan assay. The protein expression of RET and GFRα1-3 and phosphorylation of RET was analyzed by Western Blot. Morphologic changes induced by ligand exposure were observed and photographed over 7 days.

Results: IMR5 has the lowest RET expression, whereas SY5Y, SK-N-AS, NLF, NBL5 and N-E-Bc1 expressed very high levels of RET. The expression of GFRα1-3 was variable in the different lines. At least one or more GFR co-receptor was expressed at high levels in the lines with high RET expression. NB-E-Bc1 and NBL5 had the most dramatic response to ligand exposure, as determined by both Western blotting and morphologic changes. Both NB-E-Bc1 and NBL5 underwent extensive neurite outgrowth during RET and ARTN treatment. IMR5 showed no significant morphologic change in response to any ligand. Expression of the co-receptors paralleled the ligands to which the lines had the greatest response (GFRα2 for NRTN, GFRα3 for ARTN). Conclusions: Ligand activation of RET in NB cell lines required the expression of the appropriate co-receptor. RET activation caused morphologic differentiation, demonstrating that the signaling pathway was functional and may contribute to NB survival and/or differentiation.

Email: brodeur@email.chop.edu

POB104

Irrespective of ALK mutational status, neuroblastoma tumors are sensitive to Akt inhibitor perifosine

Thiele, Carol; Li, Zhijie
National Cancer Institute, National Institute of Health, Pediatric Oncology Branch, Bethesda, United States of America

Background: Akt is an intracellular serine/threonine kinase that plays a key role in survival signaling pathways. Increased Akt activity mediates survival and resistance to chemotherapy in Neuroblastoma (NB) and activated Akt is more highly expressed in tumors from patients with a poor prognosis and, Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase predominantly expressed in the developing nervous system. Mutated ALK has been identified in association with familial and sporadic NB and patients whose tumors contain amplification, mutation or express increased levels of activated ALK have a poor prognosis. Akt is frequently a downstream target of ALK. Since Perifosine inhibits Akt , the aim of this study was to assess whether perifosine was active in NB cell lines with different ALK mutational status.

Methods: Six NB cell lines were utilized; 4 with ALK mutations- SY5YALK F1174L, KCNRAL R1725Q, Leri ALK R1227Q5 and SKNFAL K5138E5 and 2 with wild type( wt) ALK- ASALKw and GNPALKw. Cell survival after perifosine treatment was evaluated by MTS assay. In representative cell lines, the effects of Perifosine on tumor xenograft growth and inhibition of ALK/Akt signaling were evaluated.

Results: In vitro, perifosine treatment caused a 50-80% decrease in growth in all NB cell lines tested. Cell lines containing ALK mutations were inhibited to the same extent as ASALKw, which expresses low levels of ALK. Moreover perifosine induces apoptosis in KCNRAL R1725Q cells, which were reported to be resistant to the ALK inhibitor TAE684. Akt activation was inhibited by perifosine in all the cell lines tested. In vivo, perifosine treatment inhibited the growth of ALK-ASALKw and GNPALKw tumors and inhibited the growth of GNPALKw tumors and KCNRAL R1725Q tumors. Perifosine inhibited Akt phosphorylation in all these tumors.

Conclusion: These data indicate that Perifosine inhibits NB tumor cell growth despite the mutational status of ALK. Perifosine may be an effective treatment for NB tumors irrespective of their ALK mutational status.

Email: zhijie@mail.nih.gov
POB105  
Enhanced effect of IFNγ on the induced-apoptosis of neuroblastoma cells by cytotoxic drugs  
Tong, Haikai1, Zhang, Jinhu2  
1The 2nd Affiliated Hospital, China Medical University, Hematological laboratory, Shenyang, China; 2The 4th Affiliated Hospital, China Medical University, Pediatric Cancer, Shenyang, China  
Background: The expressions of Caspase 8, α cysteine protease that is crucial for the apoptotic cascade, is absent in a high percentage of neuroblastoma cells. Resistance of neuroblastoma cells to cytotoxic drugs-mediated apoptosis is thought to be caused by loss of Caspase 8 expression. In this study we explored the effect of cytotoxic drugs on neuroblastoma cell line SH-SY5Y and the influence of γ-interferon(IFNγ) on the antitumor effects of cytotoxic drugs.  
Methods: The expression of Caspase 8 mRNA and protein was detected with RT-PCR and Western-blot analysis. The effects of cytotoxic drugs (adriamycin, TNFα and TRAIL) cell viability by CellTiter 96 Assay. Caspase 8 mRNA and protein were determined by real-time PCR and Western blot.  
Results: We performed a functional analysis of the JAGGED-NOTCH signalling peptide. Previously, we identified NOTCH3 as master regulator of neuroblastoma cell migration. Therefore, we investigated whether the paralogous NOTCH2 receptor controls cell migration and analyzed the function of the ligand JAGGED1 in the regulation of NOTCH2-IC signalling.  
Methods: We performed a functional analysis of the JAGGED-NOTCH signalling axis in neuroblastoma cells using lentiviral shRNA mediated gene silencing and over-expression of NOTCH2 and JAGGED1. Cell migration was tested in transwell migration assays.  
Results: Unexpectedly, JAGGED1 did not activate, but rather attenuated NOTCH2-IC signalling. Consistent with this regulation, we found that cell migration is increased after JAGGED1 silencing, but is restored to control levels by the C-terminal domain of NOTCH2. Our data reveal that the antagonistic function of JAGGED1 acts on the liberated NOTCH2-IC domain. Interestingly, JAGGED1 is strongly regulated by both NOTCH2-IC and NOTCH3-IC and attenuates apoptosis on NOTCH-IC signalling in cis.  
Conclusions: This migratory phenotype exists in vivo. Pathway analysis of genes expressed in fast migrating cell lines revealed expression of several members from the Hippo-pathway, a cascade of negatively regulating kinases that converges on the regulation of YAP1. Knockdown of this transcriptional co-activator reduces neuroblastoma cell migration.  
Results: Our data reveal a set of co-regulated genes in fast migrating neuroblastoma cell lines that correctly predicts cell migration in other cell lines. Moreover, this 'migration signature' exists in vivo in neuroblastoma tumours. Finally, we functionally implicate YAP1 in neuroblastoma cell migration.  
Email: wyang68@gmail.com

POB106  
JAGGED1 antagonizes NOTCH2 mediated cell migration  
von Groningen, Tim; Broekmans, Marloes; Akogul, Nurdan; Versteeg, Rogier; van Nes, Johan  
University of Amsterdam, Human Genetics, Amsterdam, Netherlands  
Background: Notch signalling is an evolutionary conserved mechanism of intercellular communication involved in normal development and cancer. Ligands from the DELTA/JAGGED families activate NOTCH receptors, resulting in the liberation of the NOTCH intracellular domain (NOTCH-IC) signalling peptide. Previously, we identified NOTCH3 as master regulator of neuroblastoma cell migration. Therefore, we investigated whether the paralogous NOTCH2 receptor controls cell migration and analyzed the function of the ligand JAGGED1 in the regulation of NOTCH2-IC signalling.  
Methods: We performed a functional analysis of the JAGGED-NOTCH signalling axis in neuroblastoma cells using lentiviral shRNA mediated gene silencing and over-expression of NOTCH2 and JAGGED1. Cell migration was tested in transwell migration assays.  
Results: Unexpectedly, JAGGED1 did not activate, but rather attenuated NOTCH2-IC signalling. Consistent with this regulation, we found that cell migration is increased after JAGGED1 silencing, but is restored to control levels by the C-terminal domain of NOTCH2. Our data reveal that the antagonistic function of JAGGED1 acts on the liberated NOTCH2-IC domain. Interestingly, JAGGED1 is strongly regulated by both NOTCH2-IC and NOTCH3-IC and attenuates apoptosis on NOTCH-IC signalling in cis.  
Conclusions: Our data implicate the importance of the JAGGED-NOTCH signalling axis in neuroblastoma cell migration. Furthermore, we reveal an unexpected antagonistic function of JAGGED1 on NOTCH2 signalling, that occurs in a cellular and acts on the released NOTCH2-IC. Moreover, JAGGED1 is induced by NOTCH signalling to attenuate NOTCH-IC signalling in cis, but possibly to promote NOTCH signalling in trans as well.  
Email: w.j.vannes@amc.uva.nl

POB107  
A migration signature in neuroblastoma cell lines and tumours identifies YAP1 as a regulator of cell migration  
van Nes, Johan; Indemans, Mireille; Akogul, Nurdan; Koster, Jan; Versteeg, Rogier  
University van Amsterdam, Human Genetics, Amsterdam, Netherlands  
Background: Neuroblastoma is a childhood tumour from the peripheral sympathetic nervous system, a tissue ultimately derived from migratory neural crest progenitors. Aggressive neuroblastoma tumours can be locally invasive, but also highly metastatic. Cell migration is a prerequisite for invasive behaviour. However, the genes that control migration of neuroblastoma cells remain largely unknown.  
Methods: We analyzed differential gene expression correlating with a cell migration phenotype in vitro. The predictive power of this 'migration signature' was assessed in migration of other cell lines. The existence of a 'migration signature' was analyzed in a series of neuroblastoma tumours. Functional analysis was performed to identify candidate genes that control cell migration.  
Results: Two groups of fast and slow migrating neuroblastoma cell lines were identified in vitro. Affymetrix gene expression profiles were generated from these cell lines and used to identify a set of 256 differentially expressed genes between the fast and slow migrating groups. Gene Ontology analysis revealed that this gene set was enriched for genes with annotated functions in cell migration, adhesion and chemotaxis. This 'migration signature' correctly predicted the cell migration phenotype in 6 other cell lines that had not been tested previously. Furthermore, the 'migration signature' was found to be strongly co-regulated in a series of 88 primary neuroblastoma tumours, suggesting that this migratory phenotype exists in vivo. Pathway analysis of genes expressed in fast migrating cell lines revealed expression of several members from the Hippo-pathway, a cascade of negatively regulating kinases that converges on the regulation of YAP1. Knockdown of this transcriptional co-activator reduces neuroblastoma cell migration.  
Conclusions: Our data reveal a set of co-regulated genes in fast migrating neuroblastoma cell lines that correctly predicts cell migration in other cell lines. Moreover, this 'migration signature' exists in vivo in neuroblastoma tumours. Finally, we functionally implicate YAP1 in neuroblastoma cell migration.  
Email: w.j.vannes@amc.uva.nl

POB108  
Functional microRNA library screen to identify synthetically lethal interactions in neuroblastoma  
Van Peer, Gert; Mestdagh, Pieter; Speleman, Frank; Vandensompele, Jo  
Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium  
Background: Perturbation of microRNA (miRNA) function is well established as one of the possible mechanisms contributing to cancer formation and increasing evidence suggests a role in neuroblastoma. In an effort to elucidate the role in neuroblastoma, a complementary data set was generated, containing miRNA and mRNA gene expression and copy number information in a large cohort of neuroblastoma tumors and cell lines, and next-generation sequencing based mutation data is about to complement this data set. In order to understand how altered miRNA expression levels, copy numbers or sequence variants contribute to neuroblastoma pathogenesis, functional assessment of individual miRNAs is needed.  
Method/approach: A functional miRNA library screen, containing miRNA mimics and antagonists for 470 miRNAs, will be performed on well characterized neuroblastoma cell line model systems for MYCN, ALK and TP53. Effects of library transfection on cell viability will be monitored using Real-Time Cellular Analysis (RTC) technology, measuring electrical impedance of cell cultures in 96-well plates. Using in-cell reverse transcription together with high-throughput RT-qPCR, monitored cell lines will subsequently be profiled for the expression of a set of marker genes reflecting the status of known cancer pathways. Selected miRNAs will then be further characterized, evaluating their effects on proliferation, apoptosis, differentiation and cell cycle distribution.  
Results: MiRNAs displaying differential effects in the model systems will receive further attention, as they serve as potential candidates for targeted therapy. Results on the screen will be presented.  
Conclusion: In analogy to high-throughput RNAi library screens for the identification of candidate therapeutic targets, we aim to identify synthetically lethal miRNA interactions with important cancer genes in neuroblastoma  
Email: gert.vanpeer@ugent.be
POB109
Galectin-3 protects MYCN single copy neuroblastomas cells from apoptosis by modulating the MYCN repressed Gal-3 expression. Veschi Veronica1; Gulino, Alberto1; Giannini, Giuseppe1
Children’s NHS Foundation Trust, Oncology, Liverpool, United Kingdom; Email: veronica.veschi@libero.it
NB cell to apoptosis might be closely dependent on the regulation of the MYCN repressed Gal-3 expression, suggesting that its ability to sensitize failed to induce apoptosis and sensitize MYCN single copy (MNSC) as we recently demonstrated. Surprisingly however HIPK2 overexpression sensitivity to apoptosis triggered by different events. At least in part, this is associated to neuroblastic tumor biology and its amplification (MNA) is amplified and non-amplified tumors between 4S. Andrea Hospital, Pathology, Rome, Italy
2Sapienza University of Rome, Pediatrics, Rome, Italy; 3Alder Hey Children’s NHS Foundation Trust, Oncology, Liverpool, United Kingdom; 5S, Andrea Hospital, Pathology, Rome, Italy
Galectin-3 (Gal-3) is a b-galactoside specific lectin provided with a unique carbohydrate recognition domain. Biologically, it is involved in a number of processes, largely depending on its particular localization. Nuclear Gal-3 is related to RNA processing and DNA transcription while cytoplasmic Gal-3 is involved in controlling either apoptosis or Ras signaling. Gal-3 can also be secreted in the extracellular matrix to control cell adhesion, migration and angiogenesis. Aberrant Gal-3 expression occurs in different human malignancies with potentially opposite effects on tumor progression. In thyroid tumors, Gal-3 expression may be considered diagnostic of malignancy and appears to be controlled by aberrant p53 signalling. In particular p53 phosphorylated on S15 by its proapoptotic activator kinase HIPK2 transcriptionally repress Gal-3 in response to DNA damage in order to commit cells to apoptosis. Conversely, Gal-3 suppresses HIPK2-p53 induced apoptosis. MYCN gene is strictly associated to neurotrophic tumor biology and its amplification (MNA) is the most relevant adverse prognostic marker in human neuroblastomas (NBs). Paradoxically, its expression might also be associated to increased sensitivity to apoptosis triggered by different events. At least in part, this is due to the upregulation of the proapoptotic p53 kinase HIPK2 by MYCN, as we recently demonstrated. Surprisingly however HIPK2 overexpression failed to sensitize MYCN amplified cancer cells to DNA damaging drugs, suggesting that these cells might be protected from HIPK2-p53-induced apoptosis by Gal-3. Consistent with this hypothesis, Gal-3 is strongly expressed in MNSC cells at RNA and protein level, and its depletion by specific RNA interference sensitizes these cells to apoptosis. Conversely, we detected very low levels of Gal-3 in MNA NB cells which can be made more resistant to apoptosis by transfection with Gal-3 cDNA or by constitutive Gal-3 overexpression. MYCN repressed Gal-3 expression, suggesting that its ability to sensitize NB cell to apoptosis might be closely dependent on the regulation of the HIPK2-p53-Gal-3 axis.
Email: veronica.veschi@libero.it

POB110
Genome-wide analysis of favorable-stage neuroblastoma reveals dysregulated expression and splice-site loss splitting between MYCN amplified and non-amplified tumors
Wulchenboum, Samuel L.1; Cohn, Susan L.1; George, Rani E.1
1University of Chicago, Department of Pediatric Oncology, Computation Institute, Chicago, United States; 2University of Chicago, Department of Pediatric Oncology, Chicago, United States; 3Dana-Farber Cancer Institute, Department of Pediatric Oncology, Harvard Medical School, Boston, United States
Background: Children with localized MYCN amplified hyperdiploid neuroblastomas do better than those with diploid tumors. We sought to probe the genetic signatures of these tumors to gain an understanding of their clinical behavior.
Methods: We analyzed 28 favorable stage (INSS Stage I, Ila, Iib, IVs) MYCN-amplified tumors from the COG Tumor Bank with the Affymetrix SNP 6.0 and HuEx 1.0 Expression array platforms. Exon array data was normalized with Affymetrix Power Tools and then analyzed in BioConductor. SNP genotyping was performed using Affymetrix Genotyping Console, and subsequent analysis was performed using dChip and custom scripts.
Results: SNP data was obtained on 24 tumors and 9 paired blood samples. Exon array data was obtained for 14 tumors. LOH was determined by comparison to a paired blood sample or a dataset of HapMap controls and copy number calculation by comparison to the average of blood and HapMap samples. Five samples reported to have MYCN amplification had copy numbers less than 5 and 2-4 times comparison of the diploid control. Moreover, there were too few hyperdiploid samples to allow a comparison. Unsupervised clustering of exon data revealed two groups of tumors, corresponding to MYCN status, with stage IVs tumors clustered tightly together among MYCN amplified samples. Genes whose expression contributed most to the separation along PC1 included MYCN, DDX1, PHOX2B, SYN3, and AHNAK. Analysis of alternative splicing isoforms of PHOX2B, SPARC1L, and AHNAK.
Conclusion: Using complimentary genome-wide approaches, we show that among favorable-stage tumors, MYCN amplification is associated with a discrete profile of LOH, gene expression and alternative splicing, revealing interesting tumor suppressor gene candidates and suggesting potential mechanisms for MYCN and unbalanced gain of 17q but no 11q deletion. Chromatin was isolated from cells and stabilized by cross-linking, after which immune-precipitation was carried out using specific antibodies against polymers if to isolate protein:DNA complexes. Purified RNA was then hybridized in triplicate onto the GeneChip® Human Promoter 1.0R Array and GeneChip® Human Tiling 2.0R Array (Affymetrix). Analysis was performed using Affymetrix Tiling Analysis Software. Selected target gene expression was verified by quantitative real-time PCR (qRT-PCR).
Results: We identified 102 ch 1p genes on Tiling Array analysis and 55 genes on Tiling Array analysis that showed significantly decreased Pol II binding and subsequent transcription in the LAN-6 cell line (1p-), comparing to SK-N-SH (1p+). There were 37 overlapping genes between the two arrays, of which 22 localized to 1p35-36. Among these we identified known candidate TSG genes, such as KIF1B and CDH11, the decreased expression of 17 genes in the 1p deleted cell line LAN-6, was verified by qRT-PCR (1.2 fold change, P<0.05). Decreased expression of these genes was further confirmed in four additional NB cell lines with 1p deletion compared to one cell line with intact 1p.
Conclusion: Using two complementary genomic approaches, we have identified a number of candidate tumor suppressor genes on chromosome 1p. Further functional validation will reveal whether they have a role in neuroblastoma tumorigenesis.
Email: rani.george@dfci.harvard.edu
POB113

Expression alterations in ultra-conserved non-coding RNA resulting from changes in MYCN levels and in response to ATRA-induced differentiation

Watters, Karen; Bryan, Kenneth; Foley, Niamh; Stallings, Ray
Royal College of Surgeons in Ireland, Cancer Genetics Department, Dublin, Ireland

Background: All-trans-retinoic acid (ATRA) causes the SK-N-BE neuroblastoma (NB) cell line to undergo differentiation and leads to a significant decrease in MYCN. Transcribed ultra-conserved regions (UCRs) have been shown to be differentially-expressed in cancers versus normal tissue, indicating a possible role in carcinogenesis. Here, we examine the impact of ATRA treatment and changing MYCN levels on UCR expression.

Methods: Tiling microarrays were used to profile the expression of 962 UCRs (481 in sense and antisense orientation) in ATRA treated SK-N-BE cells and in SHEP-21N cells which contain a MYCN trans-gene under the control of a tetracycline responsive repressor element. mRNA expression was analysed using mRNA expression arrays.

Results: Following ATRA induced differentiation of SK-N-BE cells, 23 UCRs had a >1.5 fold increase in response to ATRA, while 1 UCR was decreased. 19 of these UCRs were exonic and 5 were intronic, with ~70% transcribed in the same direction of the host gene. Since MYCN levels significantly decrease in these cells, we decided to determine how many of these UCRs might be undergoing expression alterations as a consequence of MYCN depletion. We profiled UCR expression in SHEP-21N cells for both high and low (Dox-treated) MYCN states. 61 UCRs were affected by changes in MYCN levels. For MYCN depleted cells, the transcriptional activity of 32 UCRs was increased by >1.5 fold. Conversely, 29 UCRs decreased by >1.5 fold. Only two UCRs that were differentially expressed in SK-N-BE cells were also differentially expressed in the SHEP-21N cells, indicating that many mechanisms are involved with UCR regulation.

Conclusions: Our results indicate that significant numbers of UCRs have increased expression levels in response to ATRA and that an even greater consequence of MYCN depletion. We profiled UCR expression in SHEP-21N cells for both high and low (Dox-treated) MYCN states. 61 UCRs were affected by changes in MYCN levels. For MYCN depleted cells, the transcriptional activity of 32 UCRs was increased by >1.5 fold. Conversely, 29 UCRs decreased by >1.5 fold. Only two UCRs that were differentially expressed in SK-N-BE cells were also differentially expressed in the SHEP-21N cells, indicating that many mechanisms are involved with UCR regulation.

Email: karenwatters@rcsi.ie

POB114

ALK signaling in neuroblastoma

Westphal, Laura1; Koniken, Peter; Carol, Hubi; Versteeg, Rogier1
1Academic Medical Center, Human Genetics, Amsterdam, Netherlands; 2Academic Medical Center, Pediatric Oncology, Amsterdam, Netherlands

Background and aims: Activating mutations of the anaplastic lymphoma kinase (ALK) gene were recently described in familial and sporadic neuroblastoma. We aimed to analyse the effects of the F1174L and R1275Q ALK mutations in neuroblastoma cell lines to identify the downstream ALK signaling routes in neuroblastoma.

Methods: Lentivirally-delivered siRNAs were used for knockdown of the ALK gene in ALK mutant and wild type neuroblastoma cells. In addition, we have constructed stably transduced neuroblastoma cell lines with doxycyclin-inducible overexpression of wild type, the F1174L mutant or R1275Q mutant ALK. The phenotypical changes and downstream signaling of these cells were extensively analyzed. The mRNA expression profiles of the knockdown and over expression experiments were created and analyzed.

Results: Introduction of the wild type, the F1174L mutant or R1275Q mutant ALK gene were found to have different effects on the downstream signaling pathways in neuroblastoma cells. Although wild type ALK protein could be very efficiently induced in our system, no increase in phosphorylation of the wild type protein was observed. Consequently, downstream signaling pathways were not noticeably affected in these cells. Expression of the F1174L or R1275Q mutant ALK protein immediately resulted in a large increase of phosphorylated ALK protein. The mutants showed highly divergent induction of major downstream signal transduction routes. These differences were reflected in the different phenotypes, growth rate, their response to inhibitors, and in the gene expression profiles of the cell lines.

Conclusions: We demonstrate differences in downstream signaling pathways between the F1174L and R1275Q ALK mutants in neuroblastoma cell lines.

Email: e.m.westphal@amc.uva.nl

POB115

Regulation of differentiation by estrogen receptors in neuroblastoma cells

Wolter, Jennifer1; Maltkin, David2; Irwin, Meredith3
1University of Toronto/Hospital for Sick Children, Department of Medical Biophysics, Toronto, Canada; 2Hospital for Sick Children, Division of Hematology-Oncology, Program in Genetics and Genome Biology, University of Toronto, Department of Medical Biophysics, Department of Pediatrics, Toronto, Canada; 3Hospital for Sick Children, Division of Hematology-Oncology, Program in Cell Biology, University of Toronto, Laboratory Medicine and Pathology, Department of Pediatrics, Toronto, Canada

Background: Although rare at diagnosis p53 mutations (mt) and other abnormalities of the p53/HDM2/ARF pathway are more commonly detected in relapsed neuroblastoma (NB). P53 plays a critical role in the response to chemotherapies and other recently has been shown to control self-renewal and differentiation in normal and malignant neural stem cells. In two patients with aggressive chemorefractory chemotherapeutic treatment we performed p53 mt and p73 expression or function in TICs results in a survival advantage in vivo. Our objective was to determine the role of these mtpt3 proteins as well as wild-type (w) p53 and p73 in chemotherapeutic response in NB and their ability to survive and proliferate in TICs (TICs) that are isolated from the bone marrow of NB patients and have cancer stem cell properties.

Methods: Adherent and TIC NB lines were transfected with plasmids encoding wtpt3, Tap73, mtpt3 (R158H and R248W) or siRNA (p53 or p73). Immunoblotting to detect target gene expression and Cl-PARP, luciferase assays, alamar blue and sphere formation assays were performed.

Results: We identified two patients with Li-Fraumeni Syndrome (LFS)- associated NB. Overexpression of mtpt3 proteins derived from these patients diminished chemotherapeutics-induced apoptosis and activation of p53-target genes (p21, BAX, NOXA). P53 siRNA knockdown of endogenous mtpt3 in fibroblasts derived from the patient with R158H mutation resulted in enhanced chemosensitivity. Both mtpt3 proteins form complexes with wtpt3 and the pro-apoptotic p73 protein Tap73, which and when overexpressed inhibit p53 and Tap73-dependent p53 target gene activation. In NB TICs (with confirmed wtpt3 transfection of either mtpt3 LFS proteins resulted in more spheres that were larger in size. Similarly, p53 or p73 siRNA also resulted in larger spheres in culture.

Conclusion: Mtppt3 decreases chemoresistance in NB and interference with wtpt3 or p73 contributes to enhanced self-renewal and proliferation of NB TICs. Taken together our data support a role for p53 and p73 in NB chemotherapy response and suggest that interference with wtpt3 expression or function in TICs results in a survival advantage in vivo.

Email: jennifer.wolter@utoronto.ca
Identification of proteomic changes associated with differentiated neuroblastoma using an in vitro differentiation system and an optimized proteomics platform based on 18O peptide labeling

Yin, Chen1; Hsu, Wen-Ming2; Tsay, Yoou-Guang3

1National Yang-Ming University, Taipei, Taiwan, Institute of Biochemistry & Molecular Biology, Taipei, Taiwan; 2National Taiwan University, Taipei, Taiwan, Department of Surgery, Taipei, Taiwan; 3National Yang-Ming University, Taipei, Taiwan, Proteomics Research Center, Taipei, Taiwan

Background: Neuroblastoma (NB) is the most common extracranial solid cancer in childhood. When tissue biomarkers indicate good differentiation, more aggressive therapeutic measures can be avoided and the overall survival of these NB patients is not affected. Here, in order to further characterize what biomarkers are associated with well differentiated neuroblastoma, we have employed a newly optimized proteomics platform based on 18O peptide labeling to examine the structural change in an in vitro cell culture system stimulated by a -secretase inhibitor (GSI)-N-(3,3-difluorophenyl) acetyl-L-alanyl-2-phenylglycine -1,1-dimethylethyl ester (DAPT).

Method/approach: The 18O peptide labeling technique is supposed to be a simple, inexpensive, and comprehensive strategy, but this approach has been long marred by the issue of incomplete labeling. To overcome this problem, we have devised a protocol that renders complete peptide labeling, which thus produces a homogeneous four-dalton mass increase in peptides with 18O labeling. This difference can be readily detected using mass spectrometry. Intriguingly, many of them have been previously identified in neuroblastoma using an in vitro differentiation system and an optimized proteomics platform based on 18O peptide labeling to examine the structural change in an in vitro cell culture system stimulated by a -secretase inhibitor (GSI)-N-(3,3-difluorophenyl) acetyl-L-alanyl-2-phenylglycine -1,1-dimethylethyl ester (DAPT).

Results: With this technique, we have found that there are a small group of proteins differentially expressed in GSI -treated SK-N-SH cells. The identities of these differentially expressed proteins have been unambiguously revealed using liquid chromatography-tandem mass spectrometry. Intriguingly, many of them have been previously identified in cell systems with other differentiation stimuli.

Conclusion: While this result highlights the prowess of this system in identification of novel biomarkers associated with neuroblastoma differentiation, it also indicates the convergence of cellular pathways in differentiating NB cell under distinct stimuli.

Email: ygtsay@ym.edu.tw

POB118
Role of Caspase 8 and Caspase 3 in TRAIL-induced apoptosis of neuroblastoma cells
Zhang, Jinhu; Tong, Haixia
1The 4th Affiliated Hospital, China Medical University, Pediatric Cancer, Shenyang, China; 2The 2nd Affiliated Hospital, China Medical University, Hematological laboratory, Shenyang, China

Objective: To study the role of Caspase 8 and Caspase 3 in TRAIL- induced apoptosis of neuroblastoma cell line CHP212 cells.

Methods: The effects of TRAIL and Caspase 8/ Caspase 3 inhibitor on TRAIL on the apoptosis of CHP212 cells was detected by flow cytometry. The relative Caspase 8/ Caspase 3 activity was measured by colorimetric assay. The morphous of the apoptosis cells was detected by using the transmission electron microscope(TEM).

Results: CHP212 cells were sensitive to TRAIL and had dose dependency. Caspase 8/ Caspase 3 inhibitor could diminish the apoptosis of CHP212 cells induced by TRAIL. The relative Caspase 8/ Caspase 3 activity of CHP212 cells increased gradually with the prolongation of TRAIL action time and reached the peak at 16h and 8h respectively. Typical features of apoptosis were seen by TEM.

Conclusion: TRAIL could induce apoptosis of CHP212 cells through Caspase - transduced signal pathway with the increase of Caspase8 and Caspase 3 activity.

Email: ywang68@gmail.com

POB119
Creation of a CHD5 knockout (KO) mouse model
Zhuang, Tianyang1; Kolla, Venkatadri; Raabe, Tobias; Koyama, Hiroshi; Higashii, Mayumi1; White, Peter S.3; Brodeur, Garrett M.4
1The Childrens Hospital of Philadelphia, Department of Pediatrics, Division of Oncology, Philadelphia, PA, United States; 2The University of Pennsylvania, Department of Genetics, Philadelphia, PA, United States; 3The Childrens Hospital of Philadelphia, Department of Pediatrics, Division of Bioinformatics, Philadelphia, PA, United States; 4The Childrens Hospital of Philadelphia and The University of Pennsylvania, Department of Pediatrics, Division of Oncology, Philadelphia, PA, United States

Background: Neuroblastomas (NBs) are characterized by deletion of 1p36.31, and CHD5 is a tumor suppressor gene (TSG) that maps to this region. Our data strongly suggest that somatically acquired inactivation of this gene contributes to the development of high-risk NBs. CHD5 is expressed exclusively in the nervous system and may play a critical role in neural development, so an animal model with CHD5-KO may provide insight into normal neural development as well as tumorigenicity.

Method/approach: We used ES cell gene targeting to insert loxP sites in introns 11 and 13 of the human CHD5 gene. For selection, we inserted PGK-neo flanked by two FRT elements between exon 13 and the second loxP site. We used both PCR and genomic Southern blotting to identify stable integration of the targeting construct. Constitutional CHD5-KO mice were generated by mating Prm-CRE (Jackson Labs) male mice with female carrying the CHD5-KO construct. To generate the CHD5 conditional KO (CKO) mice, PGK-FLP male mice (Jackson Labs) were first mated with female mice carrying the CHD5-KO construct to remove the neo gene. Then females will be mated with TH-Cre (Jackson Labs) male mice to generate the TH-Cre/CHD5-CKO mice.

Results: We have successfully generated constitutional CHD5 +/- mice. After three weeks of observation, there is no apparent difference in the phenotype or behavior between wild type and CHD5-KO heterozygous mice. We will continue to observe them and monitor for neural abnormalities and tumorigenesis until the mice reach 24 months of age. CHD5-KO heterozygous mice will be mated to determine if total absence of this gene is embryonic lethal. We will observe the phenotype and tumorigenicity of homozygous TH-Cre/CKO mice to determine the effect on neural development and tumorigenicity.

Conclusion: The CHD5 constitutional and conditional KO mice may be used to assess the role of this gene in normal development and tumorigenesis. Assuming the mice develop NBs, this model can be used to evaluate new drugs to treat NB patients. Because 1p36 deletion including CHD5 occurs commonly in many human cancers, these animals could be used to elucidate pathogenesis and treatment as well.

Email: zhuangt@email.chop.edu
**Posters – Translational**

**PO1**

**Prognostic significance of tumor and microenvironment gene expression for children with metastatic MYCN non-amplified neuroblastoma**

Sposto, Richard1; Seeger, Robert C.1; Attiyeh, Edward F.1; Hogarty, Michael D.1; Mossé, Yael P.1; Diskin, Sharon3

Children’s Hospital of Philadelphia and University of Pennsylvania School of Medicine, University of Southern California, Children’s Oncology Group, Los Angeles, United States; Children’s Hospital Los Angeles, Saban Research Institute, Los Angeles, United States; Children’s Hospital, University of Cologne, Cologne, Germany; Texas Children’s Cancer Center, Baylor College of Medicine, Houston, United States; Dana-Farber Cancer Institute, Children’s Hospital Boston, Boston, United States.

**Background:** Outcome for patients with stage 4 MYCN non-amplified (MYCN-NA) neuroblastoma who are diagnosed >18 months of age previously could not be predicted using a multigene expression model. Using epoxygenase 15,55-gene expression from the model for these patients from microarray tumor gene expression profiling (JNCI, 2006). We now present a validated 2s2Marb® Low Density Array (TLDA) assay that predicts progression-free survival (PFS) using expression of genes from the microarray signature and of genes related to inflammation.

**Method/approach:** TLDA data for 48 genes was generated using primary tumors from patients of all ages with stage 4 MYCN-NA neuroblastoma enrolled in Children’s Cancer Group (CCG) trials (training set, n=133), German Pediatric Oncology and Hematology Group (GPOH) trials (test set 1, n=46), and Children’s Oncology Group (COG)-A0737 (test set #2, n=32). After normalization, a multivariate regression model was developed for PFS for the training set using genes that did or did not correlate with age, and this was subjected to leave-one-out cross validation (LOOCV). The model was then applied to two test sets. Results were evaluated by the Receiver Operating Characteristics (ROC) of the model. A cut-off value based on the median score of the training set defined high risk (HR) and ultra high risk (UHR) tumors of patients diagnosed >18 months of age (training set n=64, test set #1 n=39, test set #2 n=32).

**Results:** Fourteen genes were selected for the final model. Expression of 4 correlated with age at diagnosis and of 10 predicted relapse independent of age. The Area Under the Curve (AUC) of the ROC for predicting PFS of children diagnosed >18 months of age in the training set (LOOCV results) and test sets #1 and #2 were 74% and 85%, and 64%, respectively. The 5-year PFS for those diagnosed >18 months of age in the molecular HR and UHR groups in these three cohorts was 42% (±5%) and 14% (±5%); 57% (±13%) and 14% (±8%); and 67% (±19%) and 22% (±8%).

**Conclusion:** The expression of 14 genes of tumor and microenvironment cells accurately identifies a ultra high risk subset of patients among those diagnosed 18 months of age with stage 4 MYCN-NA neuroblastoma.

**Email:** sasgharzadeh@chla.usc.edu

**PO2**

**Genomic characterization and targeted resequencing of high-risk neuroblastoma (the neuroblastoma TARGET)**

Altay, Eidur1; Carey, Michael D.2; Koss, Kim1; Patel P1; Diekin, Sharon J2; Hakonarson, Hakon1; Asgharzadeh, Shahab1; Spoto, Richard1; London, Wendy B.2; Gastier-Foster, Julie M.2; Gerhard, Daniela S.2; Smith, Malcolm A.2; Zhang, Jinghua1; Khan, Javed1; Seeger, Robert C.1; Mans, John M.1

1Children’s Hospital of Philadelphia and University of Pennsylvania School of Medicine, Philadelphia, PA, United States; 2Children’s Hospital Los Angeles and Keck School of Medicine/USC, Pediatrics, Los Angeles, CA, United States; 3Children’s Hospital Boston and Dana-Farber Cancer Institute, Pediatrics, Boston, MA, United States; 4Nationwide Children’s Hospital and The Ohio State University, Pediatrics, Columbus, OH, United States; 5National Cancer Institute, Pediatrics, Bethesda, MD, United States.

**Background:** Although genomic changes are associated with clinical outcome and help define a high-risk (HR) group in neuroblastoma (NB), there are few genes known to be mutated and no genomic aberrations that can be targeted. HR cases had WC losses of 3, 4, 9, 11, 14, 16, 19, and 21 while HR cases had segmental losses of 1p, 3p, 4p, 9p, 11q, 14q, 19p, and 21q. Within the HR group, cases showing only WC changes had better outcomes (p<0.01). Candidate genomic regions were large, but 117 genes and microRNAs were prioritized for resequencing in 188 HR NB samples based on CN data. Analysis to date of over 890,000 tracts revealed 841 sequence variants that passed strict filtering criteria. Non-silent sequence variants were found in 79/117 genes (range of cases with variants per gene: 1-19).

**Conclusion:** Although the same chromosomes have aberrant CN in both the lower-risk and HR groups, the aberration type (WC vs. segmental) clearly differed, with the HR group showing segmental aberrations and the lower-risk group showing WC aberrations for all overlapping regions. This suggests differing mechanisms for genomic rearrangement and reveals potential loci of interest. The low frequency of somatic mutation found by targeted resequencing underlines the need for unbiased whole genome sequencing approaches to discover mutated genes in NB (Morozova, ANR 2010).

**Email:** attiyeh@email.chop.edu

**PO3**

**Identification of miRNAs contributing to neuroblastoma chemoresistance**

Ayers, Duncan1; Mestdagh, Pieter2; Rihani, Ali1; Schramm, Alexander2; Michaelis, Martin3; Cinaf Jr., Jindrich3; Eggert, Angelika3; Day, Philip J1; Speelman, Frank1; Van Maerken, Tom1; Vandesompele, Jo2; Manchester Interdisciplinary Biocentre, University of Manchester, Manchester, United Kingdom; 2Center for Medical Genetics Ghent, Ghent University Hospital, Ghent, Belgium; 3Division of Hematology/Oncology, University Children’s Hospital, Essen, Germany; 4Institut für Medizinische Virologie, Klinikum der J.W. Goethe Universität, Frankfurt am Main, Germany.

**Background:** The emergence of the role of microRNAs (miRNAs) in exacerbating drug resistance of tumours is currently being highlighted as a crucial research field for future clinical management of drug resistant tumours (1). The purpose of this study was to identify dysregulations in expression of individual and / or networks of miRNAs which may have impact on neuroblastoma (NB) drug resistance.

**Method/approach:** Individual subcultures of chemosensitive SH-SY5Y and UKF-NB-3 cells were rendered chemoresistant to doxorubicin (SH-SY5Y, UKF-NB-3) or etoposide (SH-SY5Y). In each validated chemoresistance model, the parental and subcultured cell lines were analysed for miRNA expression profiling, using a high-throughput quantitative polymerase chain reaction (RT-qPCR) miRNA profiling platform (2,3) for a total of 668 miRNAs.

**Results:** A total of seven miRNAs were found to be differentially expressed (higher than 2-fold change) within all three NB chemoresistance models. Four miRNAs were upregulated in the subcultured chemoresistant cell lines. Three miRNAs were found to be downregulated in the chemoresistant cell lines for all models.

**Conclusion:** Based on the initial miRNA findings, this study elucidates the dysregulation of seven miRNAs in three separate NB chemoresistant cell line models, spanning two cell lines (SH-SY5Y & UFK-NB-3) and two chemotherapeutic agents (doxorubicin & etoposide). These seven miRNAs may thus be possibly linked to chemoresistance induction in NB. Such miRNAs are good candidates to be novel drug targets for future miRNA based therapies against aggressive tumours that are not responding to conventional chemotherapy.


**Email:** duncan.ayers-2@postgrad.manchester.ac.uk
**POST4**
Gene expression signatures of mutant ALK in neuroblastoma

Azarova, Anna1; Kimmeth, Kathrin1; Christensen, James1; Stieguer, Kimberly1; George, Rani E.1
1Dana-Farber Cancer Institute, Harvard Medical School, Department of Pediatric Oncology, Boston, MA, United States; 2Broad Institute of MIT and Harvard, Computational Biology Program, Cambridge, MA, United States; 3Pfizer Global Research and Development, Department of Cancer Biology, San Diego, CA, United States

**Background:** Activating mutations in the ALK tyrosine kinase receptor have been identified in both familial and sporadic neuroblastomas (NB), the most common being the ALK F1174L and R1275Q variants. Preclinical studies have identified differential regulation of NB cell lines harboring these mutations to PF2341066 (Pfizer, Inc), an ATP-competitive c-MET/ALK inhibitor which is currently in clinical trials in NB patients. The goal of this study was to identify the signaling pathways that are critical for ALK-mediated NB cell survival and whose inhibition may underlie the efficacy of PF2341066.

**Method:** We analyzed the transcriptomes of NB cell lines harboring ALK mutations; Kelly and SH-SYSY (F1174L) and SMS-KCNR (R1275Q), in which ALK signaling was abolished by treatment with PF2341066. PF2341066 doses were titrated to achieve inhibition of ALK phosphorylation without apoptosis induction. Cell lines were treated with either compound or DMSO in triplicate for 6 hours, total RNA isolated and submitted for microarray analysis. Gene expression profiles were generated using Affymetrix Human U133A HT array and Gene Pattern® software.

**Results:** Inhibition of ALK phosphorylation after treatment with PF2341066 was confirmed by western blot analysis. Gene expression profiling revealed potentially expressed genes in the treated vs. untreated cell lines (161 downregulated and 306 upregulated; p<0.05; minimum fold change of 2 and a minimum absolute difference of 50 across all the samples). The signature included known downstream effectors of ALK such as AKT and ERK and also genes involved in regulation of apoptosis, cell cycle and signal transduction. The two cell lines with the ALK F1174L mutation, Kelly and SH-SYSY, shared the highest fractional of significantly regulated genes that changed in the same direction compared to the SMS-KCNR line (R1275Q).

**Conclusion:** We have generated gene expression signatures of activated ALK in NB cell lines harboring mutant ALK. This represents a versatile tool to identify novel signaling networks regulated by ALK and to functionally validate suitable genes that may represent new therapeutic targets.

Email: rani_george@dfci.harvard.edu

**POST5**
Cytomegalovirus infection in neuroblastoma, high prevalence in tumors and reduced growth and survival in vitro and in vivo using HCVM targeted therapies

Barbey, Laura1; Wolmer-Solberg, Nina1; Fuchs, Dieter1; Verboom, Lonneke1; Rahbar, Alfsa1; Segerstrom, Lov1; Svinjbnjomard, Baida1; Johnsen, John-Inge1; Kogner, Per1; Soderberg-Naucler, Cecilia1; Karolinska Institutet, Childhood Cancer Research Unit, Department of Woman and Child Health, Stockholm, Sweden; 2Karolinska Institutet, Center for Molecular Medicine, Department of Medicine, Stockholm, Sweden

**Background:** The etiology of neuroblastomas is not completely known, but the involvement of viruses as additional trigger factors has been proposed. Neuroblastoma and other neural tumours have been related to active Human Cytomegalovirus (HCVM) infection. Recent evidence demonstrated that infection of HCVM in several cancers including tumors of the brain, colon and prostate. HCVM infects 70-90% of the world’s population and it possesses several oncomodulatory properties. HCVM infection affects cell cycle control and chromosome stability, increases COX-2 expression and influences tumor formation, and angiogenesis. Neuroblastomas express high levels of COX-2 and COX-2 inhibitors may reduce angiogenesis and tumor growth in vivo. Aim: We aimed to determine whether HCVM is present in neuroblastomas and if targeting HCVM and COX-2 reduce tumor growth in vivo and in vitro.

**Methods:** Immunohistochemistry, PCR, western blot, clonogenic assay, mouse SH-SY5Y xenografts. Results: We found HCVM proteins in 97% of neuroblastomas by immunohistochemistry (35/36) and PCR (8/11). Surprisingly, three cell lines SH-SY5Y, SK-N-BE2 and SK-N-AS were HCVM DNA positive. Co-treatment with Ganciclovir (150µM) and Celecoxib (15µM) reduced their clonogenic capacity synergistically by 50-65% (p<0.05) whereas each drug separately resulted in 10-25% reduction (p<0.05). In the mouse xenograft model, Valganciclovir significantly reduced tumor growth in a dose dependent manner. When used in combination with Celecoxib, tumor volume index was significantly reduced from 13.4±4.4 to 6.2±2.4 (p<0.05) in comparison to Valganciclovir used alone.

**Conclusion:** We found a high prevalence of HCVM in neuroblastoma tumors and cell lines supporting an oncomodulatory role of HCVM. Xenografts were positive for both HCVM and COX-2, and combined anti-tumor treatment and COX-2 inhibition resulted in reduced clonogenic ability and inhibited tumor growth in vivo and in vitro. Our data indicate novel treatment options for children with neuroblastoma.

Email: nibin.barbey@ki.se

**POST6**
Inhibition of lipoxigenases promotes retinoic acid induced cell death in neuroblastoma

Bell, Emma1; Ponthan, Frida1; Thomas, Huw1; O’Toole, Kieran1; Lovat, Penny1; Luneck, John1; Tweddle, Deborah1; Redfern, Christopher1; PF, Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, United Kingdom

**Background:** The retinoic acid (RA) derivative 13-cisRA is used in the treatment of neuroblastoma (NB) and causes growth arrest, differentiation and apoptosis. RA has previously been found to promote arachidonic acid (AA) release. We hypothesise that inhibiting release and subsequent metabolism of AA will promote RA induced cell death.

**Aims:** To determine if inhibitors of AA release and its metabolism by cyclooxygenase (COX) and lipoxigenase (LOX) pathways promote cell death after treatment with RA.

**Methods:** Cell survival was assessed in SH-SYSY, NB-69 and NGP cells using XTT assays and apoptosis measured by flow cytometry for the % of cells with sub G1 DNA content. The inhibitors used in combination with all-trans RA (atRA) were: AACC003 (PLA), Celecoxib (COX-2 and LOX-5), MK666 (LOX-5) and PD-147614 (LOX-15). An inducible COX-2 expression system was established to determine the influence of COX-2 on atRA and celecoxib induced cell death. Addition of the lipid messenger PG2 was used to test for rescue of NB cells from atRA and celecoxib induced cell death. For in vivo studies, SH-SYSY xenograft mouse models were established. Mice were treated daily with 10mg/kg atRA, 100 mg/kg celecoxib or a combination of the two drugs.

**Results:** Celecoxib synergistically promoted cell death in vitro and slowed tumour growth in vivo. Inhibiting AA release using AACC003 and inhibition of LOX-5 and to a lesser extent LOX-15 sensitised NB cells to RA induced cell death. Induced expression of COX-2 in the SH-SYSY45-COX-2 cells and addition of PG2 to the SH-SYSY-COX-2 cells had no effect on the sensitivity of NB cells to celecoxib and RA.

**Conclusion:** Celecoxib enhances the efficacy of RA for the treatment of MRD in high risk NB.

Email: e.bell@ncl.ac.uk

**POST7**
Prognosis approach of one segmental chromosome aberration in neuroblastoma

Berbegall, Ana P.1; Villamón, Eva1; Pouceras, Marta1; Tadeo, Irene1; Cañete, Adela1; Navarro, Samuel1; Castel, Victoria1; Noguera, Rosa1; University of Valencia, Medical School, Department of Pathology, Valencia, Spain; 2Hospital Universitari La Fe, Pediatric Oncology Unit, Valencia, Spain

**Background:** Neuroblastoma (NB) presenting segmental chromosome aberrations (SCA), with or without numerical chromosome aberrations (NCA), are correlated with worse outcome. However the prognostic impact of the minimal number of SCA in NB without MYCN gene amplification remains elusive. We aimed to find out any differential feature in a set of NB carrying one single SCA.

**Methods:** To detect chromosomal copy number changes, Multiplex Ligation-dependent Probe Amplification (MLPA) was carried out in 309 tumors, covering the relevant aberrant regions found in NB. In 135 cases (44%), Fluorescence In Situ Hybridization (FISH) was performed to evaluate the status and integrity of at least three chromosome regions (1p36 and/or 11q and/or 17q) and MYCN. All cases with one SCA detected by MLPA were further validated using FISH. Frozen and paraffin-embedded tissue was used.

**Results:** Informative multiplex microarray data were obtained in 285/309 (92%) cases by MLPA, of which 154/285 (54%) showed SCA while 131/285 (46%) had SCA. Out of 154 cases with SCA, 13 cases (8%) presented one SCA by MLPA. From the 131 cases with SCA, 14 (11%) had one SCA by FISH (heterogeneous cases). A total of 27/285 samples (10%) presented one SCA, the most frequent SCA detected was the gain of chromosome 17q (16/27), followed by different alterations in both arms of the chromosome 7 (5/27). Detection of 1p was found in only one sample and MYCN gain in two samples (7%) (only one patient was younger than 18 months at diagnosis). Percent frequencies of the stages diseases were as follows: stages 1, 2a and 4S, 59% (16/27); stage 3, 30% (8/27); stage 4, 7% (2/27) and stage 4S, 4% (1/27) respectively. None of the patients suffered relapse but one carrying the 1p deletion. The OS and EFS median follow-up was 48 months both, with an OS and EFS rate of 96%.

**Conclusion:** Although an extended tumor cohort study will be necessary to have more conclusive results, our data point out that one SCA in neuroblastoma is associated with young patients harbouring localised tumours with good prognosis and excellent survival.

Grants: ISSIII (RD06/2006/00102) and FAECIII (396/2009)

Email: rosa.noguera@uv.es
POST8

Relationship between ALK expression and genetic predictive factors in neuroblastoma

Berthier, Arnaud1; Piqueras, Marta2; Villamón, Eva1; Berbegall, Ana P3; Tadeo, Irene4; Castel, Victoria5; Navarro, Samuel6; Noguera, Rosa7; Cilli, Michele2; Raffaghello, Lizzia8; Pistoia, Vito9

1Centro de Investigacion Principe Felipe, Laboratory of molecular biology of cancer, Valencia, Spain; 2University School, Department of Pathology, Valencia, Spain; 3Hospital Universitario La Fe, Pediatric Oncology Unit, Valencia, Spain

Background: Neuroblastoma, the most solid extra-cranial common tumour in childhood, retains a clinical enigma. The large heterogeneity of cases translates the biological complexity and the medical diagnostic and prognostic challenge of the pathology. Recently, Anaplastic Lymphoma Kinase (ALK) has been identified as a major predisposition gene as well as a potential therapeutic target for neuroblastoma. In this way, aberrant copy number or mutations in ALK gene and overexpression of this tyrosine-kinase receptor have been related to poor prognosis indicators of the disease. However, clinical difference appears among studies and to go more ahead of these observations, we try to define and reinforce new and existing correlations between ALK expression, ALK expression, canonical genetic predictive factors and clinical outcome.

Method/approach: A total of 92 neuroblastomas was assessed for frequency of ALK status by FISH (ALK kipl probe, Dako) and expression by immunohistochemistry (anti-ALK C2667 mAb, Cell signaling technology). Data were compared with clinical criteria (age, neuroblastoma stage), MYCN gene status, mycN protein expression, 1p36 and 11q deletions or 17q gain.

Results: We observed a strong correlation between ALK protein expression and gene status or 1p36/11q chromosome alterations, but no link between ALK protein status and 17q chromosome alteration. However, contrary to previous reports, we found that ALK was equally expressed in all neuroblastoma stage excepting stage 2, and was significantly overexpressed in cases with MYCN amplification as well as in cases with mycN overexpression.

Conclusion: Our data confirm that ALK is one of the major predispositional marker for neuroblastoma but also suggest that more molecular studies are needed to really understand the biological function of ALK in neuroblastoma. This work was supported by grants from FAEC (396/2009) and ISCIII (RD06/0020/1012).

Email: rosa.noguera@ux.es

POST9

Potential role of mesenchymal stromal cells in experimental neuroblastoma cell lines

Bianchini, Simone1; Bonhomme, Laura2; Morandi, Fabio3; Antonio, Daga4; Chilli, Michele5; Raffaghello, Lizzia6; Pistola, Vita7

1G. Gaslini Institute, Laboratory of Oncology, Genoa, Italy; 2National Cancer Institute, Animal Research Facility, Genoa, Italy; 3National Cancer Institute, Laboratory of Gene Transfer, Genoa, Italy

Background/Aims: Mesenchymal stromal cells (MSC) exhibit tropism for sites of tissue damage as well as for the tumor microenvironment, where they integrate into the tumor-associated stroma supporting cancer growth. However, in vivo and in vitro effects mediated by MSC on tumor growth provided conflicting results, depending on the experimental model tested. Aim of this study was to investigate the role of MSC in the neuroblastoma (NB).

Methods: MSC were expanded in vitro from the bone marrow of healthy donors or ferrets of AU mice. In vivo NB cell proliferation was tested by SH-SY5Y transduction after co-implantation of NB cell line SH-SY5Y with or without MSC. In vitro, MSC were investigated using Matrigel invasion chamber plate with a 8-mm pore-size. Murine (m) MSC transfection with pooled mRNA from NB cells was performed using Transmessenger Transfection Reagent. In vivo therapeutic effects by MSC were evaluated in terms of survival, tumor growth, proliferation, apoptosis and angiogenic activity in subcutaneous, pseudomalignant and orthotopic NB animal models. In vivo localization of MSC labeled with the fluorescent dye SP-DiI was evaluated by immunofluorescence.

Results: MSC had heterogeneous and marginal effects on NB cell line proliferation. However, in vitro migration and invasion assays showed that MSC were significantly more invasive NB cell lines than controls. This translated as by soluble factors released by the latter cells. Moreover, after contact with MSC, NB cell lines invaded in vitro a microenvironment that mimics the bone marrow. In vivo, pseudomalignant NB models, MSC localized specifically to NB metastases, but did not affect primary tumor growth. In contrast, in a subcutaneous NB model, MSC injected inside of the tumor mass induced an antineoplastic effect through inhibition of proliferation and induction of apoptosis. Finally, marginal immunomodulating and antimetastatic activities of mMSC transfection with NB mRNA were observed in a pseudomalignant immunocompetent NB model.

Conclusion: We suggest that MSC may control NB growth depending on the experimental model tested. Email: prucci@libero.it

POST10

Combinatory effect of 5-AZA-cyldin and Octreotide on neuroblastoma cell proliferation and apoptosis

Björklund, Peyman1; Oskarsson, Anna2; Helman, Per1

1University Hospital, Department of Surgical Sciences, Uppsala University, Uppsala, Sweden; 2University Hospital, Department of Surgery, Uppsala University

Introduction: Neuroblastomas have several similarities to pheochromocytomas, mostly but not exclusively, developing in adults. Knowledge from treatment of these tumors may be applied to neuroblastomas. Since apoptosis is an essential mechanism for inactivation of neuroblastomas, and activation of these has been demonstrated to induce apoptosis in pheochromocytoma. Methylation of promoter regions in DNA may reduce expression of tumor suppressor genes involved in cell proliferation and apoptosis. We have studied effects of combinations of somatostatin analogs and the demethylating agent 5-AZA-cyldin on activation of apoptosis pathways and cell proliferation in neuroblastoma cell lines.

Materials and methods: Neuroblastoma cell lines SK-N-SH, SH-SY5Y, SK-N-AS, IMR-32, SK-N-DZ and KELLY were grown in normal conditions recommended by ATCC. Expression of SSTR was measured by quantitative PCR (Q-PCR). Methylation status of CpG island in SSTR type 2 (SSTR2) gene promoter was analyzed using methylation specific PCR (MS-PCR). Proliferation and apoptosis in cells treated by either the somatostatin analog Octreotide or 5-AZA-cyldin alone or in combination were analyzed by ELISA based methods.

Results: Octreotide induced apoptosis in all cell lines, (SK-N-SH 63%, SH-SY5Y 16%, SK-N-AS 19%, IMR-32 69%, SK-N-DZ 73% and KELLY 59%). Q-PCR analysis revealed significant lower expression of SSTR2 in low-responsive compared to high-responsive cell lines (0.32 and 0.26 in SH-SY5Y and SK-N-DZ respectively). SK-N-DZ and KELLY PCRs showed high methylation level of CpG islands in the SSTR2 gene promoter, while this disappeared after treatment with 5-AZA-cyldin. Proliferation rate in these cells were reduced by 28% and apoptosis was induced in 11%. Treatment with both 5-AZA-cyldin and Octreotide with minimal concentrations for 12 hours demonstrated increasingly reduced proliferation rate to > 90%. Apoptosis was induced in almost all cells.

Conclusion: Combinatory use of 5-AZA-cyldin and Octreotide at low doses can be an effective and mild choice in treatment of neuroblastoma. Further studies to reveal cytotoxic and systemic effects of this combination are required.

Email: per.helman@surgsci.uu.se

POST11

ALK and PHOX2B mutations in neuroblastic tumours with highly suspected predisposition: Rare events and unexpected clinical features

Bourdeau, Franck1; Ribeiro, Agnès2; Gauthier-Villars, Marion3; Michon, Jean1; Perel, Yves4; Schleiermacher, Gudrun5; Amiel, Jeanne6; Pierron, Gaëlle7; Janoueix-Lerosey, Isabelle8; Delattre, Olivier9

1Institut Curie, INSERM U830, Paris, France; 2Institut Curie, Unité de Génétique Somatique, Paris, France; 3Institut Curie, Oncogenetique, Paris, France; 4Institut Curie, Pediatrics, Paris, France; 5CHU Bordeaux, Onco-hematologie pediatric, Bordeaux, France; 6Institut Curie, Pediatric, INSERM U830, Paris, France; 7Hopital Necker, Genetic, Paris, France; 8Institut Curie, Unite de Genetique Somatique, Paris, France; 9Institut Curie, INSERM U830, Unite de Genetique Somatique, Paris, France

Neuroblastic tumours (NBt) may occur in a predisposition context. Two main genes are involved: PHOX2B and frequently associated with other neurocrystopathies (Onedine's and Hirschsprung's disease), and ALK, mostly in familial tumours. We have assessed the frequency of mutations of these two genes in NBt patients with highly suspected predisposition. The whole coding sequences of the 2 genes were analysed in tumour and/or constitutional DNAs. Methods: We sequenced both genes in 5 multilocal neonatal (<1 month) (MNN), 25 unifocal neonatal (NN), 10 multifocal (MF) and 12 syndromic NBt. Syndromes included 1 familial trunci arteriosus (TA), 1 familial patent ductus arteriosus (PDA), one Tetralogy of Fallot (TOF), one Hirschsprung disease (HSCR), one Ondine's curse (CCS), 2 obesity with late onset hypothymosis and hypothyollasia (SLOS) and 1 (SOSHD), 2 growth defects and 1 mental retardation. Results: The 5 MNN tumours showed no mutation. ALK variants were found in 2/25 NN (R1275Q and P228del3bp), 3/10 MF (R1275Q, NbT at 3 months, R1192G at 5 months and R1192G at 2years) and 1/11 syndromic NBt (M596T in AT). PHOX2B variants were found in 1/10 MF (766insC), 1 CCS (618insC) and 1 LO/CHS/ HD (C17Y); the patient with HSCR had no mutations. Patients with the ALKR1192G and the PHOX2B676insG variants showed neuroblastoma and multiple sub-cutanueous ganglioneuromas. The PHOX2B676insG patient also showed an intestinal ganglioneuromatosis, whereas hyperplasia of myenteric plexus was observed in the case with the ALKT1151R mutation. Conclusion: ALK and PHOX2B deleterious mutations are rare events (6/52) in patients with a high probability of predisposition. Sub-cutanueous ganglioneuromatosis is linked to both ALK and PHOX2B alterations. The intestinal phenotype associated with such mutations deserves closer attention. The search for other predisposing genes is warranted.

Email: franck.bourdeau@curie.fr

ANR 2010, June 21–24 2010
POT12
TLR9 expression and functionality in neuroblastoma delineate a novel prognostic marker and therapeutic target.

Brignole, Chiara1; Marimpietri, Danilo1; Di Paolo, Daniela1; Morandi, Fabio2; Pastorio, Fabio2; Zorzoli, Alessia; Pagnan, Gabriella; Loi, Monica; Caffa, Irene1; Ennio, Giuseppe1; Haupt, Riccardo2; Gambini, Claudio2; Perri, Patrizia1; Pistola, Vito1; Ponzoni, Mirco1

1G. Gaslini Children’s Hospital, Experimental Therapies Unit, Laboratory of Oncology, Genoa, Italy; 2G. Gaslini Children’s Hospital, Laboratory of Oncology, Genoa, Italy; 3G. Gaslini Children’s Hospital, Epidemiology and Biostatistics Section Scientific Directorate, Genoa, Italy; 4G. Gaslini Children’s Hospital, Department of Pathology, Genoa, Italy

Background: The immune system to cope with pathogens. TLR9 expression has been reported in several tumors.

Aims: To evaluate TLR9 expression in Neuroblastoma (NB), both in vitro and in vivo, and to determine its functionality and biological impact.

Methods: TLR9 expression was evaluated by RT-qPCR and flow cytometry. NB cells and peripheral blood mononuclear cells were treated with CpG oligonucleotides, either free (CpG) or Lipofectamine-complexed (L-CpG).

Cell proliferation was assessed by 3H-Thymidine incorporation, apoptosis by phosphatidylinositol exposure, mitochondrial membrane potential depolarization and detection of caspase 3 and 7 activity. Evaluation of TLR9 functionality was assessed using inhibitory oligonucleotides (iODN) and RNA interference. In a pseudometastatic mouse model of human NB, mice received intravenously either CpG-containing NB-lysosomes (TL-CpG) or CpG. Immunohistochemistry was applied to detect TLR9 in NB specimens.

Results: Treatment of TLR9-expressing NB cells with L-CpG inhibited cell proliferation and induced cell death, differently to cells of hematopoietic origin, in which the same treatment triggered cell proliferation. Caspase-dependent apoptotic events were induced in NB cells with L-CpG. iODN abrogated L-CpG-mediated anti-proliferative and pro-apoptotic effects, confirming TLR9 functionality. RNA interference experiments still left sufficient amount of TLR9 expression enabling functional responses to CpG. Compared to CpG, TL-CpG administration prolonged significantly survival of NB-bearing mice (P<0.0154). TLR9 expression in human primary NB specimens was demonstrated, and found to inversely correlate (P<0.0001) with disease stage.

Conclusion: This study demonstrates the functional expression of TLR9 in NB and suggests that it might represent a novel prognostic and/or therapeutic target.

Email: mircoponzoni@ospedale-gaslini.ge.it

POT14
Molecular imaging of MYCN-amplified neuroblastoma tumorigenesis in orthotopic xenograft and transgenic TH-MYCN murine models.

Cantelli, Erika1; Pasolli, Antonio1; Quarta, Carmelo1; Mezzanotte, Laura1; Serravalle, Salvatore1; Di Leo, Korinne1; Nanni, Cristina1; Fanti, Stefano2; Roda, Aldo1; Weiss, William A6; Hrelia, Patrizia1; Pession, Andrea1; Tonelli, Roberto1

1Pediatric Hematology-Oncology, University of Bologna, Pediatric Gynaecological Obstetric Science, Bologna, Italy; 2University of Bologna, Department of Nuclear Medicine, Bologna, Italy; 3University of Bologna, Department of Pharmaceutical Sciences, Bologna, Italy; 4University of Bologna, Pediatric Gynaecological Obstetric Science, Bologna, Italy; 5Pediatric Hematology & Oncology University of Bologna, Pediatric Gynaecological Obstetric Science, Bologna, Italy; 6University of California San Francisco, Department of Helen Diller Family Cancer Research Building, San Francisco, United States; 7University of Bologna, Department of Pharmacology, Bologna, Italy; 8Pedicatrie Hematologie & Oncologie University of Bologna, Department of Pharmacology, Bologna, Italy

Background/aims: MYCN amplification (MA) in Neuroblastoma (NB) is associated with a poor prognosis. MYCN plays an important role in the disregulation of a wide number of genes involved in cancer progression and resistance against classic chemotherapies. Thus, it is important to obtain predictive preclinical models and accurate procedures to follow up the tumor progression and response to new therapies in vivo. In this regard, we report a real-time monitoring of the tumorigenesis by non-invasive molecular imaging in two complementary MA-NB mice models: orthotopic xenograft and homozygous transgenic TH-MYCN models (Weiss WA,’97).

Methods: Orthotopic model was established by injection of 4 Luciferase-positive MA-NB cell lines in NOD/SCID mice. Bioluminescence Imaging (BLI) was used to detect any tumor burden formation starting from the day of injection, once a week. PET was performed on homozygous TH-MYCN mice with 18FDG. We analyzed tracer uptake in the tumors with the Standardized Uptake Value (SUV) from the 4th week of age, once every 4 days. All animals were sacrificed and each sample was used for histology, immunohistochemical and molecular analysis of MYCN and N-Myc levels.

Results: Orthotopic model created from all the 4 cell lines showed a 100% NB incidence. IMR-5 (a IMR-32 clone with higher MYCN copies) and SK-N-Be2(c) (from relapsed patient) cell lines showed the shorter latency (2 weeks) and progression periods (5 weeks). Homozygous TH-MYCN mice showed a 100% incidence, a latency of 28 days and progression period of 5 weeks. Histology confirmed the accordance between imaging results and tumor presence. Tumor samples showed MYCN amplification and overexpression.

Conclusion: The real-time monitoring by non-invasive molecular imaging allowed the early detection and evolution of MA-NB in two complementary mouse models. Moreover, the definition of the tumor signal trend offers the possibility to define optimal ranges in which it will be possible to evaluate the efficacy of new therapies against MYCN-amplified neuroblastoma.

Email: erika.cantelli@gmail.com
**POT15**

Microarray-based pathway analysis leads to the identification of potential cellular mechanisms underlying gamma-secretase inhibitor-induced neuronal differentiation of neuroblastoma cells.

Chang, Hsiu-Hao1; Hsu, Wen-Ming2; Juan, Hsueh-Fen3; Liao, Yung-Feng4; Valentina2; Carlini, Barbara3; Corrias, Maria Valeria4; Ferrini, Silvano2

*1The Institute of Cancer Research, Paediatric Oncology, Sutton, United Kingdom; 2The Institute of Cancer Research, Clinical Magnetic Resonance Research Group, Sutton, United Kingdom; 3The Institute of Cancer Research, Paediatric Oncology, Sutton, United Kingdom; 4The Institute of Cancer Research, Clinical Magnetic Resonance Research Group, Sutton, United Kingdom*

**Background:** Gamma-secretase inhibitor (GSI) in block Notch signaling and thus evaluation in neuroblastoma (NB) cells. However, other membrane proteins, including some receptors tyrosine kinase, could be also the substrates of gamma-secretase and therefore involve in the mechanisms underlying the GSI-induced differentiation of NB cells.

The aims of this study are to investigate the major signaling pathways by which GSI induced NB cells differentiation.

**Methods:** The time-course cDNA microarray experiments (Agilent system) were conducted. Human NB cell line SK-N-SH were treated with 10 μM DAPT, a GSI, dissolved in 0.1% DMSO or vehicle alone (0.1% DMSO) for 12, 24, 48, or 72 hours. The clarified lysates derived from DMSO- or DAPT-treated cells as well as untreated cells were collected, and mRNAs were isolated and reverse-transcribed for the cDNA microarray analysis.

**Results:** NB cells became morphologically differentiated after DAPT treatments. Genes with a 2-fold change in mRNA transcript levels at any time points between control and DAPT- or DMSO-treated cells were selected. These genes exhibited alterations to DMSO treatment, comparing to untreated control, were excluded for subsequent pathway analysis. Through this approach, we found 2767 differentially expressed genes in response to inhibition of gamma-secretase, and 1648 out of those genes were mapped in Ingenuity Pathway Analysis (IPA) tool. Ten canonical signaling pathways were identified to display significant changes (P < 0.05) in expression of their component genes by IPA. The 10 pathways, in order of statistic significance, included Wnt, GΩM-SF, hepatic fibrosis, neuroinfl, IL-2, synapic-long potentiation, circadian rhythm, Notch, insulin-like growth factor-1 (IGF-1), fibroblast growth factor (FGF) signaling pathways.

**Conclusion:** The canonical signaling pathways could play crucial roles in modulating GSI-induced neuronal differentiation of NB cells. The present findings could pave the way for the identification of novel therapeutic targets for anti-NB therapy.

**Email:** changhh2001@ntu.edu.tw

**POT16**

IL-21-based immunotherapy of neuroblastoma in combination with lymphophotodynamic therapy.

Croce, Michele1; Oreno, Anna Maria1; Brizolara, Antonella1; Rigo, Valentina1; Carlini, Barbara1; Cornai, Maria Valeria1; Ferrini, Silvana2

1Italian NB Foundation/Gaslini Institute, Laboratory of Immunological Therapy, Genoa, Italy; 2Italian National Cancer Research Laboratory, Laboratory of Immunological Therapy, Genoa, Italy; 3Gaslini Institute, Laboratory of Oncology, Genoa, Italy

**Background:** Interleukin (IL)-21, the lastly discovered member of the cytokine IL-6 family, is a pleiotropic cytokine produced by CD4+ T cells. IL-21 has shown anti-tumor activity in several pre-clinical tumor models. Clinical phase I-I trials indicated that IL-21 has an acceptable toxicity and induces immune-activation resulting in some objective clinical responses. Thus we evaluated whether IL-21-based immunotherapy (IT) may affect NB growth in a syngeneic metastatic murine model.

**Method/approach:** IL-21-transfected Neuro2a cells (Neuro2a/IL-21) alone or in combination with depleting anti-CD4, anti-CD25 or anti-CD8 mAbs were used as vaccine in a therapeutic setting in order to evaluate the role of different immune populations in eliciting anti-NB effects.

**Results:** Neuro2a/IL-21 IT cured about one third of syngeneic mice bearing disseminated NB, through a CD8+ T cell-dependent response. The co-administration of an anti-CD25 monoclonal antibody (mAb), targeting immune-suppressive CD4+CD25+FoxP3+ regulatory T (Treg) cells, slightly augmented its efficacy. Conversely, the co-administration of an anti-CD4 mAb produced a significant increase in the cure rate (80%). The potent synergetic effect achieved by the anti-CD4 mAb was related to a complete depletion of CD4+CD25+FoxP3+ Treg cells and possibility of other tumour-conditioned suppressive CD4+ T cell subsets. Mice receiving the IL-21-releasing vaccine + anti-CD4 mAb developed an immune-activation resulting in some objective clinical responses. Thus we developed IL-21-based immunotherapy in conjunction with transient CD4+ lymphodepletion in human stage 4 NB.

**Email:** michelacrocce@libero.it

**POT17**

Characterisation of tumour progression, vascularisation and response to chemotherapy in transgenic mouse models of Neuroblastoma (TH-MYCN and TH-MYCN/p53ERTam)° using magnetic resonance imaging.

Cullis, Elizabeth1; Jamin, Yann1; Vaughan, Lynsey2; Popp, Sergey3; Koh, Dow-Mur, Pearson, Andrew1; Chester, Louis1; Frericks, Simon1

1The Institute of Cancer Research, Paediatric Oncology, Sutton, United Kingdom; 2The Institute of Cancer Research, Clinical Magnetic Resonance Research Group, Sutton, United Kingdom

**Background:** The TH-MYCN mouse model of neuroblastoma (NB) faithfully replicates high-risk-human MYCN amplified NB by targeted overexpression of MYCN to the neural crest. Tumours arise most commonly from the adrenal medulla and are highly vascular. As a prelude to development of novel therapeutic strategies for NB, the TH-MYCN and the TH-MYCN/p53ERTam°-haploinsufficient model have been characterised by MRI.

**Method/approach:** Mice were imaged on a 7T Bruker system pre- and post-treatment with cyclophosphamide (CP) (100mg/kg, two doses, 24 hours apart), or methotrexate (MTX) (25mg/kg). MRI relaxation rates, T1, T2 and R1, were measured for each tumour with dynamic contrast-enhancement (DCE) using intravenous Gd-DTPA.

**Results:** Anatomical T1-weighted coronal images revealed that TH-MYCN mice developed tumours originating from the adrenal glands and displacing abdominal organs, with enlargement of the abdominal aorta and vena cava. Histologically, untreated tumours grew as sheets of cells, divided into lobules and surrounded by thin fibrovascular septa, inflicting all surrounding tissues. 48 hours after treatment with CP, tumours were undetectable or difficult to identify, consistent with the clinical sensitivity of NB to CP. Preliminary data suggest that TH-MYCN/p53ERTam° mice show evidence of early tumour relapse. There was no response to MTX, consistent with the clinical sensitivity of NB to MTX. Normalisation of T1 relaxometry rates revealed a heterogeneous spatial distribution of T1 (1748 ± 14ms), T2 (55 ± 1ms) and R1 (115 ± 3s). The relatively fast baseline R1* is indicative of a large tumour blood volume, which was corroborated by DCE (IRA) tool as diffuse parenchymal enhancement across the tumour (IAUC60 = 0.13 ± 0.01ms).

**Conclusion:** This study reinforces the TH-MYCN and TH-MYCN/p53ERTam° models as useful tools that replicate high-risk human NB in its anatomical and radiological appearance, and resistance to chemotherapy. MRI of TH-MYCN models may accelerate development of novel therapeutics by allowing simultaneous evaluation of MRI biomarkers of treatment response and resistance.

**Email:** lizziegullis@doctors.org.uk
**POT19**
The DNA-binding protein, YB-1 is a direct N-Myc target and influences cell death in neuroblastoma via ALK inhibition.

Degen, Stephanie1; Kuhfittig-Kulke, Steffi2; Schulte, Johannes H.1; Westermann, Frank3; Schramm, Alexander2; Eggert, Angelika1; Astrahantseff, Kathy2
1University Children's Hospital Essen, Dept. of Pediatric Oncology, Essen, Germany; 2German Cancer Research Center, Department of Tumor Genetics, Heidelberg, Germany.

**Background:** YB-1 is a member of the shock family of proteins, and regulates gene transcription and translation. It has been shown to bind DNA repair proteins and cisplatin-modified DNA in vitro, and increase chemosensitivity of prostate cancer cells to cisplatin and paclitaxel. Increased expression and nuclear localization of YB-1 has been associated with aggressive phenotypes of several cancers.

**Method/approach:** YB-1 expression was examined by western blotting and immunocytochemistry. Effects of YB-1 on cell viability after cisplatin treatment were assessed in MTT assays with and without siRNA-mediated YB-1 knockdown. Capacity for double-strand break repair was assessed by counting γH2AX-positive foci. The effect of MYCN on YB-1 expression was assessed in 2 regulatable MYCN cell models (4-OHT-inducible SHEP-MYC-ER and SHEP-MYC tet-off), MYCN binding to the YB-1 promoter was analyzed using ChiP-chip and ChiP-seq, including markers for transcriptional activation, repression and elongation.

**Results:** YB-1 was expressed in all 7 NB cell lines examined. Elevated expression in the nuclei and cytoplasm of a cisplatin-resistant cell line was observed in comparison to parental cells. Cisplatin treatment induced YB-1 protein expression and nuclear translocation in both cell lines. YB-1 knockdown increased sensitivity to cisplatin in both cell lines, as well as the number of γH2AX foci, indicating reduced repair capacity for double-strand break repair. Expression increased or decreased with MYCN activation or repression, respectively, in cell culture models. MYCN binding to the YB-1 promoter was observed in all 7 NB cell lines examined, together with epigenetic marks for active transcription and elongation.

**Conclusion:** In NB cells, YB-1 is upregulated and translocated to the nucleus after cisplatin treatment, and enhances capacity for double-strand break repair. We show that MYCN regulates YB-1 expression, implicating it as a possible target for therapeutic intervention. YB-1 may be involved in mechanisms of resistance development in NB, and expression is currently being assessed on an NB tissue microarray to correlate protein expression with tumour phenotype.

Email: kathy.astrahantseff@uni-du.de

**POT21**
ALK and pALK protein levels in NBL cell lines correlate with ALK mutation status and responsiveness to ALK inhibition.

Duijkers, Floor1; Gaal, Joseph1; Adriaal, Pieter1; Pieters, Rob1; Meijerink, Jules1; Krüger, de, Ronald2; Noesel, Max1
1Erasmus MC, Pediatric Oncology-Hematology, Rotterdam, Netherlands; 2Erasmus MC, Pathology, Rotterdam, Netherlands.

**Background:** Responsiveness to Anaplastic Lymphoma Kinase inhibitor (ALK) molecules is excellent in ALK mutated neuroblastoma (NBL) cell lines with high phosphorylated ALK (pALK) protein levels. Recently, it was shown that higher total ALK levels correlates to better prognosis in NBL patients, regardless of mutation status (Passoni et al. 2009). We examined the correlation between ALK and pALK protein levels and response to ALK treatment in ALK mutated (MUT) and wild type (WT) NBL cell lines.

**Method/approach:** ALK (α-ALK, Thermo Fisher Scientific) and pALK (α-pALK Y1604, Epitomics) protein levels were measured in 22 NBL cell lines by western blot. Two pALK products (both around 200 kDa) were identified and quantified together. The mutation status of the cell lines was further characterized by sequencing the ALK gene (exon 20, 22-25) and performing multiplex ligation-dependent probe amplification (MLPA) of the 2p arm. Sensitivity to the ALK inhibitor TAE684 (Axon Medchem) was tested in 6 NBL cell lines (3 WT, 3 MUT) by cell viability measurement with MTS/MTS after 72 hours of incubation.

**Results:** ALK and pALK protein levels were significantly higher in mutant than WT cell lines (p=0.003 and p=0.03, respectively). These mutant cell lines showed a significantly higher sensitivity towards ALK (LC50 values 3.4 fold lower) than WT cell lines. For mutant cell lines, ALK and pALK protein levels strongly correlated with responsiveness to ALK treatment (ALK 200 kDa r=0.74 , ALK 140 kDa r=0.95 and pALK r=0.80), whereas only pALK levels but not ALK levels correlated with response to ALK treatment in WT cell lines (ALK 200 kDa r=0.42 , ALK 140 kDa r=0.35 and pALK 200 kDa r=0.68).

**Conclusion:** In conclusion, ALK gene mutation is strongly correlated with high ALK and pALK protein levels and high ALK responsiveness. For ALK WT cell lines ALK response was best predicted by the pALK protein level.

Email: f.duijkers@erasmusmc.nl

**POT22**
Development of a DNA methylation array normalization method for analyzing demethylating treatment effects in paired neuroblastoma cell lines

Duijkers, Floor1; Iseron, van, Maarten1; Meijerink, Jules1; Admiral, Pieter1; Pieters, Rob1; Boer, de, Judith2; Menezes, de, Renee1; Noesel, van, Max1
1Erasmus MC, Pediatric Oncology-Hematology, Rotterdam, Netherlands; 2LUMC, Genetics, Leiden, Netherlands.

**Background:** DNA methylation is important in normal development and neuroblastomaigenesis. Demethylating agents (Mi) are potential therapeutic drugs. We studied genome-wide demethylating effects of epigenetic drugs in paired neuroblastoma (NB) cell lines, before and after treatment. Conventional normalization methods correct for experimental differences between samples, however large differences can also be erased as ‘noise’. We developed a normalization technique for analyzing mass methylation data after treatment in paired samples.

**Method/approach:** We treated 23 neural crest cell lines (NB and PNET) with 30 nM Decitabine (Mi) for 72 hours, and 25 nM Trichostatin A (HDAC inhibitor) for the last 48 hours. DNA samples were cut using MseI, linker-containing Mi for 72 hours, and 25 nM Trichostatin A (HDAC inhibitor) for the last 48 hours. DNA samples were cut using MseI, linker-containing Mi for 72 hours, and 25 nM Trichostatin A (HDAC inhibitor) for the last 48 hours.

**Results:** Conventional normalization: Loess and VSN two channel normalization showed an increase of methylation in 8% and a decrease in 10% of CpG islands after treatment. Novel normalization: Our new normalization technique is based on Miel fragments without methylation sensitive restriction enzymes according to the Differential Methylation Hybridization technique (Yan et al. 2002). Labeled ampicillins, enriched for methylated regions were hybridized to 244 K CpG island arrays (Agilent).

**Conclusion:** We developed a normalization technique for high resolution methylation array data, based on the equal distribution of uncut DNA fragments after methylation sensitive restriction digestion. We showed that this technique preserves demethylating effects in paired samples and better compares to the proven biological effect.

Email: f.duijkers@erasmusmc.nl
POT23
Expression of chemokine CCL21 and its receptor CCR7 in neuroblastoma
Dunphy, Josiah1; Upkarar, Urmita2; Bates, David1; Ramani, Pramila2
1Bristol Royal Infirmary, Pathology, Bristol, United Kingdom; 2Bristol Royal Infirmary, Paediatric Oncology, Bristol, United Kingdom

Background: Chemokines are small (8–14kDa) soluble proteins that play an important role in lymphatic metastasis by binding to their G-Protein coupled receptors. The aim of this study was to evaluate the expression of the chemokine CCL21 and its receptor CCR7 in neuroblastoma (NB) cell lines and tumours.

Methods: Protein and RNA were assessed using Enzyme-linked immunoadsorbent assay (ELISA) and reverse transcriptase polymerase chain reaction (RT-PCR) in NB cell lines, before and after treatment with 10μM aTRA and from 53 frozen neuroblastic tumours, including ganglioneuroblastoma and ganglionearomas. Immunohistochemical staining for CCL21 and CCR7 was performed in formalin-fixed, paraffin-embedded tissue samples.

Results: RT-PCR showed expression of CCR7 and CCL21 in all the NB cell lines and tumour samples. ELISA assay showed CCL21 protein in all the cell lines, which decreased after treatment with aTRA. CCL21 expression increased with stage, with G1 containing the lowest (0.28pg/μg protein) and Stage 4 the highest (4.09pg/μg protein) protein levels, although there was no significant difference in levels across the stages. There was no statistical difference in CCL21 protein levels in MYCN non-amplified and MYCN-amplified NBs. Weak cytoplasmic staining for CCL21 was identified in ganglion cells and neuroblasts, while undifferentiated neuroblasts were negative. The neuropil showed weak expression. Endothelial staining was identified in <3% lymphatic channels. Strong membrane staining for CCR7 was detected in <10% poorly-differentiated neuroblasts.

Conclusion: Higher levels of CCL21 protein in advanced neuroblastomas indicate that functional studies are essential to investigate the biological role of CCL21/CCR7 axis in lymph node metastases in neuroblastoma.

Email: pramila.ramani@bristol.ac.uk

POT24
Abstract withdrawn

POT25
Hypoxia gene signature as a prognostic factor in neuroblastoma patients
Fardin, Paolo1; Cornejo, Andrea2; Acquaviva, Massimo1; Barla, Annalisa2; Mosci, Sofia1; Versteeg, Rogier4; Molenaar, Jan J.1; Ora, Ingrid3; Caron, Hub N.4; Varesio, Luigi1
1Gianna Gaslini Institute, Laboratory of Molecular Biology, Genoa, Italy; 2University of Genoa, Department of computer and information science, Genoa, Italy; 3University of Amsterdam, Department of Human Genetics, Academic Medical Center, Amsterdam, Netherlands; 4University of Amsterdam, Department of Pediatric Oncology, Academic Medical Center, Amsterdam, Netherlands

Background: Neuroblastoma is the most common extracranial solid tumor in childhood and shows notable heterogeneity with regard to both histology and clinical behavior. Hypoxia, a decrease of oxygen tension, is crucial for tumor progression inducing angiogenesis and inhibiting apoptosis and cell differentiation. Hypoxia is related to poor prognosis in cancer and it has a strong impact on neuroblastoma aggressiveness.

Our aim is to define the hypoxia signature from in vitro controlled system and to test its prognostic value on the gene expression of a cohort of neuroblastoma patients.

Method/approach: H1–H2 regularization framework has been applied on gene expression profiles of 11 neuroblastoma cell lines to define the neuroblastoma hypoxia signature (NBHS). We applied k-means clustering on the expression level of the 62 probesets of NBHS to separate 88 neuroblastoma patients and subgroups obtained by common risk factors stratification. We analyzed the classes by Kaplan-Meier curves and log-rank test for overall survival (OS) and event-free survival (EFS). Multivariate Cox analysis was performed to define the predictive power of the signature.

Results: The NBHS distinguished two groups of neuroblastoma patients classifying them as poor prognosis, those having OS rate of 25.5% and EFS rate of 27.7%, and as good prognosis, those having OS rate of 73.2% and EFS rate of 67.7%. The poor prognosis patients show an over-expression of the hypoxia probesets. Multivariate Cox analysis revealed that the NBHS is a significant independent predictor after controlling for commonly used risk factors. When applied to MYCN not amplified patients, the NBHS stratifies patients with OS rate of 24.2% and EFS rate of 27.3% for patients with poor prognosis, compared with OS rate of 81.4% and EFS rate of 74.8% for patients with good prognosis.

Conclusion: We demonstrate that the NBHS is a significant prognostic factor capable of stratifying neuroblastoma patients. Furthermore, we obtained the proof of principle that the approach of hypoxia genes selection from in vitro controlled tumor cell lines is applicable to identify specific contribution of microenvironment to the tumors biology.

Email: paolofardin@ospedale-gaslini.ge.it

POT26
Analysis of cytotoxic drugs that selectively target cells with MYC overexpression
Frenzel, Ann1; Albohm, Ami1; Zirath, Hanna1; Vita, Marina1; Arsenian Henriksson, Marie1
Karolinska Institutet, Department of Microbiology Tumor and Cell Biology, Stockholm, Sweden

Background: Expression of MYC is deregulated in a wide range of human neoplasias, and is often associated with aggressive, and poorly differentiated tumors in children such as Burkitt’s lymphoma (100% c-MYC translocation) and neuroblastoma (~40% MYCN amplification). MYCN-amplification is strongly correlated with poor clinical outcome of neuroblastoma, with low survival rates despite advances in treatment strategies. Therefore, novel treatments are urgently needed and one approach is to identify compounds with selectivity for cells over-expressing MYCN.

Method: Neuroblastoma and Burkitt lymphoma like cells with conditional over-expression of MYCN and c-MYC, respectively, were used to screen a library of 80 conventional cytotoxic drugs and small compounds for their ability to reduce tumor cell viability in a MYC dependent manner.

Results: We found that 21% of the analyzed compounds induced apoptosis and/or inhibited proliferation in a MYC-specific manner, with a large overlap between the MYC and MYCN over-expressing cells. The majority of the positive hits were compounds belonging to two classes: tubulin targeting agents and topoisomerase inhibitors. This indicates that MYC over-expression potentiates tumor cell killing by cytotoxic drugs in a mechanism-specific manner. Treatment of the cells with topoisomerase inhibitors led to down-regulation of MYC protein levels whereas no effect was observed using the tubulin stability effectors. One of the drugs was also found to be able to disrupt MYC-Max DNA binding in an EMSA assay.

Conclusion: The MYC pathway is only targeted by a subset of conventional cytotoxic drugs currently used in the clinic. Elucidating the mechanisms underlying their specificity towards MYC may be of importance for optimizing treatment for tumors with MYC deregulation. Our data also underscores that MYC is an attractive target for novel therapies using cellular library screenings.

Email: anna.frenzel@ki.se
**POST72**

Expression of the neuron-specific protein CHD5 is an independent marker of outcome in neuroblastoma

Garcia, Idilia1; Mayol, Gemma1; Rodriguez, Eva1; Suñol, Maria1; Gershon, Timothy1; Plos, José1; Cheung, Nai-Kong1; de Torres, Carmen1; Kienast, Mark1; Mora, Jaume1; Lavernia, Cristina1; del Bosch, Jaume1; Rebollo, Carles1; de Viola, Francesc1; de Duè1, Oncologia, Barcelona, Spain; 1Hospital Sant Joan de Déu, Pathology, Barcelona, Spain; 2University of North Carolina, Neurology, Barcelona, Spain; 3Hospital Clinic, Biostatistics & Epidemiology, Barcelona, Spain; 4Memorial Sloan Kettering Cancer Centre, Pediatrics, New York, United States; 5Pediatric Oncology, Dana-Farber Cancer Institute, Boston, United States

Background: The chromodomain, helicase DNA-binding protein 5 (CHD5) is a neuron-specific protein that is absent in gliomas, with a region recently deleted in high risk neuroblastoma (NB). CHD5 mRNA expression has been reported in normal neural tissues and in low risk NB, nevertheless, the distribution of CHD5 protein has not been explored. The aim of this study was to investigate CHD5 protein expression as an immunohistochemical marker of outcome in NB. With this propose, CHD5 protein expression was analyzed in normal neural tissues and neuroblastic tumors (NTs). Reactivation of CHD5 expression in response to induced differentiation processes was investigated in high risk tumors and NB cell lines.

Results: We report that CHD5 is a neuron-specific protein, absent in glial cells, with diverse expression amongst neuron types. Within NTs, CHD5 immunoreactivity was found restricted to differentiating neuroblasts and ganglion-like cells, and absent in undifferentiated neuroblasts, and stromal Schwann cells. An immunohistochemical analysis of 90 primary NTs highlighted a strong association of CHD5 expression with favorable prognostic variables (age, tumor stage, histology and ploidy; P<0.001 for all), with overall survival (P<0.001) and event-free survival (P<0.001). Multivariate analysis and Predictive Value analysis showed that CHD5 prognostic value is independent of other clinically relevant parameters and represents an independent marker of outcome in NB. The prognostic value of CHD5 was confirmed in an independent validation cohort of 25 NB tumors. Reactivation of CHD5 expression after induction chemotherapy was observed only in high risk tumors with evident neuroblastation maturation features. These NB tumors showed a clinical response and prolonged patient survival. None of these tumors harbored 1p deletion or MYCN amplification. In vitro, retinoic acid (RA) induced neuronal differentiation demonstrated that CHD5 expression is sensitive to RA treatment in NB cell lines lacking 1p deletion and MYCN amplification.

Conclusion: The neuron-specific protein CHD5 may represent a marker of outcome in NB that can be tested by conventional immunohistochemistry.

Email: clavarino@fsjd.org

**POST79**

Omega-3 fatty acid supplementation delays the progression of neuroblastoma in vivo

Gleissman, Helena1; Segenströrm, Lov1; Hamberg, Mats2; Ponthan, Frida2; Lindskog, Magnus2; Johnsen, John Inge1; Kogner, Per1; Karolinska Institutet, Women’s and Children’s Health, Stockholm, Sweden; 2Karolinska Institutet, Medical Biochemistry and Biophysics, Stockholm, Sweden; 3Newcastle University, Northern Institute for Cancer Research, Newcastle upon Tyne, United Kingdom; 4Karolinska Institutet, Oncology and Pathology, Stockholm, Sweden

Background: Epidemiological and preclinical studies have revealed that omega-3 fatty acids have anti-cancer properties. We have previously shown that the omega-3 fatty acid docosahexaenoic acid (DHA) induces apoptosis of neuroblastoma cells in vitro by mechanisms involving intracellular peroxidation of DHA by means of 15-lipoxygenase or autodioxidation. In the present study, the effects of DHA supplementation on neuroblastoma tumor growth in vivo were investigated using two syngeneic models.

Method/approach: For the purpose of prevention, DHA as a dietary supplement was fed to athymic rats before the rats were xenografted with human neuroblastoma cell lines. For therapeutic purposes, athymic rats with established neuroblastoma xenografts were given DHA daily by gavage and tumor growth was monitored. DHA levels in plasma and tumor tissue were analyzed by gas liquid chromatography.

Results: DHA delayed neuroblastoma xenograft development and inhibited the growth of established neuroblastoma xenografts in athymic rats. A revised version of the Pediatric Preclinical Testing Program (PPTP) evaluation scheme used as a measurement of treatment response showed that untreated control animals developed progressive disease, whereas treatment with DHA resulted in stable disease or partial response, depending on the DHA concentration.

Conclusion: In conclusion, prophylactic treatment with DHA delayed development of neuroblastoma in rats, suggesting that DHA could be a potential therapeutic agent in the treatment of minimal residual disease and should be considered for prevention in selected cases. Treatment results on established aggressive neuroblastoma tumors suggest further studies aiming at a clinical application in children with high-risk neuroblastoma.

Email: helena.gleissman@ki.se

**POST82**

Sphingosine-1-phosphate signaling is a mechanism of fenretinide resistance and provides a novel therapeutic target

Ghent, Matthew V.; Chen, Taylor; Kim, Youngleum; Jakimeno, Ana; Kang, Min; Reynolds, C. Patrick

Texas Tech University/ Aflac Children’s Cancer Center, Pediatric Oncology, Lubbock, United States

Background: Fenretinide (4-HPR), is a retinoid cytotoxic to cancer cells via increased dihydroceramide levels that has clinical activity against recurrent neuroblastoma (NB). Sphingosine kinase 1 (SPHK1) generates via increased dihydroceramide levels that has clinical activity against recurrent neuroblastoma (NB). Sphingosine kinase 1 (SPHK1) generates sphingosine-1-phosphate (S1P) that acts via S1PR signaling which is a mechanism of chemoresistance in NB. We asked whether SPHK1 is over-expressed in NB cell lines and if targeting SPHK1 can sensitize NZB/F1 (4-HPR resistant) NB cells to 4-HPR.

Methods: We first confirmed that NZB/F1 cells had increased SPHK1 expression compared to low risk NB cell lines. We then determined if SPHK1 over-expression was due to increased SPHK1 mRNA translation or protein translation. We then determined if SPHK1 over-expression is due to increased S1PR expression in the cell membrane.

Results: NZB/F1 cells had increased SPHK1 expression compared to NB106 (low risk NB cell line). The translation of SPHK1 was not increased in NZB/F1 cells. However, the translation of SPHK1 to the membrane was increased. The S1PR1 and S1PR2 expression were increased in NZB/F1 cells compared to NB106 cells.

Conclusion: These data suggest that increased SPHK1 expression is through increased S1PR expression which may lead to increased S1P signaling. This increased S1P signaling may be a mechanism of chemoresistance in NZB/F1 NB cells.

Email: matt.ghent@ttuhsc.edu

**POST80**

"BH3 profiles" identify neuroblastomas with exquisite ABT-737/chemotherapy sensitivity in vivo and Bim signaling is a critical determinant

Goldsmith, Kelly C.; Gross, Michelle1; Lu, Xueyuan1; Peirce, Susan K.; Prince, Chengyu1; Vu, Annette2; Chen, Niel2; Reynolds, C. Patrick3; Hogarty, Michael D.

1Emory University/ Aflac Children’s Cancer Center, Pediatric Oncology, Atlanta, GA, United States; 2Children’s Hospital of Philadelphia, Pediatric Oncology, Philadelphia, PA, United States; 3Texas Tech University Health Sciences Center, Pediatric Oncology, Lubbock, TX, United States

Background: Apoptosis evasion contributes to therapy resistance in neuroblastoma (NB). NB mitochondria release cytochrome c selectively to pro-death BH3 peptides. Such "BH3 profiles" identify Bcl2 family addiction patterns and predict sensitivity to Bcl2 antagonists (Cell Death Diff, 2010). We now show BH3 profiles are preserved in xenografts (XG) and predict XG therapy response.

Method/approach: Mitochondrial responses to diverse BH3 peptides were obtained from >15 NBs and XGs and clustered (unsupervised). Cell lines were assessed for in vitro/in vivo response to small molecule Bcl2 antagonists and predictions were validated by siRNA and colo.

Results: Clustering identified three groups: Mcl1-dependent (cyto c release to Noxa), Bcl2 dependent (cyto c release to Bik), and BH3 resistant (minimal cyto c release). XGs clustered with their cell lines suggesting cellular signaling. Notably, BH3 resistant NBs appeared to be insensitive to BH3 peptides. XGs were derived at relapse. Most NBs had activated Bim bound to pro-survival proteins, yet relapsed NBs show little activated Bim on Mcl1, Bcl2 or BclxL by colo. This "Loss of Bim priming"may explain therapy resistance seen clinically. Accordingly, siRNA knockdown of Bim in "primed" NB cells led to chemoresistance. XGs from each BH3 profile group were treated with cyclophosphamide (CPM), + ABT737 (targets Bcl2/xl/w), or +/-AT101 (targets Mcl1). ABT737 alone regressed BclxL dependent SMS-SAN XGs (9/10), while ABT737/CPM regressed all tumors and cured >50% after a single treatment (p<0.001 v. CPM). CPM cured no mice. Profiles from the most resistant (BH3 profiles) showed that untreated control animals developed progressive disease, whereas treatment with DHA resulted in stable disease or partial response, depending on the DHA concentration.

Conclusion: In conclusion, prophylactic treatment with DHA delayed development of neuroblastoma in rats, suggesting that DHA could be a potential therapeutic agent in the treatment of minimal residual disease and should be considered for prevention in selected cases. Treatment results on established aggressive neuroblastoma tumors suggest further studies aiming at a clinical application in children with high-risk neuroblastoma.

Email: kgoldsm@emory.edu
POT31
Cepharanthine reverses multidrug resistance and sensitizes neuroblastoma cells to vincristine-induced cell death

Graham, Regina1; Thompson, John1; Guest, James1; Webster, Keith1; Vanni, Steven1
1University of Miami, Neurological Surgery, Miami, United States; 2University of Miami, Neurology, Miami, United States; 3University of Miami, Molecular and Cellular Pharmacology, Miami, United States

Background: Acquired multidrug resistance (MDR) is a contributing factor to the poor prognosis faced by neuroblastoma (NB) patients. Cepharanthine (CEP), an alkaloid extracted from Stephania Cepharantha Hayata, has been used in Japan to treat both acute and chronic diseases without serious side effects. We sought to determine the efficacy of CEP on NB and MDR.

Methods: The effect of CEP alone or in combination with vincristine (Vin) was evaluated in NB cell lines: SMSKCNR (SMSR), SK-N-BE2c (BE2c), and SH-SY5Y (SY5Y). Viability was determined by MTS assay after 72 hours of treatment. The effect of CEP on MDR was evaluated using fluorescent microscopy. Cells were pretreated with 10μM CEP for 1hr followed by 1hr DOX (3μM). DOX levels were determined 18 hours after drug exposure.

Results: CEP (10μM) significantly reduced viability to 11±2.2% (SMSR); 8.2±1.1% (BE2c); 0.4±1.4% (SY5Y) compared to vehicle-treated controls. To determine if CEP could potentiate Vin-induced cytotoxicity, NB cells were treated with 3μM or 300μM CEP, 50μM Vin, or a combination of CEP and Vin. At 72 hours, 3μM CEP alone reduced viability to 72±3.4% (SMSR), 83±3.7% (BE2c), and 62±7.5% (SY5Y) of vehicle-treated controls. Vin had no effect on viability in 92±4.3% (SMSR), 104±4.7% (BE2c), and 94±7.5% (SY5Y). Addition of 3μM CEP to Vin reduced viability to less than 10% of vehicle-treated controls (SMSR 7.2±3.8%, BE2c 8.9±2.7%, SY5Y 3.8±9.7%). Furthermore, 300μM CEP, a concentration that had no effect on viability, plus reduced viability to approximately 50% of controls (SMSR 45±2.1%, BE2c 54±2.7% and SY5Y 58±5.4%). To determine if CEP modulated Vin-induced cell death by reversing MDR, we examined the effect of CEP on DOX cellular accumulation. DOX signal was approximately 3-fold higher in BE2c cells treated with CEP (3µM DOX): 59±19.2% vs. 20±19.1 arbitrary units (AU); p<0.001 suggesting an inhibition of MDR by CEP.

Conclusion: CEP can directly induce NB cell death as well as sensitize cells to Vin at concentrations reported to be clinically achievable. CEP represents a novel addition to NB treatment regimens and may potentially improve clinical outcome.

Email: rgraham@med.miami.edu

POT33
Computer vision in neuroblastoma: computer-aided prognosis

Gurcan, Metin1; Bertel, Olcay2; Shimada, Hironuki2
1The Ohio State University, Department of Biomedical Informatics, Columbus, OH, United States; 2Children’s Hospital Los Angeles, Department of Pathology, Los Angeles, CA, United States

Background: The diagnosis, prognosis, and treatment planning of neuroblastoma depend on classification of the tumor, which in turn depends on morphological feature differences. A key component of the Neuroblastoma prognostic classification process involves a morphology-based scheme: International Neuroblastoma Pathology Classification System (INPC). Currently the Children’s Oncology Group Neuroblastoma Biology Study utilizes the INPC system for patient stratification and protocol assignment. In a recent study, it has been shown that there is 20% disagreement between central and institutional reviewers using the INPC system.

Method/approach: In this study, we built upon our preliminary work in the area of image analysis for cancer to further develop computerized techniques to analyze the morphology of neuroblastoma histopathology slides. Specifically, we analyzed the the degree of Schwannian stromal development and the grade of neuroblastic differentiation to categorize the tissue sample as either favorable or unfavorable histology. We developed multi-resolution image analysis, feature selection and novel classification techniques. In addition to conventional texture features, we introduced a novel way of constructing structural features that captures the high-level perceptual patterns.

Results: The developed system was tested with an independent set of 34 whole-slide images and achieved a classification accuracy of 94.1% (32/34).

Conclusion: A combined computer-assisted neuroblastoma prognosis system can be developed. The latest cooperative effort of INFRG (International Neuroblastoma Risk Group) in developing a consensus approach to risk stratification has determined to incorporate a part of the INPC criteria; such as “Schwannian stromal development” and “Grade of neuroblastic differentiation”, in the factors significantly predicting clinical outcome of neuroblastoma patients. This requires a standardized/ unified histopathology evaluation by participating pathologists in different countries in North America, Europe, and Japan. Computer-assisted tools once established, could play a critical role in supporting this activity.

Email: metin.gurcan@osumc.edu

POT34
Analysis of aggressive human and mouse ALK neuroblastoma mutations.

Halebding, Bengt; Kamaraj, Satu; Schönheerr, Christina; Ruuth, Kristina; Axelsson, Cecilia; Palmer, Ruth
1University of Pennsylvania School of Medicine, Department of Pediatrics, Philadelphia, United States; 2University of Miami, Neurological Surgery, Miami, United States; 3Our Lady’s Children’s Hospital, National Center for Medical Genetics, Dublin, Ireland; 4University of Pennsylvania School of Medicine, Department of Pediatrics, Philadelphia, United States; 5University Hospital of Geneva, Department of Oncology, Geneva, Switzerland

Background: Evidence suggests that chromosome 17q plays a role in neuroblastoma (NB) biology. ATM gene resides at 11q22-23 and ATM protein mediates a kinase cascade linking DNA damage to cell-cycle progression and apoptosis. The aim of this project is to determine the prevalence of ATM alterations in 16 NB cell lines and 50 primary NB samples.

Methods: We determined the relative tumoral DNA copy number for the 64 ATM exons, using the MLA assay (P04/P042) in NBs. ATM mRNA expression was measured by quantitative real-time PCR.

Results: No intragenic deletion/duplication was detected in NB. Six NB cell lines (38%) and 14 NB (28%) had a complete ATM deletion (del), while 1 NBT had a complete ATM duplication. ATM mRNA levels were significantly decreased in ATM del cell lines (p<0.005). ATM del was more prevalent in stage 4 vs. stages 1, 2, 3 NBT (11/24; 95% CI 26-67% vs. 3/26; 95% CI 2-30%); p=0.011). ATM del appears not to be associated with MYCN amplification status, only 1 of the 11 stage 4 NBT samples with ATM del was found with MYCN amplification vs 5 of the 13 stage 4 NB samples without ATM del.

Conclusions: We show here that ATM del is a frequent event in NB, that it is associated with decreased ATM mRNA expression, and that it correlates to advanced clinical stages independently from MYCN amplification status. Further work has documented an indirect link between MYCN overexpression and ATM downregulation in NB cell lines. Our observations provide additional evidence for ATM contribution in NB biology, suggesting that in non-amplified MYCN NB, ATM downregulation could directly result from ATM gene deletion.
**POT35**

**Rapamycin upregulates osteoprotegerin and increases time to pathologic fracture in a mouse neuroblastoma bone metastasis model**

Hartwich, Joseph; Myers, Adriann; Ng, Cathy; Daviddoff, Andrew M

St Jude Children’s Research Hospital, Surgery, Memphis, United States

**Introduction:** Osteoprotegerin (OPG) is a soluble decoy receptor for RANK ligand (RANKL) that can inhibit osteoclastogenesis and slow the progression of osteolytic bone lesions. Rapamycin is an immunosuppressive and anti-neoplastic agent that has been shown to upregulate OPG in human bone marrow stroma. We tested the hypothesis that rapamycin could inhibit osteolytic neuroblastoma bone metastases through its action on OPG.

**Methods:** Mouse bone marrow cells were co-cultured with human neuroblastoma cells (CHLA-255 or NB1691) and treated with rapamycin. Supernatant was collected for OPG ELISA and cells were stained to detect osteoclasts. For in vivo studies, an orthotopic model of bone metastasis was created by injecting neuroblastoma cells intra-femorally in SCID mice. Mice with established disease were treated with one cycle of standard chemotherapy, with or without rapamycin (5mg/kg IP, QD). X-rays were obtained twice a week to detect pathologic (Grade IV) fractures.

**Results:** OPG in co-culture medium was increased when cells were treated with 100nM rapamycin compared to control in CHLA 255 (52.19 pg/mL +/-1.12, vs. 11.42 pg/mL +/-0.50, p = 0.0168) and NB1691 (153.8pg/mL +/-5.05 vs. 50.2pg/mL +/-1.01 p=0.0395). The mean number of osteoclasts was significantly decreased compared to control in wells containing either CHLA255 (14.3+/-.50 vs 30.07+/-.304, p= 0.004) or NB1691 (17.72+/-.6 vs. 38.36+/-.4.9, p=0.001). In vivo, tumor-bearing mice treated with rapamycin had a significantly increased survival level of OPG (82.11pg/mL +/-4.002 vs. 56.63pg/mL +/-3.63 p=0.005) and longer median time to pathologic fracture compared to control with CHLA 255 (103 days +/-12.03 p=0.0139) and NB1691 (93 days +/-11.24 vs 62 days +/-8.39 p=0.0086).

**Conclusion:** Rapamycin treatment increased OPG expression in both bone marrow cells and tumor cells, and delayed the time to pathologic fracture in a mouse neuroblastoma xenograft model, thus demonstrating a novel anti-tumor mechanism of action for rapamycin. These results support the continued exploration of the role of rapamycin in the treatment of children with neuroblastoma, particularly if bone metastases are present.

**Email:** Joseph.Hartwich@stjude.org

---

**POT37**

**Screening at 18 months of age using the new serum marker for reducing the mortality of neuroblastoma: Simulation using Japanese population based cohort study**

Hiyama, Eriko; Kamitsuse, Arata; Kamei, Naomi; Masujima, Tsutomu; Hiyama, Kengo; Ohaki, Megu

1. Hiroshima University Hospital, Pediatric Surgery, Hiroshima, Japan; 2. Hiroshima University, Graduate School of Biomedical Science, Hiroshima, Japan; 3. Hiroshima University, Research Institute for Radiation Biology and Medicine, Hiroshima, Japan

**Background:** Infantile screening for neuroblastoma (NBL) reduced the incidence rate (IR) of advanced disease or mortality rate (MR) due to NBL but the significant increase of IR. Then, we proposed the NBL screening at 18-months of age.

**Methods:** Japanese nationwide mass screening was conducted using a quantitative HPLC in 1990-2003. Cumulative IRs and MRs of NBLs diagnosed before 6 years of age were compared between children born in 1990-58 (n = 10,878,918, participation rate: 85.9%) to historical control born in 1980-1984 (n = 7,620,203) as well as between screened and unscreened children. Then, we simulated the IRs and MRs of NBLs under NBL screening at 18-months of age with the same participation rate. Moreover, the new candidate marker for unfavorable NBLs was surveyed using serum and urine samples of 45 NBL patients by LC-MS-MS using ESI-TOF-MS. The candidate markers for detecting unfavorable NBLs were extracted using Mass Mapping (MM) software.

**Results:** The IRs of the screening cohort became significantly higher than that in the historical control (1.91 vs 30.30, P < .0001), while the IRs in unscreened subgroup of the screening cohort was similar to that in historical control. The MRs of these two cohorts were 2.82 and 5.14 (P<0.001). Simulation analysis under NBL screening at 18-months of age showed the significantly decrease of IR (40%) without increase of MR. After surveyed the urine substances and the serum metabolites in NBL patients using LC-MS, MM revealed the specific candidate serum marker for unfavorable NBLs (peptide, MW 1500). This marker increased only in pretreatment sera of 13 unfavorable NBLs patients. Serum levels of this marker decreased after effective chemotherapy and reseption of tumor but increased at the time of tumor progression or recurrence.

**Conclusion:** There is a possibility that HPLC-quantitative screening for NBL at 18 month of age result in significant decrease in MR by NBL of children without the increase of IR. The new marker for detecting unfavorable NBLs specifically might attribute to more effective NB screening as well as evaluation of therapeutic effects in unfavorable NBL.

**Email:** eiso@hiroshima-u.ac.jp

---

**POT38**

**Effect Phosphoinositide-3-Kinase (PI3K) and mTOR dual inhibitors in Human Neuroblastomas (NBs)**

Hsu, Ruth; Minturn, Jane; Brown, Valerie; Iyer, Madhika; Sheen, Cecilia; Halfin, Jessica; Simpson, Anisha; Varela, Carly; Mangino, Jennifer; Kolla, Venkatadri; Brodeur, Garrett

The Children’s Hospital of Philadelphia, Oncology Department, Philadelphia, United States

**Background:** The PI3K-AKT-mTOR pathway is constitutively activated by different receptor tyrosine kinases (RTKs), such as TrkB, TrkA, IGF-I, EGFR, and ALK in NB cells. It plays an important role in proliferation and tumorigenesis. Blocking RTK signaling by inhibiting the PI3K-AKT-mTOR pathway with specific inhibitors improves the growth of NB cells. We studied the effect of direct targeting key signaling proteins with inhibitors of either PI3K (GDC-0941), mTOR (rapamycin-Rap) or both with dual inhibitors, such as PI-103, NVP-BEZ235 (BEZ235) in NBs. We also investigated the effect of inhibiting both PI3K-AKT and Trk receptors with the Trk inhibitor lestaurtinib.

**Methods:** We used a panel of NB cells, including TrkA and TrkB transfectants of SY5Y and NLF. We assessed changes in cell viability by inhibitors with MITT and RT-CES assays. We determined the expression, activation and inhibition of Trk, AKT and S6 by immunoblotting with specific antibodies.

**Results:** The IC50 of GDC-0941 was 2µM, and the IC50 of Rap was over 10µM. In contrast, the IC50 of PI-103 averaged 0.5µM (range 0.5 to 2µM) and BEZ235 was the most potent at inhibiting growth of wild type NB cells in vitro, compared to other inhibitors tested. The effect of the dual inhibitors did not correlate with expression of Trk receptors. Lestaurtinib and PI-103 showed no additive effect on SKNAS (endogenous Trk) or on SY5Y-TrkB G8 (TrkB low) cells. PI-103 (1µM) abolished AKT but not Trk phosphorylation. Lestaurtinib (200 nM) completely inhibited Trk but only partially inhibited AKT phosphorylation in G8 cells. No additive effect on phosphorylation was observed in combination of lestaurtinib and PI-103.

**Conclusion:** Dual inhibitors of PI3K and mTOR effectively inhibited the growth of NB cells in vitro regarding PI3K-AKT-mTOR pathway antagonism to the most effective among the signaling inhibitors. PI-103 and lestaurtinib showed no additive effect on Trk-expressing NB cells. In summary, targeting PI3K-AKT-mTOR pathway alone is an effective way to potency inhibit NB cell growth.

**Email:** hor@email.chop.edu
**POT39**

Analysis of molecular interactions between the GD2 ganglioside-specific mouse monoclonal antibody 14G2a and GD2-mimicking peptides  

Horiawczyk, Irena1; Kowalczyk, Aleksandra1; Bzowska, Małgorzata2; Czaplicki, Dominik1; Rokita, Hanna1  
1The Jagiellonian University, Laboratory of Molecular Genetics and Virology, Krakow, Poland; 2The Jagiellonian University, Department of Immunology, Krakow, Poland  

**Background:** Children with high risk neuroblastoma (NB) are at the risk for relapse, which addresses the need for new treatment protocols to control minimal residual disease. Over-expression of GD2 ganglioside (GD2) distinguishes NB from benign neural tumors and most normal cells. Anti-GD2 antibodies are used in NB diagnosis and tested in passive immunotherapy approaches. GD2 can also be targeted by active immunization strategies. Our goal is to design GD2-targeting active immunotherapy of NB by replacing the weakly immunogenic GD2 with its peptide mimotopes isolated with application of a phage display technology and the mouse anti-GD2 monoclonal antibody 14G2a (mAb).  

**Method/approach:** We performed in vitro competition tests and vaccination studies on mice to characterize the 14G2a-binding of the peptides, evaluate, and optimize anti-GD2 specific responses induced with our mimotopes.  

**Results:** We showed that despite clear dissimilarities in amino acid (aa) sequences all five isolated peptides have overlapping binding sites on the 14G2a mAb. Moreover, these peptides mimic a unique GD2 epitope, as they do not cross-react with other ganglioside-specific mAbs. We elucidated molecular mechanism of the observed mimicry for one of our best binding peptides (#94 RCNPMPERPRCF). With consecutive aa replacements by A, we identified aa indispensable for the observed GD2 mimicry by the #94 peptide. Additionally, we compared several analogs of the #94 peptide with further replacements, truncations, or elongations, and found new longer peptides, which showed significant improvement of the 14G2a binding in the competition assays. Finally, we showed that vaccines containing our peptides conjugated to KLH induce GD2-targeting antibodies in mice, and we analyzed the level and specificity of the peptide-induced humoral responses.  

**Conclusion:** The accumulated data allowed us to gain insight into the molecular mechanism of the observed GD2 mimicry, and can lead to improvement of anti-tumor activity of our peptides. Acknowledgments: This work was financed in years 2006 - 2008 from the research grant No. N302 034 31/3063 (awarded to Irena Horawczyk).  

Email: irena.horawczyk@uj.edu.pl

**POT40**

NLRR2 is a novel regulator of neuroblastoma cell death via ER stress  

Hossain, Md. Shamim1; Takatori, Atsushi1; Akter, Jesmin1; Hasan, Kamrul1; Nakagawa, Akira1  
1Chiba Cancer Center Research Institute, Division of Biochemistry & Innovative Cancer Therapeutics, Chiba, Japan  

**Background:** To understand the pathogenesis of neuroblastoma (NB), we previously screened for human NB cDNA libraries we generated, and identified NLRR2 (neuronal leucine rich-repeat protein 2) among the NLRR family (neuronal leucine rich-repeat proteins). We have so far found that, among the family members, functions of NLRR1 and NLRR3 are associated with cell proliferation and differentiation, respectively. However, the role of other member, NLRR2, remains elusive.  

**Method/approach:** Immunocytochemistry was performed to check the localization of NLRR2. FACs, Western blot and transcription assays were employed to assess ER mediated effects. RT-PCR and Western blot were performed to check the expressions of mRNA and protein, respectively. NLRR2 promoter activity was measured by dual lucerase assay. Tunicamycin (TM) and Thapsigargin (TG) were used to induce endoplasmic reticulum (ER) stress.  

**Results:** There was no difference in expression levels of NLRR2 mRNA among the subsets of NB (n=32, p=0.05). However, immunohistochemical analysis showed that NLRR2 proteins localize in the cytoplasm of primary NB cells and mainly in the ER of NB as well as other cell lines. To know the functional role of NLRR2 in the neuronal cell fate, we tested several stress responses in cells. Interestingly, NLRR2 overexpression led to cellular apoptosis upon ER stress (TM and TG treatments) as well as oxidative stress (H₂O₂ treatment), whereas siRNA-mediated knockdown of NLRR2 resulted in the resistance to those stresses. Moreover, the expression of endogenous NLRR2 was induced upon ER stress, which was confirmed by the lucerase reporter assay using the core promoter region of NLRR2. A previous study showing that ER stress enhanced transcription of NLRR2. Indeed, the expression of proapoptotic genes (CHOP, BAX and BAK) were induced by NLRR2 overexpression.  

**Conclusion:** Different from the functions of NLRR1 and NLRR3, NLRR2 was found to be a stress-inducible gene to cause apoptosis in cells.  

Email: mshossain2@yahoo.com

**POT41**

Positive feedback loop of Mycn-nlrr1-egf/egfr signals in aggressive neuroblastomas to accelerate cell growth  

Hossain, Md. Shamim2; Takatori, Atsushi1; Akter, Jesmin1; Suena, Yusuke1; Ozaki, Toshinori1; Nakagawa, Akira1  
1Chiba Cancer Center Research Institute, Division of Biochemistry & Innovative Cancer Therapeutics, Chiba, Japan; 2Chiba Cancer Center Research Institute, Laboratory of Anti-tumor Research, Chiba, Japan  

**Background:** Neuroblastoma (NB) is one of the most common solid tumors in children. To understand the pathogenesis of NB, we screened for human NB cDNA libraries we generated, and identified NLRR2 gene for its transcriptional factor may be one of the main regulators for activating MYCN expression in the aggressive NB cells. Since NLRR1 is a downstream target gene of MYCN, NLRR1 may form a positive regulatory loop with MYCN in NBs. These findings might help to develop novel chemotherapeutic tools to cure aggressive NBs.  

Email: mshossain2@yahoo.com
Expression and gene status of HER2 in neuroblastic tumors

Izycka-Swieszewska, Ewa; Wozniak, Agnieszka; Kot, Jacek; Grajewska, Wieslawa; Drozynska, Elzbieta; Balcerska, Anna; Perek, Danuta; Dembowska, Bozena; Limon, Janusz

1 Medical University of Gdansk, Pathomorphology, Gdansk, Poland; 2 University Hospitals Catholic University of Leuven, Laboratory of Experimental Oncology, Department of General Medical Oncology, Leuven, Belgium; 3 Medical University of Gdansk, Department of Hyperbaric Medicine and Sea Rescue, Gdynia, Poland; 4 Children’s Health Memorial Institute, Department of Cyclin, Gdansk, Poland

Background: HER2 is essential for normal embryonic development of autonomic and peripheral nervous system and plays a critical role in oncogenesis and progression of some types of cancer. The biological and prognostic role of HER2 in neuroblastic tumors is not well established.

Method/approach: In the current study we evaluated HER2 expression, its prognostic significance and clinicopathological correlations in series of 79 neuroblastoma. The immunohistochemical assessment of HER2 as well as FISH for HER2 copy number status were performed on tissue microarrays.

Results: In the examined group 20% of patients died of disease from 4 to 107 months (median 18) from the diagnosis and the survivors were followed up for 14 to 149 months (median 59). Sixteen cases were HER2-immunonegative. HER2 expression characterized 63 tumors (34 low and 29 high level) showing either membranous or mixed membranous-cytoplasmic pattern within neuroblastic component. Schwannian stroma disclosed low level HER2 expression. The pattern of immunolabeling depended on the maturity of neuroblastic cells. None of tumors revealed HER2 amplification. Patients’ age, stage of disease, tumor location, MKI and presence of HER2 expression were statistically significantly related to survival probability as detected by the Cox proportional hazard model. In the univariate analysis Kaplan-Meier curves revealed significantly poorer outcome of HER2-negative than HER2-positive tumors (either low or high expression). The immunonegativity was associated with adverse clinicopathological parameters, including poor survival, metastatic stage of disease, un- or poorly differentiated histology and high MKI.

Conclusion: HER2 expression, not accompanied by gene amplification, is common in neuroblastic tumors. HER2-positive tumors seem to have a positive prognostic significance. HER2 expression with a variable pattern is a marker of the stage of neuroblastic cells differentiation and is connected to Schwannian stroma development.

Email: eczis@wp.pl

Ki-67 proliferation index is marker of poor prognosis in neuroblastoma especially in patients aged over 18 months

Izycka-Swieszewska, Ewa; Lipska, Beata Stefania; Drozynska, Elzbieta; Balcerska, Anna; Perek, Danuta; Grajewska, Wieslawa; Dembowska, Bozena; Klepacka, Teresa; Wozniak, Wojciech; Chybicka, Alicja; Limon, Janusz

1 Medical University of Gdansk, Department of Pathology, Gdansk, Poland; 2 University Hospitals Catholic University of Leuven, Laboratory of Experimental Oncology, Leuven, Belgium; 3 Medical University of Gdansk, Department of Biology and Genetics, Gdansk, Poland

Background: Proliferation index measured immunohistochemically with Ki-67 (PI KI67) is the accepted prognostic factor in some types of cancer. Its significance in neuroblastoma (NB) is not well established. The aim of our study was to assess the prognostic impact of PI KI67 and its pathoclinical relations in a series of NB tumors.

Methods: 103 patients followed-up from 4 to 149 (median 46) months [m] were enrolled in the study. 34 patients died of disease. Analyzed data included: patients’ age, tumor localization, stage, overall survival, tumor histology, MKI, MYCN and ploidy status. Ki-67 immunostaining was performed on representative tissue slides and counted as percent of positive nuclei for 100-1000 neoplastic cells.

Results: Patients’ age ranged 1-169 m, including 63 children older than 18 months (>18m). There were 74 neuroblastoma, 20 ganglioneuroblastoma and 9 ganglioneuroma cases. High MKI characterized 28 cases. PI KI67 ranged from 0 to 72% (median 15%). PI was lower in >18m children (median 10%; p=0.0002).

Conclusion: Ki-67 proliferation index has a prognostic significance in NB tumors based on patients’ age. Two cut-off values were identified as markers of poor prognosis: 10% for >18m children and 30% for the entire group. We propose to include the assessment of PI KI67 to the standard histological assessment of NB tumors.

Email: b.lipska@gumed.edu.pl
Background: The increasing knowledge on genetic alterations associated with neuroblastoma tumors brings also an increasing need for a method that identifies these alterations in a diagnostic multi-genomic approach. The Neuroblastoma specific salma-MLPA (Multiplex Ligation-dependent Probe Amplification) probe is able to identify gains or losses of different genomic loci covering the ten chromosomal regions of highest interest within a given sample. The aim of this work was to compare/validate the MLPA results with a panel of already well established FISH assays.

Methods: A total of 62 tumor samples from patients diagnosed and treated in the SCMCi were analyzed by MLPA, 47 of them were evaluated in parallel with interphase FISH (MYCN, 1p, 17q, 11q) and DNA index by Flow Cytometry.

Results: Segmental alterations were associated with higher stage neuroblastoma, whereas in the low stages gain and/or loss of whole chromosomes could be detected. In 25/47 cases, the structural aberrations were detected by both methods. In 16/47 tumors, no structural aberrations were detected by any of the methods. In 3 cases aberrations were detected by MLPA alone, and in another 3 cases aberrations were seen only by FISH. These discordances could be explained by sample error and low percentage of tumor cells, respectively. McNemar pairs comparison test was applied to statistically determine the significance of differences in the detection of segmental alterations by both methods. The two-tailed P value equals 0.683 which is considered to be not statistically significant, by conventional criteria.

Conclusion: MLPA collected wider information on gain and losses than the classical cytogenetic and FISH methods. The use of this new routine technique with such customized probes sets would be a simple and easy molecular adjunctive tool in the classification of neuroblastoma tumors. Email: iyaniv@clair.illinois.edu

POT45

MLPA (Multiplex Ligation-dependent Probe Amplification) and FISH comparison/validation for genetic characterization of neuroblastoma xenografts

Kaneko, Setsuko; Kaneko, Michio

University of Tsukuba, Pediatric Surgery, Tsukuba, Japan

Background: In neuroblastoma (CPT-11) is highly effective against neuroblastoma xenografts. However, the combination of CPT-11 alone cannot completely abolish tumor. Cytotoxicity (COX-2) which promotes tumor progression is overexpressed in NB. We evaluated the anti-tumor effect of CPT-11 combined with low-dose celecoxib, a selective COX-2 inhibitor, against human NB xenografts.

Methods: NB xenografts used were drug-sensitive lines, NB09, NS-N-5nu, and a multidrug-resistant line, NS-N-2nu. Five different treatment schedules were evaluated: celecoxib alone at 5 mg/kg daily for 20, low-dose (5.9mg/kg) CPT-11 × celecoxib daily for 20 days, and conventional-dose (59mg/kg) CPT-11 in 3 doses at 4-day intervals × celecoxib for 12 consecutive days. Tumor growth inhibition was evaluated for mean tumor doubling time. Cell proliferation, angiogenesis, apoptosis, and protein expression in tumor tissues were analyzed.

Results: CPT-11 alone on a low-dose prolonged schedule was equally or more effective than a conventional-dose intermittent one. Celecoxib administered daily at 5 mg/kg could not prevent the growth of any NB xenografts. However, the combination of daily low-dose CPT-11 and simultaneous celecoxib reduced in highly significant suppression of tumor growth in all xenografts (p<0.001) compared not only with low-dose CPT-11 alone but also with the combination of intermittent conventional-dose CPT-11 and celecoxib, accompanied by decreased proliferation and increased induction of apoptosis in tumor cells. Induction of apoptosis was associated with the up-regulation of Bax and the down-regulation of Bcl-2. The enhanced anti-tumor effect of the combination of the two drugs against the NB xenografts might be partially COX-2 independent and was probably mediated through multiple factors including diminished expression of VEGF and activation of the caspase-dependent mitochondrial apoptotic pathway.

Conclusion: Prolonged low-dose CPT-11 treatment combined with low-dose celecoxib showed promising anti-tumor activity through the blockage of multiple critical targets related to NB tumor cell survival and proliferation.

Email: mkaneko@md.tsukuba.ac.jp

POT46

Prolonged low-dose administration of the cyclooxygenase-2 inhibitor celecoxib enhances the antitumor activity of irinotecan against neuroblastoma xenografts

Kaneo, Setsuko; Kaneko, Michio

University of Tsukuba, Pediatric Surgery, Tsukuba, Japan

Background: Irinotecan (CPT-11) is highly effective against neuroblastoma xenografts. However, the combination of CPT-11 alone cannot completely abolish tumor. Cytotoxicity (COX-2) which promotes tumor progression is overexpressed in NB. We evaluated the anti-tumor effect of CPT-11 combined with low-dose celecoxib, a selective COX-2 inhibitor, against human NB xenografts.

Methods: NB xenografts used were drug-sensitive lines, NB09, NS-N-5nu, and a multidrug-resistant line, NS-N-2nu. Five different treatment schedules were evaluated: celecoxib alone at 5 mg/kg daily for 20 days, low-dose (5.9mg/kg) CPT-11 × celecoxib daily for 20 days, and conventional-dose (59mg/kg) CPT-11 in 3 doses at 4-day intervals × celecoxib for 12 consecutive days. Tumor growth inhibition was evaluated for mean tumor doubling time. Cell proliferation, angiogenesis, apoptosis, and protein expression in tumor tissues were analyzed.

Results: CPT-11 alone on a low-dose prolonged schedule was equally or more effective than a conventional-dose intermittent one. Celecoxib administered daily at 5 mg/kg could not prevent the growth of any NB xenografts. However, the combination of daily low-dose CPT-11 and simultaneous celecoxib reduced in highly significant suppression of tumor growth in all xenografts (p<0.001) compared not only with low-dose CPT-11 alone but also with the combination of intermittent conventional-dose CPT-11 and celecoxib, accompanied by decreased proliferation and increased induction of apoptosis in tumor cells. Induction of apoptosis was associated with the up-regulation of Bax and the down-regulation of Bcl-2. The enhanced anti-tumor effect of the combination of the two drugs against the NB xenografts might be partially COX-2 independent and was probably mediated through multiple factors including diminished expression of VEGF and activation of the caspase-dependent mitochondrial apoptotic pathway.

Conclusion: Prolonged low-dose CPT-11 treatment combined with low-dose celecoxib showed promising anti-tumor activity through the blockage of multiple critical targets related to NB tumor cell survival and proliferation.

Email: mkaneko@md.tsukuba.ac.jp

POT47

CHD5 is part of the nucleosome remodeling and histone deacetylation (NuRD) complex in neuroblastoma (NB) cell lines.

Kolla, Venkatadri1; Zhuang, Tiangang1; Koyama, Hiroshi1; Naraparaju, Kounoudi; Higashi, Mayumi1; Bloibe, Gerd A.2; White, Peter S.3; Brodeur, Garrett M.4

1The Children's Hospital of Philadelphia, Department of Pediatrics, Division of Oncology, Philadelphia, United States; 2The Children's Hospital of Philadelphia and The University of Pennsylvania, Department of Pediatrics, Division of Hematology, Philadelphia, United States; 3The Children's Hospital of Philadelphia, Department of Pediatrics, Division of Bioinformatics, Philadelphia, United States; 4The Children's Hospital of Philadelphia and The University of Pennsylvania, Department of Pediatrics, Division of Oncology, Philadelphia, United States

Background: Eukaryotic gene expression is developmentally regulated by chromatin remodeling, and its dysregulation has been linked to cancer. CHD5 is a tumor suppressor gene that maps to a region of consistent deletion on 1p36.31 in NBs. It is preferentially expressed in neural tissues, whereas expression is consistently low or undetectable in NB cell lines. The CHD5 gene encodes a protein with chromatin remodeling, helicase and DNA-binding motifs. CHD5 is highly homologous to CHD3 and CHD4, which are core subunits of the NuRD chromatin-remodeling complex. We performed studies to determine if CHD5 forms a similar complex.

Method/approach: NLf cells were stably transfected with CHD5 cDNA in the sense or antisense orientation. Immunofluorescence microscopy was used to demonstrate nuclear localization of CHD5 protein. NuRD components were identified by immunoprecipitation and by using GST-Fog as an affinity reagent to purify the NuRD complex. Proteins were detected by SimplyBlue staining and by Western blot. LC-MS was used to confirm the presence of CHD5 protein in the complex.

Results: We examined 50 cell extracts from CHD5-transfected NLf cells to determine if CHD5 forms a NuRD complex similar to CHD4. V5/His-tagged CHD5 was immunoprecipitated from nuclear extracts with a V5 or CHD3 antibody. Pull-down with GST-Fog after CHD4 depletion. CHD5 associated with all NuRD subunits, including MTA1/2, P66, HDAC1/2, RbAp46/48 and MBD3) as determined by Western blotting and LC/MS.

Conclusion: Our data suggest that CHD5 encodes an ATP-dependent chromatin-remodeling protein that forms a NuRD complex similar to CHD4. The CHDS/NuRD complex presumably plays an important role in chromatin remodeling and tumor suppression. This may occur by transcriptional repression (or activation) of genes important in regulating neuroblast growth or differentiation.

Email: KOLLA@EMAIL.CHOP.EDU

POT48

Mechanisms of CHD5 inactivation in neuroblastomas

Koyama, Hiroshi1; Zhuang, Tiangang1; Light, Jennifer E.1; Kolla, Venkatadri1; Higashi, Mayumi1; London, Wendy B.2; Brodeur, Garrett M.3

1Children's Hospital of Philadelphia, Department of Pediatrics, Division of Oncology, Philadelphia, PA, United States; 2Boston Children's Hospital, Department of Pediatrics, Division of Oncology, Boston, MA, United States

Background: Neuroblastomas are known to have genetic, biological and clinical heterogeneity. High-risk neuroblastoma is defined by several genetic changes, including 1p36.31 deletion, and we have recently identified the chromatin-remodeling gene CHDS as a tumor suppressor gene that maps to this region. Low or absent CHD5 expression is associated with all NuRD subunits, including MTA1/2, P66, HDAC1/2, RbAp46/48 and MBD3) as determined by Western blotting and LC/MS.

Results: We examined 50 cell extracts from CHD5-transfected NLf cells to determine if CHD5 forms a NuRD complex similar to CHD4. V5/His-tagged CHD5 was immunoprecipitated from nuclear extracts with a V5 or CHD3 antibody. Pull-down with GST-Fog after CHD4 depletion. CHD5 associated with all NuRD subunits, including MTA1/2, P66, HDAC1/2, RbAp46/48 and MBD3) as determined by Western blotting and LC/MS.

Conclusion: Our data suggest that CHD5 encodes an ATP-dependent chromatin-remodeling protein that forms a NuRD complex similar to CHD4. The CHDS/NuRD complex presumably plays an important role in chromatin remodeling and tumor suppression. This may occur by transcriptional repression (or activation) of genes important in regulating neuroblast growth or differentiation.

Email: KOLLA@EMAIL.CHOP.EDU
Neuroblastoma cell lines, phenotype and susceptibility towards natural killer cells
Kraal, Kathelijne CJM; Ostaijen ten Dam, MM; Bal, LM; van Tol, MJH; Egeler, RIM
LUMC. Pediatric Immunology-Hematology-oncology and bone marrow transplantation, Leiden, Netherlands

Background: Currently the prognosis for relapsed/ refractory high-risk (HR) neuroblastoma (NBL) patients is poor, warranting new treatment strategies such as immunotherapy. NBL tumour cells show low or absent expression of HLA class I, and NBL cell lines are resistant to specific, HLA restricted cytotoxic T cells. Natural killer (NK) cells are bone marrow derived lymphocytes, that are cytotoxic against tumour cells and virus infected cells. The interaction of NK cells with target cells is controlled by a balance of inhibition (KIR) and activating (e.g. DNAM-1, NKG2D) receptors on the NK cells.

Aim: Preclinical study to investigate whether NBL tumours are sensitive targets for NK cells and whether the NK cell cytolitic potential towards NBL tumours can be enhanced.

Methods: NBL cell lines have been tested for in vitro sensitivity of killing by NK cells using cytotoxicity assays. Cell lines have been phenotyped using flow cytometry (FACS) analysis and molecularly typed for HLA to document presence of possible inhibitory KIR ligands. Blocking experiments using monoclonal antibodies (MoAbs) were performed to assess the pathways involved in killing. Results: in vitro experiments using purified NK cells have indicated that NBL cell lines are sensitive to killing mediated by allogeneic cytokine (IL-15)-activated NK cells, but are barely lysed by resting NK cells. NBL cell lines variably express HLA class I and express some NKG2D ligands, all express DNAM-1 ligands. Preliminary experiments suggest that interaction of activated NK cells and NBL cells is partially mediated by MoAb directed against DNAM-1. Blocking of HLA class I did not result in enhanced killing of NBL cell lines by resting NK cells.

Conclusion: NBL cells express DNAM-1 ligands, making them a possible target for immunotherapy by infusion of activated NK cells. These findings will need to be extended using primary tumour material, in order to further support the potential contribution of infusion of allogeneic IL-15-activated NK cells for the treatment of NBL cells in vivo.

Email: K.Kraal@lumc.nl

UCHL1-Upregulation correlates with reduction of vital neuroblastoma cells by fenretinide and doxorubincin treatment
Kuehnel, Sandraa; Cernaianu, Grigorie; Stuehler, Kai; Meyer, Helmutb; Butel, Albrechtc; Koeller, Manfredb; Sitek, Barbaraa; Troebs, Ralf-Bodoa
1Marienhospital Herne, Department of Pediatric Surgery, Herne, Germany; 2Ruhr-University Bochum, Medizinisches Proteom-Center, Bochum, Germany; 3Kliniken der Westfälischen Universitätsmedizin Münster, Department of Surgery, Bochum, Germany

Background: Neuroblastoma (NB) disseminating need an intensive cytotoxic chemotherapy. Studies prove the effectiveness of retinoic acid derivates (RA) as differentiating agents on NB cells. We studied the effect of the combined treatment of the synthetic retinoid fenretinide (F) with the cytotoxic drug doxorubicin (D) on the vitality of the human NB cell line SH-SY5Y.

Methods/approach: SH-SY5Y cells were incubated for 6 days with 50% inhibitory concentrations of F and D Treatment groups: control (CO), F, D, F+D. Subsequently, measurements of vitality with MultiTox-Fluor Multiplex Cytotoxicity assays were performed. Differentially regulated proteins were identified by 2D-difference gel electrophoresis (2D-DIGE) and mass spectrometry (MS). Transcriptomic regulation of these proteins and the expression profiles of cell cycle regulators, apoptosis and differentiation markers were analyzed by real time PCR (RT-PCR).

Results: Single (F or D) or the combined treatment (F+D) of SH-SY5Y cells resulted in the following vitality rates: F 60±2.2%, D 69±9.2±7% and F+D 42±2.4% (p<0.01, n=7). 23 differentially regulated proteins (p<0.05 and average ratio <2 or >2) were identified by MS. Among these UCHL1, KHSRP, THARAP3, ENO2, TPM2, MAPRE1, GRB2 and PATL1 were of particular interest. RT-PCR results for UCHL1, KHSRP, THARAP3, ENO2 and TPM2 were confirmed by Western Blot analysis. The markers of Schwann-cell differentiation GFAP and S100B were not influenced.

Conclusion: RAs are commonly considered as differentiating agents which inhibit the activity of cytostatic drugs. In contrast, the new synthetic retinoid F combined with D decreases the vitality of SH-SY5Y cells and induces neuronal differentiation. Both substances alone and in combination alter the regulation of different genes on protein and/or mRNA level. A combination of UCHL1 was identified as a selective target of the combination therapy (F+D).

Email: sandra.kuehnel@rub.de

Low dose metronomic (LDM) administration of oral topotecan and pazopanib as an effective preclinical antiangiogenic therapy in neuroblastoma
Kumar, Sushilb; Mokhtari, Rezaa; Wu, Bingc; Zhang, Libod; Mann, Sharr; Kerbel, Robertb; Yeger, Hermann; Bardeyn, Sylviana; McGrady, Patrick Wa; London, Wendy Bb; Brodeur, Garrett Mba; Children’s Hospital of Philadelphia, Department of Pediatrics, Division of Oncology, Philadelphia, PA, United States; 2Hospital For Sick Children, laboratory medecine, Toronto, Canada; 3Hospital For Sick Children, hematologyst and Oncology, Toronto, Canada; 4BG-Kliniken Bergmannsheil GmbH, Department of Surgery, Surgical Research, Bochum, Germany

Background: Angiogenesis plays a critical role in neuroblastoma (NB) growth and metastasis. Low dose metronomic (LDM) chemotherapy, combine with VEGF pathway inhibitors is an emerging treatment strategy.

Objectives: To establish the efficacy, PK/PD of LDM topotecan (TP) with/ without pazopanib (PZ) an oral antiangiogenic tyrosine kinase inhibitor (TKI) in NB mouse model.

Methods: SK-N-BE(2) and SH-SY5Y cell lines were used to establish IC50 and NOD-SCID mice model to both subcutaneous primary tumour and metastatic experiments. Mice were randomized into 5 groups: control group, LDM TP (1.0mg/Kg), PZ (30mg/kg and 150mg/Kg) and the combination (1.0mg/Kg TP + 150mg/Kg PZ). Localized tumor model, animals were treated daily till 56 days; metastatic model, were treated until death. Angiogenic makers, circulating Endothelial cells (CECs) and circulating Endothelial Progenitor cells (CEPs) were determined by flow cytometry. PK studies were conducted to determine the plasma concentration-time profiles of both the drugs.

Results: IC 50 of topotecan on cells was 129.9 ng/ml(SK-N-BE(2)) and 4.0mg/ml (SH-SY5Y). Pazopanib did not induce cytotoxicity up to 10ug/ml. In xenograft model, statistically significant efficacy was observed for single agent (TP or PZ) and combination with repsetive p values. Combination (PZ 150mg/kg)(p<0.0002) > LDM TP (p<0.0008)> than PZ >than control in the SK-N-BE(2) metastatic model. The three treatment regimens significantly prolonged animal overall survival compared to control group. P (p<0.0005) > TP (p<0.0006) > P+Z (p<0.0006) > than PZ. No toxicity was observed in any of the groups. The Cmax of PZ was 135.5µg/ml(PZ) and 125.6µg/ml (PZ+TP). PZ plasma concentration was maintained above the optimal concentration for up to 18 h. The combination of LDM TP and PZ reduced the levels of viable CEP (p=0.125) and CEC (p=0.0005) after 7 days treatment.

Conclusion: Daily oral LDM TP and PZ and combination are effective and safe regimens in both localized and metastatic neuroblastoma mouse models. The reduction in CEC/CEP levels supports the anti-angiogenic activity of these drugs regimen.

Email: sylvain.bardeyn@email.chldkids.ca

Clinical significance of TRK family gene expression in neuroblastomas
Light, Jennifer E.; Koyama, Hiroshi; Gordin, Eli; Minturn, Jane E.; iyer, Radhika; Ho, Ruth; Simpson, Anisha M.; Kolla, Venkatareddy; McGrady, Patrick W.; London, Wendy B.; Brodeur, Garrett M.; Children’s Hospital of Philadelphia, Department of Pediatrics, Division of Oncology, Philadelphia, PA, United States; 2Boston Children’s Hospital, Department of Pediatrics, Division of Oncology, Boston, MA, United States

Background: NBs are characterized by clinical heterogeneity, from spontaneous regression or differentiation to relentless progression. Evidence from our laboratory and others suggests that the pattern of TRK family gene expression can be used to distinguish amongst the disparate clinical outcomes. TrkA is expressed in favorable NBs, whereas TrkB and its ligand BDNF are coexpressed in unfavorable NBs, representing an autocrine survival pathway. We determined the expression pattern and clinical significance of TRK family genes in a large set of primary NBs.

Patients and Methods: We analyzed the expression the following genes in 610 representative NBs using quantitative real-time RT-PCR with TaqMan low-density array cards: TRK/NTKR1, TrkB/NTKR2, TrkC/NTKR3, NGF, BDNF, and P75/NGFR. Expression (high vs. low) for each was compared to clinical and biological variables as well as outcome. Patients were categorized into one of two groups, dichotomized by the median expression value of each gene. A Kruskal-Wallis test was used to test for association of expression of each gene with each of the dichotomized risk factors. Life table analysis and log rank tests were performed to compare the event-free survival (EFS) or overall survival (OS) of the two groups for each gene.

Results: High TrkA expression was strongly correlated with favorable age, stage, MYCN, histology and risk group (p<0.0001 for all), and weakly with favorable ploidy (p=0.004). NGF expression was correlated with favorable risk group, but no other TRK family gene correlated significantly with clinical/biological variables. TrkC expression did not correlate with outcome, but low NGF was correlated with favorable EFS and OS.

Conclusion: We conclude that the high expression of TrkA was very strongly correlated with all clinical and biological risk factors except ploidy, but surprisingly did not correlate with EFS or OS in this analysis. Also, high TrkB and/or BDNF expression have correlated with unfavorable outcome in the past but did not correlate with EFS or OS in this analysis. The high expression of TrkA and TrkB expression will identify tumors that are likely to respond to TrkB inhibitors alone or in combination with conventional agents.

Email: brodeur@email.chop.edu
**POT54**

**Induction of miR-183 via an epigenetic mechanism suppresses neuroblastoma malignancy**

Lodrel, Marco1; Schulte, Johannes H1; Castoldi, Mitco2; Drehne, Ina3; Muckenthaler, Martina1; Witt, Olaf4; Deutzner, Hedwig E5

1German Cancer Research Center (DKFZ), Clinical Cooperation Unit Pediatric Oncology, Heidelberg, Germany; 2University of Essen, Department of Pediatric Hematology and Oncology, Essen, Germany; 3EMBL, Molecular Medicine Partnership Unit, Heidelberg, Germany

**Background and Aim:** Therapy of high-risk neuroblastoma has remained difficult. Despite high-dose chemotherapy and peripheral stem cell transplantation, resistant relapses frequently occur. Histone deacetylase inhibitors (HDACi) cause differentiation of neuroblastoma cells in preclinical models. However, little is known about the underlying molecular events. Here, we investigated the role of microRNAs, known to play a key role in promoting neural development, in mediating a differentiated phenotype by HDACi.

**Methods and Results:** We employed ChIP-on-Chip and real-time quantitative PCR methodologies, we identified miR-183 as strongly induced microRNA by both the cyclic tetrapeptide Helminthosporium carbonum (HC)-toxin and the carboxylic vapecolic acid (VPA), siRNA-mediated silencing of the 11 HDACs belonging to classes I, II and IV showed that knockdown of HDAC2 induced miR-183 expression, whereas the plasmid-mediated enforced expression of HDAC2 repressed miR-183. Transient transfection of miR-183 into MYCN amplified and single copy neuroblastoma cell lines inhibited both metabolic activity and cell cycle progression and induced cell death. In MYCN single copy tumors, high miR-183 expression was found to be significantly associated with event-free survival of children.

**Conclusions:** Induction of miR-183 via inhibition of HDAC2 suppresses neuroblastoma malignancy. The data highlight both HDAC2 as relevant target for an HDACi-mediated intervention and the administration of miR-183 as potential therapeutic agent against high-risk neuroblastoma.

Email: h.deubzer@dkfz.de

---

**POT55**

**Telomere elongation and chromosomal instability in non-MYCN amplified clinically aggressive neuroblastoma**

Lundberg, Gustaf1; Hjorth, Henrik2; Frigyesi, Attila3; Castel, Victoria1; Varela, Carly1; Evans, Audrey1; Broder, Garrett1

1Children's Hospital of Philadelphia, University of Pennsylvania, Philadelphia, United States; 2The Children's Hospital of Philadelphia, University of Pennsylvania, Pediatría, Division of Oncology, Philadelphia, United States; 3The Children's Hospital of Philadelphia, University of Pennsylvania, Pediatric, Division of Cardiology, Philadelphia, United States

**Background:** NB is a childhood tumor that is characterized by heterogeneous clinical behavior, and the TRK neurotrophin receptors probably play a role. We have used the TRK-selective inhibitor lestaurtinib to treat NBs in xenograft models. Although a phase II trial with lestaurtinib was well tolerated and demonstrated efficacy at higher doses, some toxicities were seen. Here we explore the use of NPs for targeted delivery of lestaurtinib to improve efficacy and decrease toxicity.

**Method/Approach:** We used the TRK-null SY5Y line transplanted with Trk8 for our studies. NPs were synthesized using polyacrylic-PEG with the far-red fluorophore BODIPY (650/665X) incorporated for optical imaging. We used the RT-CES system (AceABio) to monitor cell proliferation. Lestaurtinib (Cephalon, Inc) was either given as free or NP-encapsulated drug. NP-lestaurtinib was delivered IV (20 mg/kg) once every 1-2 weeks, whereas free drug was given sub-Q (20 mg/kg) twice a day, five days a week. We also performed optical imaging studies to assess the systemic distribution of NP-lestaurtinib given IV.

**Results:** Initially we tested 0.05 to 10 µM of either free or NP-lestaurtinib on Trk8-expressing NB cells in vitro. <0.2 µM, the highest concentration we tested, had an equivalent effect. However, >0.3 µM, NP-lestaurtinib was significantly better at inhibiting proliferation. We saw almost complete inhibition at 1 µM with NP-lestaurtinib compared to continued proliferation with the same concentration of free drug. Western analysis of Trk phosphorylation showed almost complete inhibition of phosphorylation with 0.1 µM NP-lestaurtinib, whereas it took 0.5-1.0 µM free drug to achieve similar inhibition. Greater imaging intensity of NP-lestaurtinib correlated with better tumor response.

**Conclusion:** Our preliminary data show that NP-lestaurtinib was at least as effective as free drug in vitro, possibly due to protein binding of the free drug to protein carriers in the fetal calf serum. We also show that NP-lestaurtinib concentrates in subQ tumors, and response correlates with intensity of uptake. These findings support further investigation of NPs to deliver TRK inhibitors as well as other agents to treat NBs.

Email: m�nngio@ema.ilion.co.edu

---

**POT56**

**Novel SINGLE (NP) delivery of the Trk inhibitor lestaurtinib in neuroblastoma**

Mangino, Jennifer1; Iyer, Radhika2; Chorny, Michael3; Allenier, Ivan4; Minturn, Jane1; Ho, Ruth1; Simpson, Anisha1; Varela, Carly1; Evans, Audrey1; Levy, Robert4; Broder, Garrett1

1The Children’s Hospital of Philadelphia, University of Pennsylvania, Philadelphia, United States; 2The Children’s Hospital of Philadelphia, University of Pennsylvania, Pediatric, Division of Oncology, Philadelphia, United States; 3The Children’s Hospital of Philadelphia, University of Pennsylvania, Pediatric, Division of Cardiology, Philadelphia, United States

**Background:** NB is a childhood tumor that is characterized by heterogeneous clinical behavior, and the TRK neurotrophin receptors probably play a role. We have used the Trk-selective inhibitor lestaurtinib to treat NBs in xenograft models. Although a phase II trial with lestaurtinib was well tolerated and demonstrated efficacy at higher doses, some toxicities were seen. Here we explore the use of NPs for targeted delivery of lestaurtinib to improve efficacy and decrease toxicity.

**Method/Approach:** We used the Trk-null SY5Y line transplanted with Trk8 for our studies. NPs were synthesized using polyacrylic-PEG with the far-red fluorophore BODIPY (650/665X) incorporated for optical imaging. We used the RT-CES system (AceABio) to monitor cell proliferation. Lestaurtinib (Cephalon, Inc) was either given as free or NP-encapsulated drug. NP-lestaurtinib was delivered IV (20 mg/kg) once every 1-2 weeks, whereas free drug was given sub-Q (20 mg/kg) twice a day, five days a week. We also performed optical imaging studies to assess the systemic distribution of NP-lestaurtinib given IV.

**Results:** Initially we tested 0.05 to 10 µM of either free or NP-lestaurtinib on Trk8-expressing NB cells in vitro. <0.2 µM, the highest concentration we tested, had an equivalent effect. However, >0.3 µM, NP-lestaurtinib was significantly better at inhibiting proliferation. We saw almost complete inhibition at 1 µM with NP-lestaurtinib compared to continued proliferation with the same concentration of free drug. Western analysis of Trk phosphorylation showed almost complete inhibition of phosphorylation with 0.1 µM NP-lestaurtinib, whereas it took 0.5-1.0 µM free drug to achieve similar inhibition. Greater imaging intensity of NP-lestaurtinib correlated with better tumor response.

**Conclusion:** Our preliminary data show that NP-lestaurtinib was at least as effective as free drug in vitro, possibly due to protein binding of the free drug to protein carriers in the fetal calf serum. We also show that NP-lestaurtinib concentrates in subQ tumors, and response correlates with intensity of uptake. These findings support further investigation of NPs to deliver TRK inhibitors as well as other agents to treat NBs.

Email: m�nngio@ema.ilion.co.edu
POT58
Inhibition of PARP-1 enhances the efficacy of [131I]MIBG/Topotecan combinations in vitro. McCleskey, Anthony G1; Sorensen, Annette1; Tesson, Mathias1; Mairs, Robert J2; Boyd, Marie2
1University of Strathclyde, Strathclyde Institute of Pharmacy and Biomedical Sciences, Glasgow, United Kingdom; 2University of Glasgow, Centre of Oncology and Applied Pharmacology, CRUK Beatson Laboratories, Glasgow, United Kingdom

Background: [131I]MIBG targeted radiotherapy induces favourable remissions in a number of neuroblastoma patients as a single agent. However, combining [131I]MIBG with other agents may enhance its potential.
Previously, we reported that inhibition of DNA repair and supra-additive toxicity to NAT-expressing cells and xenografts were achieved by [131I]MIBG and topotecan (topoisomerase I poison) in combination. Here, we assess the potential of PARP-1 inhibition to further enhance [131I]MIBG/ topotecan efficacy.

Methods: Combinations of topotecan, PJ34 (PARP-1 inhibitor) and [131I]MIBG were carried out in the laboratory in SK-N-Be(2c) (neuroblastoma), EJ138-CMV/NAT (NAT-transfected bladder cancer) and UWW-CMV/NAT (NAT-transfected glioma). Topotecan/PJ34 combinations were assessed using three schedules: topotecan given 24h before [i], after [ii] or simultaneously with [iii] PJ34. In a similar manner, PJ34/[131I]MIBG and PJ34/[131I]MIBG/topotecan combinations were also assessed.

Results: Topotecan/PJ34: In SK-N-Be(2c) cells, supra-additive toxicity was observed following schedules [i] and [iii], but not schedule [ii]. In EJ138-CMV/NAT cells, schedule [ii] was most effective, while schedules [i] and [iii] induced supra-additivity. In UWW-CMV/NAT cells, all schedules induced supra-additive effects. PJ34/[131I]MIBG: In SK-N-Be(2c) cells, supra-additivity was observed following schedule [iii] treatment, but not schedule [i]. EJ138-CMV/NAT cells: EJ138-CMV/NAT-supra-additivity was induced by all three schedules. In UWW-CMV/NAT cells, schedules [iii] and [ii] induced supra-additive responses. PJ34/[131I]MIBG/topotecan: Supra-additivity was observed in SK-N-Be(2c) and EJ138-CMV/NAT cells following all three schedules, but only schedule [iii] induced synergy in UWW-CMV/NAT. All three agents induced G2 arrest as single agents. However, triple combinations caused less cell cycle disruption than treatment by single agents.

Conclusions: PARP-1 inhibition enhances the toxicity of [131I]MIBG/ topotecan in vitro. This suggests that anti-PARP-1/[131I]MIBG/topotecan combinations may be beneficial in patients with neuroblastoma and other NAT-expressing tumours.

Email: anthony.mccleskey@strath.ac.uk

POT59
CDK inhibitors Roscovitine and CR8 trigger Mcl-1 Down-regulation and apoptotic cell death in neuroblastoma cells Meijer, Laurent; Delehousse, Claire; Loaëc, Nadège
CNRS, Protein Phosphorylation & Human Disease, Station Biologique, Roscoff, France

Background: Protein kinases are widely investigated as therapeutic targets in a variety of diseases. We have developed pharmacological inhibitors of cyclin-dependent kinases (CDKs), with potential applications against cancer and neurodegenerative diseases (Alzheimer, Parkinson, stroke), renal diseases (glomerulonephritis, PKD), inflammation, etc... Neuroblastoma (NB) is highly malignant paediatric cancer, and the identification of candidate therapeutic agents with neuroblastoma cells with different MYCN status (SH-SY5Y, SK-N-Be(2), IMR-32, SK-N-DZ, T21N containing inducible MYCN) was carried out for cytotoxicity in six cell lines with different MYCN status (SH-SY5Y, SK-N-Be(2), IMR-32, SK-N-DZ, T21N containing inducible MYCN) when treated with substances from the tyrosine kinase inhibitor library.

Results: Initial screening identified three tyrosine kinase inhibitors with potential interest which were evaluated further since they all had an inhibitory effect on cell survival and cell proliferation. Tyrophostin 9 was more effective on the MYCN amplified cell lines SK-N-Be(2), SK-N-DZ, IMR-32 and T21N expressing MYCN with EC50 values below 10 µM respectively. Tyrophostin 9 targets PDGFR and to some extent EGFRR proven to be important in tumour development of neuroblastoma. Treatment with Tyrophostin 9 resulted in apoptosis in the multidrug resistant MYCN amplified cell line SK-N-Be(2), ZMA49829 and ZM39923 targets JAK/STAT pathway, not previously studied in neuroblastoma and both compounds were highly cytotoxic to all neuroblastoma cell lines tested.

Conclusion: Evaluation of a kinase inhibitor library revealed significant activity of substances that may contribute to a more specific therapy for high-risk neuroblastoma, along with potential chemotherapeutic drugs. Our results potentially highlight JAK/STAT signalling as an important target for future neuroblastoma therapy.

Email: ebba.palmberg@ki.se

POT60
Preclinical testing of novel kinase inhibitors in high-risk neuroblastoma Palmberg, Ebba1; Rickardson, Linda1; Wickstrom, Malin1; Lindskog, Magnus2; Johnsen, John Inge1; Kogner, Per1
1Karolinska Institutet, Childhood Cancer Research Unit, Department of Women’s and Children’s Health, Stockholm, Sweden; 2Uppsala University, Division of Clinical Pharmacology, Department of Medical Sciences, Uppsala, Sweden; 3Karolinska Institutet and Uppsala University, Childhood Cancer Research Unit, Department of Women’s and Children’s Health, Division of Clinical Pharmacology, Department of Medical Sciences, Stockholm and Uppsala, Sweden

Background and aims: Novel treatments targeting MYCN amplified neuroblastoma tumours are highly warranted since a amplification of the MYCN oncogene constitutes the single most important predictor of bad prognosis. The purpose of this study was to, from a library containing 80 kinase inhibitors, highlight the most effective substances and their targets in MYCN amplified neuroblastoma cells.

Methods: Methods used include fluorometric microculture cytotoxicity assay (FMCA), Western blot for target inhibition and the Arrayscan to determine caspase 3 cleavage, fragmentation and cell density. The morphology of the cells was studied using the Incucyte, an automated imaging system to monitor live cells in culture. Kinase inhibitors were tested for cytotoxicity in six cell lines with different MYCN status (SH-SY5Y, SK-N-Be(2), IMR-32, SK-N-DZ, T21N containing inducible MYCN) when treated with substances from the tyrosine kinase inhibitor library.

Results: Initial screening identified three tyrosine kinase inhibitors with potential interest which were evaluated further since they all had an inhibitory effect on cell survival and cell proliferation. Tyrophostin 9 was more effective on the MYCN amplified cell lines SK-N-Be(2), SK-N-DZ, IMR-32 and T21N expressing MYCN with EC50 values below 10 µM respectively. Tyrophostin 9 targets PDGFR and to some extent EGFRR proven to be important in tumour development of neuroblastoma. Treatment with Tyrophostin 9 resulted in apoptosis in the multidrug resistant MYCN amplified cell line SK-N-Be(2), ZMA49829 and ZM39923 targets JAK/STAT pathway, not previously studied in neuroblastoma and both compounds were highly cytotoxic to all neuroblastoma cell lines tested.

Conclusion: Evaluation of a kinase inhibitor library revealed significant activity of substances that may contribute to a more specific therapy for high-risk neuroblastoma, along with potential chemotherapeutic drugs. Our results potentially highlight JAK/STAT signalling as an important target for future neuroblastoma therapy.

Email: ebba.palmberg@ki.se

POT61
Balance of pro- versus anti-angiogenic splice isoforms of vascular endothelial growth factor as a regulator of neuroblastoma growth Peiris, Maria1; Bates, David Owen1; Ramani, Pramila1
1University of Bristol, Cellular and Molecular Medicine, Bristol, United Kingdom; 2University of Bristol, Physiology and Pharmacology, Bristol, United Kingdom; 3Bristol Royal Infirmary, Department of Pathology, Bristol, United Kingdom

Background: VEGF is a key mediator of angiogenesis and is upregulated by a variety of tumours. An endogenous family of anti-angiogenic isoforms, VEGFΔb, has been identified in normal, non-angiogenic tissues, and in contrast with the angiogenic VEGFxxx isoforms is down-regulated in epithelial tumours including colo-rectal and prostate carcinoma. This is the first study of VEGFΔb in neuroblastomas, ranging from malignant neuroblastoma (NB) to benign ganglioneuroma (GN).

Method/approach: Twenty tumour samples and 5 NB cell lines, BE(2) C, IMR-32, SHIN, SY5Y and SHEP, with different tumorigenic potential and MYCN amplification status were assessed for VEGFΔb and VEGFxxx expression by RT-PCR and ELISA. To determine if VEGFΔb inhibits NB in vivo, 13 nude mice were injected subcutaneously with NB cell, bi-weekly s.c. injections of 100ug of VEGFΔb or saline were initiated in each group when tumours reached a diameter of 4-5 mm.

Results: VEGFΔb, but not VEGFxxx, was up-regulated in NB compared to GN at mRNA level. At the protein level NB showed a significantly lower ratio VEGFΔb/total VEGF (0.2±0.1) than GN (1.0±0.2). All NB cell lines expressed VEGFΔb and VEGFxxx by RT-PCR and ELISA. To determine if VEGFΔb inhibits NB in vivo, 13 nude mice were injected subcutaneously with NB cell, bi-weekly s.c. injections of 100ug of VEGFΔb or saline were initiated in each group when tumours reached a diameter of 4-5 mm.

Conclusion: Taken together these results suggest that the clinical drug rosuvastine and its novel analogue CR8 induce apoptotic tumor cell death by down-regulating Mcl-1, a key survival factor expressed in all NB cell lines. CDK inhibition may thus constitute a new approach to treat refractory high-risk NB.

Email: Maria.Peiris@bristol.ac.uk

Abstract Book 193
Predictive consequences of risk stratification neuroblastoma patients using the new INRG classification system.

Piqueras, Marta; Navarro, Samuel; Cañete, Adela; Castel, Victoria; Noguera, Rosa

*University of Valencia, Medical School, Department of Pathology, Valencia, Spain.*

**Background:** Risk classification and treatment stratification of neuroblastoma patients is mandatory because the clinical heterogeneity behaviour of this neurotumour is complex. Understanding of neuroblastoma genetics will improve with genome-wide techniques, which are recommended in samples with <60% neuroblastic cell content. Our aim was to evaluate the use of FISH on tissue microarrays (TMA) to detect aberrations in MYCN gene, 1p36, 11q and 17q chromosome regions, and for patient stratification.

**Method:** 369 tumors were included in TMA, 291 were primary tumors. We performed FISH assays to determine the status of MYCN gene and 1p36 region on TMA and compared with routine diagnosis previously known in 139 tumors to evaluate the feasibility of these assays. After this validation, 11q and 17q alterations were analyzed in 369 samples. Partial genetic instability (PGI) was defined as the ratio between segmental chromosome aberrations (SCA) detected and number of genetic markers diagnosed in each tumor. prognostic value of currently clinical and biological variables used was evaluated to know if our cohort was statistically representative. We compared patient risk using SIOPEN classification with estimated risk using the new INRG classification system.

**Results:** No discordance between status of MYCN gene and 1p36 region by FISH on TMA and previously routine diagnosis was observed. PGI was established in 260 primary tumors, 67 of them contained <60% neuroblasts, including always MYCN gene status and at least 2 of the others. poorer outcome was statistically worse for patients whose tumors presented high PGI (p<0.0001). Risk estimation was established in 280 patients. 32 and 13 patients with intermediate risk by SIOPEN presented low and high risk by the new INRG classification respectively.

**Conclusion:** In our cohort, PGI established by FISH on TMA was associated with patient outcome. PGI is a useful method to identify high risk patients whose tumors have <60% neuroblasts. Grants: Instituto Carlos III, Spain (RD0600201012) and FAECC (369/2009). Email: rosa.noguera@uv.es

Targeting neuroblastoma and neuroblastoma tumour initiating cells with the oncolytic viruses rhodamine and VSV.

Potter, Richard; Annis, James; Grandori, Carla; Park, Julie

*Alberta Cancer Research Institute/University of Calgary, Department of Surgery, Calgary, Canada; 1Hospital for Sick Children/University of Toronto, Department of Molecular and Medical Genetics, Toronto, Canada; 2University of Florida, Department of Molecular Genetics and Microbiology, Gainesville, United States; 3University of Ottawa, Ottawa Regional Cancer Centre, Ottawa, Canada; 4Alberta Cancer Research Institute/University of Calgary, Department of Biochemistry and Molecular Biology, Calgary, Canada; 5Alberta Children’s Hospital/Alberta Cancer Research Institute/University of Calgary, Department of Surgery, Calgary, Canada.

**Background:** Neuroblastoma is the second most common pediatric extracranial malignant tumor. High-risk patients do poorly and little progress has been made in improving their outcome. Implications of tumor cells called Tumor Initiating Cells (TICs) may drive aggressive tumor behavior and treatment resistance. A neuroblastoma TIC has been identified, but their role in tumor behavior is not yet characterized and their susceptibility to existing and novel cancer treatments is unknown. Myxoma and Vesicular Stomatitis Virus (VSV) are two oncolytic viruses that have been shown to effectively destroy brain tumor cells, which share a common ancestry with neuroblastoma in that both are derived from neural crest cells. It is unknown if these oncolytic viruses can effectively destroy neuroblastoma cells or neuroblastoma TICs.

**Objectives:** Characterize the ability of VSV and Myxoma to target and destroy 1) neuroblastoma cells; and 2) neuroblastoma TICs.

**Methods:** Viability assays on infected neuroblastoma and neuroblastoma TIC lines were performed including infection and viral protein production assessed by intergenic fluorescent protein expression, Western blot detection of viral proteins from infected cell lysates, and measurement of infected cell lysis and viral activity by plaque assay. In vivo viral activity was measured by intratumoral injection in an established human neuroblastoma mouse xenograft model.

**Results:** Both myxoma and VSV infect and kill neuroblastoma cells in vitro. Cytopathic effects and green fluorescent protein (Myxoma) or rhodamine (VSV) expression were both seen after infection. Infection of neuroblastoma cells was further confirmed by Western blot detection of myxoma and VSV viral protein expression. Most neuroblastoma TIC lines are also infected and destroyed by myxoma but appear resistant to VSV. Finally, both myxoma and VSV were found to effectively inhibit the growth of neuroblastoma in subcutaneous xenografts.

**Conclusion:** Myxoma and VSV effectively destroy several neuroblastoma cell lines while myxoma is able to target neuroblastoma tumour initiating cells. Oncolytic viruses may offer a novel approach to treatment of high-risk neuroblastoma.

Email: paul.beaudry@albertahealthservices.ca

Survival pathways of high-risk neuroblastoma identified by functional genomics.

Richard, Lauren; Arnis, James; Grandori, Carla; Park, Julie

*University of Washington, Molecular and Cellular Biology, Seattle, United States; 2University of Washington, Department of Pediatrics, Seattle, United States; 3Queens High Throughput Screening Core, Institute for Stem Cells and Regenerative Medicine, Seattle, United States; 4University of Washington, Fred Hutchinson Cancer Research Center, Pharmacology, Cancer Biology, Seattle, United States; 5University of Washington Children’s Hospital, Seattle, United States; 6University of Calgary, Department of Biochemistry and Molecular Genetics, Calgary, Canada; 7Hospital for Sick Children/University of Toronto, Department of Molecular and Medical Genetics, Toronto, Canada; 8Alberta Cancer Research Institute/University of Calgary, Department of Biochemistry and Molecular Biology, Calgary, Canada.

**Background:** Neuroblastoma is a molecularly heterogeneous disease with amplification of the MYCN oncogene detected in about one third of high-risk cases.

**Method/approach:** Our aim was to identify critical survival pathways and therapeutic targets for high-risk NB utilizing functional genomics. A parallel high throughput siRNA screen was performed in high-risk neuroblastoma cell lines with and without MYCN amplification. The screen included a set of 43 genes previously implicated in neuroblastoma pathogenesis and a collection of siRNA targeting the entire human kinome (~750 genes). Network pathway analysis was utilized to integrate the gene “hits” from the screen with available microarray data (Oncometomics website link: http://pob.abcc.ncifcrf.gov/cgi-bin france.uk).

**Results:** The screen highlighted differential sensitivities between MYCN amplified and non-amplified lines to inhibition of key players of WNT and NRG signaling, as well as to inhibition of mitotic kinases, such as STK6 (Aurora A), NEK2, NEK4 and WE1. However, additional genes, not previously implicated in NB were also revealed. A complete list of “hits” will be presented and linked to both known and novel NB pathways, which could constitute potential therapeutic targets.

**Conclusion:** This study identified druggable genes and pathways required for proliferation of high risk NB cells in vitro. In vivo validation of these genes through stable RNAi or using available small molecule inhibitors, will provide evidence for the potential development. Furthermore, our study indicates the importance of combining functional genomics with gene expression data to select within the patient population which patients could benefit from specific biologic therapies.

Email: lincl@uw.edu

194

ANR 2010, June 21–24 2010
Tumor angiogenesis is an important prognostic factor in neuroblastoma. Presence of high vascularity, sprouting of new vessels by angiogenesis growth factors, and integration of circulating endothelial cells from bone marrow origin in aggressive and disseminated disease suggest the use of anti-angiogenic agents for the treatment of high risk neuroblastoma. We evaluated the new oral pan-VEGFR tyrosine kinase inhibitor axitinib (AG-013736) against neuroblastoma cell lines and the subcutaneous and orthotopic xenograft model IGR-N91 derived from a primary neuroblastoma bone marrow metastasis. Axitinib reduced cell proliferation in a dose-dependent manner with IC50 doses between 667 and 1069 nmol/L. Oral treatment with 30 mg/kg BID during 2 weeks at advanced tumor stage yielded significant tumor growth delay in median time to reach 5 times initial tumor volume of 11.4 days compared to controls (p=0.0006; Mann-Whitney test) and significant reduction in bioluminescence, respectively. mTOR inhibition using rapamycin 20 mg/kg q2d x 5 resulted in significantly decreased microvessel density (MVD) and overall surface fraction of tumor vessels (OSFV), respectively, at 14 days were 21.27 +/-10.03 and 0.56% compared to 39.44 +/-14.50 and 1.36% in controls (p=0.0006). We further explored the effects of axitinib on circulating mature endothelial cells (CECs) and endothelial progenitor cells (CEPs) measured by flow cytometry. While a transient reduction was observed for CECs, CEPs were significantly reduced during and at least 14 days after end of treatment. Thus, axitinib is a potent anti-angiogenic new agent targeting tumor angiogenesis in neuroblastoma and demands further evaluation.

Conclusion: This study strengthens the independent effect of ploidy and genomic profile in children <18 months; SCA were equally distributed in the tumors independently of their ploidy (p=0.517).

Results: The analysis of 343 Japanese sporadic NBs showed that 322 (93.9%) possessed no aberration of ALK gene (mutation 4.7%; amplification 1.5%). We then examined the expression levels of ALK, RPTP/bz and PTN mRNAs in 78 NBs with wildtype of ALK. The high expression of ALK mRNA was significantly associated with favorable outcome of NB (p=0.04). Notably, the levels of ALK mRNA expression were significantly high in stage 4s tumors (n=5) as compared with other stages, that was further confirmed by using additional 9 samples in stage 4s (p<0.01). The high levels of PTN and RPTP/bz mRNA expression were also significantly associated with better survival (p=0.02 and p=0.01), and the combination of low expression of both genes showed the worst survival rate (p<0.01). The expression of mutant as well as wildtype of ALK enhanced cell migration, growth and invasion in NB cell lines. On the other hand, elevated wildtype ALK in PC12 cells enhanced neuritis outgrowth. These suggested that wildtype ALK may regulate cell growth and survival as well as differentiation in neuronal cells. The intracellular adaptor proteins, Shh and BMCC1 we previously identified, interacted with both TrkA and ALK, suggesting the presence of crosstalk between TrkA and ALK signaling. Surprisingly, the ALK expression was induced by MYCN and Sp1, that was confirmed by reporter and ChIP assays.

Conclusion: Wildtype of ALK signaling may be regulated bi-directionally among the different subsets of NBs.
POT70

Dendritic cell-based immunotherapy using sendai virus vector - a preclinical efficacy study against neuroblastoma - An advanced report

Tanaka, Sakura; Tajiri, Tatsuro; Yonemitsu, Yoshikazu; Tatsuta, Kyouku; Souza, Ryota; Ueda, Yasash; Koga, Yukki; Sumine, Akiko; Hara, Toshijiro; Taniguchi, Tadayoshi

1Graduate School of Medical Sciences, Kyusyu University, Department of Pediatric Surgery, Fukuoka, Japan; 2Graduated School of Pharmaceutical Sciences, R&D Laboratory for Innovative Biotherapeutics, Department of Gene Therapy, Fukuoka, Japan; 3DNAVEC corporation, DNAVEC corporation, Tsukuba, Japan; 4Graduate School of Medical Sciences, Kyusyu University, Department of Pediatrics, Fukuoka, Japan

Background: We have recently reported that the induction of efficient antitumor immunity to C1300 murine neuroblastoma (C1300) using DCs treated with recombinant Sendai virus (SeV/DC) is the aim of the current study to look into the potential of SeV/DC to treat less immunogenic neuroblastoma as a preclinical efficacy study. Methods: A/J mice were subcutaneously inoculated with C1300. Bone marrow-derived DCs treated with SeV (SeV/DC) were administered intratumorally after irradiation of tumor (4Gy/day for 3 days). Human peripheral monocyte-DCs derived from SeV, 48h after the transfection of SeV, were examined the expression of the surface markers of DCs and gene transduction efficiency by flow cytometry, and the expression of inflammatory cytokines in the medium of cultured DCs by ELISA. Results: Use of SeV/DC without preirradiation showed some efficacy on established C1300, but antitumor effect against vascularized/established tumor was weakened. The combination with irradiation and SeV/DC was effective against vascularized/established tumor (> 5 mm), and dramatically enhanced the ratio of complete elimination of established tumor (5/6=83%). Antitumor effect of SeV/DC with preirradiation was enhanced further compared with the established tumor specific long term memory against C1300. The result of the effector cell-depletion experiment confirmed that CD8+ T cells were predominant effector cells in antitumor immunity against C1300 in the early phase and CD6+1 T cells in the secondary phase. Specificity of SeV to human peripheral monocyte-derived DCs were sufficient (average>70%) and some inflammatory cytokines were induced by SeV transfection without any remarkable changes in the expression of CD surface markers. Conclusion: These results indicate that less immunogenic neuroblastoma could be a potential target of SeV/DC-based immunotherapy. Therefore we conclude that SeV/DC system is warrant to further investigation to treat patients with intractable malignancies including far advanced neuroblastoma in clinical setting.

Email: sakura@pathol1.med.kyushu-u.ac.jp

POT71

DD3, a large non-coding RNA against the pro-apoptotic BMCC1 gene, is a candidate target for treating neuroblastoma

Tatsuro, Yonemitsu; Yokoyama, Tomaoki; Ooo, Miy Lin; Takano, Ryo; Takagi, Datsumu; Ohno, Miki; Nakagawara, Akira

Chiba Cancer Center Research Institute, Division of Biochemistry & Innovative Cancer Therapeutics, Chiba, Japan

Background: We previously identified a novel gene BMCC1 from our neuroblastoma cDNA library in human NBs (BMCC1-Id, 1996). The BMCC1 mRNA was preferentially expressed in human nervous tissues and prostate. BMCC1 is a pro-apoptotic protein expressed at high levels in favorable NBs and is up-regulated during the NGF withdrawal-induced apoptosis in mouse neuroblastoma cells. Here, we have identified DD3 as a large non-coding RNA targeting BMCC1. Methods: The expressions were examined by semi-quantitative RT-PCR, real-time RT-PCR and immunoblot. The reciprocal co-immunoprecipitation was performed for protein interaction assay. Results: BMCC1 mRNA was down-regulated in tumors tissues as compared with the corresponding normal tissues in many cancers, suggesting that it appears to be a tumor suppressor. BMCC1 physically interacted with both TrkA and ErbB2, and the activation of its GAP activity at the BCh domain attenuated the signals for differentiation and proliferation by inhibiting ERK phosphorylation. From the genomic database, we found an antisense gene, DD3, located at the intron 6 of the BMCC1 gene. DD3 was supposed to be a large non-coding RNA of 3.7 kb in size. Expression of both BMCC1 and DD3 mRNA was significantly decreased in unfavorable NBs (n=100; p=0.011 and p=0.001, respectively). Decreased expression of both BMCC1 and DD3 mRNA was significantly associated with MYCN amplification (p=0.001), low expression of TAA4 (p=0.001) and DNA ploidy (p=0.001). However, overexpression of DD3 down-regulated BMCC1, whereas siRNA-mediated knockdown of DD3 increased BMCC1 expression. In addition, accumulation of p38 alpha mRNA was also abrogated by cisplatin treatment. Of further interest, the cell death induced by cisplatin was significantly enhanced by knockdown of DD3 by using siRNA-DD3 in both NB and prostate cancer cell lines. Conclusion: DD3 functions like an oncogene by targeting BMCC1 tumor suppressor in cancer cells. In NBs, the BMCC1 and DD3 negative regulatory loop may contribute to the reduction of regression. In addition, targeting DD3 may enhance the therapeutic sensitivity to anti-cancer drugs.

Email: ylatsumi@chiba-cc.jp

POT72

The novel PKD1 inhibitor OSU03012 and the dual PI3k/mTOR inhibitor PI103 target high in neuroblastoma

Segeteström, Lova; Baryawnow, Nibin; Sveinbjörnsson, Baldur; Elfman, Lotta; Kogner, Per; Johnsen, John Inge

Karolinska Institutet, Childhood Cancer Research Unit, Department of Women's and Children's Health, Stockholm, Sweden

Background and aims: Proteins regulating signalling through the phosphoinositide-3-kinase (PI3K/Akt) pathway are frequently altered in human cancers, including neuroblastoma. We investigated two compounds inhibiting key proteins in PI3K/mTOR signalling: a phosphoinositide-dependent protein kinase-1 (PKD1) inhibitor, OSU03012, and the dual class IA PI3K/mammalian target of rapamycin (mTOR) inhibitor, PI103, on neuroblastoma in vitro and in vivo. Methods: Primary tumours were investigated for the presence and activation status of components of the PI3K/Akt pathway by immunohistochemistry. In vitro, the efficacy of these inhibitors was investigated by proliferation, cell cycle and apoptosis assays whereas PI3K/Akt pathway activation/inhibition was detected by western blotting. Tel211 cells were used to examine the efficacy of the inhibitors in a low/high-MYC setting. In vivo, athymic mice engrafted with human neuroblastoma cells were randomised to treatment with either inhibitor and the tumour growth was followed. Results: Immunohistochemical analysis showed the presence and activation status of components of the PI3K/Akt pathway in human neuroblastomas. Both OSU03012 and PI103 inhibited neuroblastoma growth in vitro. In treated cells, OSU03012 induced apoptosis and an S phase cell cycle arrest, but only minor apoptosis was detected in PI103 treated cells together with a G1 arrest. Both OSU03012 and PI103 down regulated phosphorylation of Akt and its downstream targets, GSK3β and S6K1, as well as cyclin D1 and MYCN protein expression. Cell lines expressing high levels of MYCN were more sensitive to OSU03012 or PI103 compared to cells expressing low MYCN levels. Both OSU03012 and PI103 significantly inhibited the growth of established MYCN-amplified xenografts.

Conclusion: Our results suggest that key proteins in the PI3K/Akt signalling cascade represent a clinically relevant target for high-risk MYCN-amplified neuroblastoma.

Email: Lova.Segetström@ki.se

POT73

NK cells engineered to express the chimeric receptor scFv(ch14.18)-zeta specifically lyse GD2 expressing neuroectodermal tumors

Seidel, Diana; Huebner, Nicole; Mueller, Tina; Pfreundtenges, Doerthe; Shibina, Anaselasia; Tanaka, Sakura; Tajiri, Tatsuro; Yonemitsu, Yoshikazu; Tatsuta, Kyosuke

Karolinska Institutet, Childhood Cancer Research Unit, Department of Pediatrics, Experimental Oncology, Berlin, Germany; TÜHU Sc, Dept. of Cell Biology and Biochemistry, Lubbock, Texas, United States; Chemotherapeutisches Forschungsinstitut, Georg-Speyer-Haus, Frankfurt, Germany; Institute for Transfusion Medicine and Immunohematology, Institute for Transfusion Medicine and Immunohematology, Frankfurt, Germany; University Hospital, Pediatric Hematology and Oncology, Frankfurt, Germany

Introduction Neuroblastoma (NB) is a neuroectodermal tumor of childhood characterized by a poor prognosis. The T cell independent antigen ganglioside GD2 is highly expressed on most NB which makes it an interesting target for immunotherapeutic strategies. In order to specifically direct the cytotoxic abilities of NK cells towards NB cells, the human NK cell line NK-92 was genetically engineered to express a chimeric receptor, consisting of a GD2-specific ch14.18scFv-antibody fragment and the signal transducing zeta-chain of the CD3 complex (NK-92-scFv(ch14.18)-zeta).

Methods: In order to determine specificity of NK-92-scFv(ch14.18)-zeta (NK-92facs), FACS analysis was performed. For this, we used an anti-idiotypic-antibody (anti-id-Ab), which mimics the GD2 epitope and is directed against the binding domain of ch14.18. In vitro cytotoxicity assays measuring chromium (Cr)51 release with GD2+ and GD2–NB and melanoma cell lines were performed, in order to prove functionality of transduced NK-92 cells. Furthermore, cytotoxic activity of NK-92facs was blocked using the α-lf-Ab in a blocking assay from the genomic database, we found an antisense gene, DD3, located at the intron 6 of the BMCC1 gene. DD3 was supposed to be a large non-coding RNA of 3.7 kb in size. Expression of both BMCC1 and DD3 mRNA was significantly decreased in unfavorable NBs (n=100; p=0.011 and p=0.001, respectively). Decreased expression of both BMCC1 and DD3 mRNA was significantly associated with MYCN amplification (p=0.001), low expression of TAA4 (p=0.001) and DNA ploidy (p=0.001). However, overexpression of DD3 down-regulated BMCC1, whereas siRNA-mediated knockdown of DD3 increased BMCC1 expression. In addition, accumulation of p38 alpha mRNA was also abrogated by cisplatin treatment. Of further interest, the cell death induced by cisplatin was significantly enhanced by knockdown of DD3 by using siRNA-DD3 in both NB and prostate cancer cell lines. Conclusion: DD3 functions like an oncogene by targeting BMCC1 tumor suppressor in cancer cells. In NBs, the BMCC1 and DD3 negative regulatory loop may contribute to the reduction of regression. In addition, targeting DD3 may enhance the therapeutic sensitivity to anti-cancer drugs.

Email: diana.seidel@ttuhsc.edu

ANR 2010
June 21–24 2010
196
197
POT74
Synergistic inhibition of neuroblastoma tumor development by targeting ornithine decarboxylase and topoisomerase II
Sholler, Giselle1; Currier, Erik2; Bachmann, Andre3
1University of Vermont, Pediatrics, Burlington, United States; 2University of Vermont, Vermont Cancer Center, Burlington, United States; 3University of Hawaii, Cancer Research Center of Hawaii, Honolulu, United States

Background: Neuroblastoma (NB) is a deadly childhood cancer that arises from neural crest cells of the sympathetic nervous system. MYCN amplification occurs in a large number of NBs and is associated with poor prognosis. Since MYCN controls a number of genes involved in ornithine decarboxylase (ODC), we proposed that ODC should be considered as a novel target for MYC-driven tumors such as NB (Hawaii Med J. 2004, 63:371-4, 2005, 24:560-8B). We showed that ODC and polyamines are markedly elevated in NBs and targeted inhibition of ODC by alpha-difluoromethylornithine (DFMO) resulted in polyamine pool depletion and subsequent p27/Rb-mediated G1 cell cycle arrest.

Methods: We utilized in vitro calciom AM cell viability testing to assess the effect of DFMO drug combinations on several NB cell lines. Nude mice were injected with 10^6 human NB cells subcutaneously and treated with 1) vehicle, 2) 40mg/kg etoposide on day 1 and day 3 intraaperitoneally, 3) 2% DFMO in drinking water, or 4) the combination of etoposide and DFMO.

Results: We identified a synergistic interaction in NB cells with the combination of DFMO and etoposide, a topoisomerase II inhibitor that is commonly used in front-line therapy of NB patients. Remarkably, DFMO and etoposide, in combination, synergistically reduced the tumor burden in mice and extended tumor-free survival.

Conclusion: Given the current lack of effective therapies for relapsed/refractory NB patients, the preclinical effectiveness of this combination and high safety profile of DFMO we have advanced DFMO and etoposide into an FDA approved Phase Ib clinical trial.

Email: abachmann@crr.hawaii.edu

POT75
The genetic and clinical implications of MYCN gain in neuroblastoma
Sousaki, Ryota1; Tajiri, Tatsuro2; Teshiba, Risa3; Kinoshita, Yoshiaki4; Tanaka, Sakura5; Taguchi, Tomoaki6; Nakamura, Yuichi7; Oda, Tomohiro8
1Kyushu University, Department of Pediatric Surgery, Fukuoka, Japan

Purpose: The MYCN gene is located in chromosome 2p24, and MYCN amplification (MYCN-A) is a strong prognostic factor in neuroblastoma (NB). MYCN-gain which is a low level of MYCN-A as determined by FISH is identified as less than 4-fold additional copies of MYCN signals in relation to the number of chromosome 2. It is unclear whether the MYCN-gain is the pre-status of MYCN-A. Furthermore, the clinical significance of MYCN-gain is unclear. This study assessed the correlation of MYCN-A and MYCN-gain, and the clinical implication of MYCN-gain in NB.

Methods: The status of the MYCN gene was determined by FISH and quantitative polymerase chain reaction (Q-PCR) in 47 primary NB samples and the status of chromosome 2p in all cases was analyzed using a single nucleotide polymorphism (SNP) array.

Results: Eight of the 47 cases analyzed using FISH showed MYCN-A, 7 cases showed MYCN-gain and 32 cases showed no MYCN amplification (NMA). A SNP array analysis showed that 6 of 8 cases with MYCN-A by FISH had amplification of the MYCN region without distal 2p gain and other 2 cases had both amplification of MYCN region and distal 2p gain. All 7 cases with MYCN-gain by FISH had distal 2p gain without amplification of the MYCN region, and all 32 cases with NMA by FISH demonstrated neither the amplification of the MYCN region nor the 2p gain. Three of 7 cases with MYCN-gain showed slight increase of MYCN gene dosage by Q-PCR. The 5-year overall survival rate of patients with MYCN-gain (n=7, 71.4%) were poor in comparison to that of patients with NMA (n=32, 90.6%) by FISH. However, no significant difference was observed (p=0.11). The SNP array analysis showed that NBs with MYCN-gain had more other genetic aberrance such as 1p loss, 11q loss and 17q gain had more other genetic aberrance such as 1p loss, 11q loss and 17q gain without NMA by FISH.

Conclusion: These results suggest that the MYCN-gain represents the distal 2p gain and is not the pre-status of MYCN-A. NB with MYCN-gain by FISH have other genetic aberrances associated with the prognosis.

Email: ryotas@pedsurg.med.kyushu-u.ac.jp

POT76
A new syngeneic MYCN-overexpressing neuroblastoma mouse model and MYCN-DNA vaccine
Bianco, Fabio1; De Vecchi, Carla1; Garaventa, Alberto2; Teshiba, Risa3; Sholler, Giselle1; Currier, Erika2; Bachmann, Andre3; Stigliani, Sara1; Coco, Simona1; Moretti, Stefano1; Oberthuer, André4; Anastasia2; Fest, Stefan3; Lode, Holger N.4
1University of Genoa, Department of Pediatric Oncology, Genoa, Italy; 2INFN Genoa, Department of Oncology and Genetics (DOGIB), Genoa, Italy; 3National Cancer Research Institute (IST), Molecular Epidemiology, Genoa, Italy; 4Institut Curie, Paris, France; 5University Children's Hospital Cologne, Department of Pediatric Oncology, Cologne, Germany; 6Gaslini Institute, Department of Hematology-Oncology, Genoa, Italy; 7IFCCS San Raffaele Pisana, Clinical and Molecular Epidemiology, Rome, Italy

Background: Fifty percent of children with neuroblastoma (NB) have high-risk (HR) metastatic disease and show a long-term survival <30%. In order to identify novel molecular prognostic markers useful to refine current criteria of patients' relapse risk estimation, we performed genome-and transcriptome-wide analyses in stage 4 HR-NB.

Methods: Patients older than 1 year of age at diagnosis were categorized as "short-survivors" (SS) (dead of disease within 5 years from diagnosis) and "long-survivors" (LS) (alive with an overall survival time > 5 years). Array-CGH was performed in 91 NBs, gene expression profiling in 75 NBs, and the expression study of 481 Transcribed-Ultra Conserved Regions (t-UCRs) by qPCR and of 723 miRNAs by arrays in 34 NBs.

Results: All NBs were characterized by structural aberrations on chromosomes 1, 2, 3, 7, 11, 17. The number of these alterations was significantly higher (P<0.0005) in SS compared to LS. Tumors with MYCN amplification showed a simpler pattern of alterations compared to MYCN-single copy NBs (P=0.0008), dominated by 17q gain and 1p loss. ROC and Kaplan-Meier survival analyses showed that at least 4 structural aberrations are needed to discriminate (P=0.0001) LS from SS. In tumors of SS, genes involved in cell cycle are up-regulated, whereas Rho/Ras pathway is down-expressed. Among the genes upregulated in SS, regulators of neuronal differentiation (DPYSL3, NTRK1, CHD8, FYN) are enriched. An inverse correlation between expression level of t-UCRs and their complementary miRNAs targeting neuronal differentiation genes was observed in SS patients.

Conclusion: a) Structural aberrations are significantly associated with fatal outcome; b) Deregulation of Rho/Ras-mediated progression through cell cycle may explain the increase of tumor aggressiveness in SS; c) Integrative expression analysis of non-coding RNAs and host genes suggests that changes in primary transcription rates are responsible for switching off the neuronal differentiation in HR-NB pathogenesis.

Funding: Fondazione Italiana per la Lotta al Neuroblastoma, MIUR. Participation of P. S. to ANR 2010 is supported by NuGEN Technologies, Inc.

Email: paola.scaruffi@istge.it
POT78 Tumor cell detection in autologous stem cell harvests in patients with high risk neuroblastoma

Stutterheim, J1; Vree, F2; Hero, B3; Zappej - Kannegieter, L1; Voermans, C1; Schumacher-Kuckelkorn, R2; Koehl, U2; Schulte, J H3; Niggli, F4; Fruhwald, M C5; van Noesel, M C6; Niemeyer, C M7; Bode, U8; Schilling, F H9; Schultz, C10; Graf, M10; Nathrath, M10; Schilling, F9; Baron, H N7; van der Schoot, C E11; Tytgat, G A M12

1Sanquin, Experimental Immunohematology, Amsterdam, Netherlands; 2Emma Children’s Hospital, Academic Medical Center, Department of Pediatric Oncology, Amsterdam, Netherlands; 3Children’s Hospital, University of Cologne, Pediatric Hematology and Oncology, Cologne, Germany; 4Children’s Hospital, University of Frankfurt/Main, Department of Pediatric Hematology and Oncology, Frankfurt, Germany; 5Children’s Hospital, University of Essen, Department of Pediatric Hematology and Oncology, Essen, Germany; 6Children’s Hospital, University of Zurich, Department of Pediatric Hematology and Oncology, Zurich, Switzerland; 7Children’s Hospital, University of Muenster, Department of Pediatric Hematology and Oncology, Muenster, Germany; 8Sophia Children’s Hospital, Department of Pediatric Hematology and Oncology, Rotterdam, Netherlands; 9Children’s Hospital, University of Freiburg, Department of Pediatric Hematology and Oncology, Freiburg, Germany; 10Children’s Hospital, University of Bonn, Department of Pediatric Hematology and Oncology, Bonn, Germany; 11Children’s Hospital, University of Luebeck, Department of Pediatric Hematology and Oncology, Luebeck, Germany; 12Children’s Hospital, University of Homburg, Department of Pediatric Hematology and Oncology, Homburg, Germany; 13Technical University of Munich, Department of Pediatrics, Munich, Germany; 14Children’s Hospital, University of Munich, Department of Pediatric Hematology and Oncology, Munich, Germany

Introduction: The presence of minimal residual disease (MRD) detected by real-time quantitative (RQ) -PCR in autologous stem cell harvests in children with high risk neuroblastoma (NBL) seems to be associated with an unfavourable outcome, however to date only small studies have been performed. Moreover, these studies suffered from lack of specificity of the assay due to background amplification of the PCR target in normal bone marrow (BM) and peripheral blood stem cells (PBSC). In this retrospective multicenter study, harvests of a large patient cohort are studied using a recently described optimal panel of PCR targets (1).

Methods: In total, 37 BM harvests, 75 PBSC harvests and 55 CD34+ selected harvests from 167 high risk patients were retrospectively collected at 2 Dutch and 12 German centers. In 137 patients the harvest was reinjected. Of those, 25 PBSC before CD34+ selection, 44 CD34+ selected harvests and 68 unselected harvests (28 BM and 40 PBSC) were tested. RQ-PCR was performed with six NBL-specific markers: PHOK2B, TH, DCC, GAP43, CHRNA3 and DBH. The prognostic impact of MRD in autologous harvests and the reinfection of contaminated harvests was assessed using Kaplan-Meier plots and log-rank tests.

Results: Presence of NBL mRNA was detected in 46% (17/37) BM harvests, 12% (9/75) PBSC harvests and 14% (8/55) CD34+ harvested cells (p<0.001). This was associated with poor survival (5 years overall survival (5-y-OS), 23.4±5.2% versus 45.6±4.5%; p=0.008). In 21% (24/112) of the patients an MRD positive harvest was reinjected, which was associated with poor outcome (5-y-OS 30.5±10.5% versus 55.4±4.5%; p=0.03). Remarkably, there was no difference in survival in patients after reinfection of BM harvests compared to PBSC harvests (1+CD34 selection) (p=0.66).

Conclusion: Our series of autologous stem cell harvests is the largest series described up till now. In this series, BM harvests were more often contaminated than PBSC or CD34+ selected harvests. Both the presence of MRD in the harvests and reinfection of a contaminated harvest were associated with worse outcome.

Reference List

E-mail: j.stutterheim@sanquin.nl

POT79 Functional analysis of the p53 pathway in neuroblastoma cells using the smallmolecule MDM2 antagonist, RG7112

Van Maeren, Tom1; Rihanli, Ali2; Drexiax, Daniel3; De Clercq, Sarah4; Yigit, Nurten5; Marine, Jean-Christophe6; Westermann, Frank7; De Paepe, Anne8; Speleman, Frank9; Vandesompele, J10

1Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium; 2German Cancer Research Center, Department of Tumor Genetics, Heidelberg, Germany; 3Flanders Interuniversity Institute for Biotechnology, Laboratory of Molecular Cancer Biology, Ghent, Belgium

Background: Inactivation of the p53 pathway is essential for tumor cells to survive and thrive, and improved understanding of the mechanisms behind p53 inactivation may guide the development of targeted therapeutic strategies. Functional analysis of the nature of p53 pathway detects in a large panel of neuroblastoma cell lines using the selective MDM2 antagonist RG7112 as a tool to directly activate p53.

Methods: The entire coding region of the p53 gene from 34 human neuroblastoma cell lines was amplified by direct sequencing. Functional integrity of the p53 pathway was probed by measuring the reduction in cell viability after treatment with nutlin-3. Activation of the p53 pathway in experiments that aimed at identification of modulators of the response to nutlin-3 was assessed by real-time quantitative RT-PCR analysis of p53 target genes and by cell viability and caspase assays.

Results: We identified 9 cell lines (26.5%) with a mutation in the p53 gene, including 6 missense mutations, 1 nonsense mutation, 1 in-frame deletion, and 1 homozygous deletion of the 3’ end of the p53 gene. Sensitivity of p53 reporter assays was highly dependent on the presence of p53 mutation. Cell lines with wild-type p53 were subject to marked nutlin-3-induced cytotoxicity in 23 out of 25 cases, indicating that p53 downstream signaling pathways are functionally intact in the vast majority of neuroblastoma cell lines. The presence of a homozygous DCKX2A (p16INK4a/p14ARF) deletion in one of both nutlin-3-refractory cell lines with wild-type p53 (i.e., in SHEP cells) prompted us to investigate the role of p14ARF and p16INK4a in the response to nutlin-3. The nutlin-3-resistant phenotype of SHEP cells could not be reversed by reintroduction of p14ARF or p16INK4a, but knockdown and overexpression experiments in several other neuroblastoma cell lines pointed to a stimulatory effect of p14ARF on the response to nutlin-3.

Conclusions: Mutational inactivation of p53 is not uncommon in neuroblastoma cell lines, whereas defects in effectors pathways downstream of p53 are rare. Expression levels of p14ARF may modulate the response to nutlin-3, dependent on the cellular context.

E-mail: Ali.Rihanli@UGent.be

POT80 Detection of microRNAs in bone marrow from children with high-risk neuroblastoma predicts survival; a UK CCLG study.

Vijep, Viprey1; Corrias, Maria2; Gregory, Walter3; Brock, Penelope4; Burnell, Susan1

1Leeds Institute of Molecular Medicine, Children’s Cancer Research Group, Leeds, United Kingdom; 2Gaslini Institute, Paediatric Oncology, Genoa, Italy; 3University of Leeds, Clinical Trials Research Unit, Leeds, United Kingdom; 4Great Ormond Street Hospital, Paediatric Oncology, London, United Kingdom

Background: MicroRNAs (miRNAs) are differentially expressed in tumours compared to normal tissues, resulting in an aberrant expression signature that is a hallmark of cancer. We have therefore tested the hypothesis that miRNAs might constitute a new class of biomarkers for detection of disease in bone marrow (BM) from children with neuroblastoma (NB), and potentially provide an informative signature for disease prognosis and monitoring.

Methods: miRNAs were isolated from BM samples stabilised in PAXgene® Blood RNA tubes. The expression profile of 380 miRNAs was assessed using high-throughput stem-loop QRT-PCR (Low Density Arrays, Applied Biosystems). BMs positive (n=30) and negative (n=10) for NB cells detected by QRT-PCR were taken from children at diagnosis were analysed. BMs (n=5) from healthy children were also analysed.

Results: Eight miRNAs predicted for survival; 7 ranging from 5.08 to 9.01 (p<0.024) identified using ranked significance levels from Cox model analyses. High expression of miR-519a was most significantly (p<0.003) associated with increased risk of death (Relative Risk >7.85 [95% CI 2.03-30.11]). Of the 8 miRNAs identified, miR-450-5p and miR-708 were also detected in a 67 miRNA signature that distinguishes BM containing NB cells from that with no NB cells, and from BM of healthy children (unequal variance t-test p< 0.01, fold expression change > 4). Of these 67 miRNAs 11 were down-regulated and 56 up-regulated; miR-223 and miR-145 were amongst the most significantly down-regulated (p<0.00002) and expression of miR-137, miR-149, miR-375 and mir-10b were up-regulated >1000-fold (p<0.003).

Conclusions: We have identified a panel of miRNAs that when detected in BM can predict survival. We also established a 67 miRNA signature that distinguishes BM containing NB cells from that with no NB cell contamination. This signature includes miRNAs that regulate cell cycle progression, angiogenesis and metastasis, providing novel insight into the biology of the circulating NB cell. These pilot observations require study in a larger group of children with NB.

E-mail: v.vijep@leeds.ac.uk
POT81
SKP2-mediated neuroblastoma dedifferentiation is triggered by MYCN through CDK4 induction
Westermann, Frank1; Muth, Daniel1; Drexel, Daniel1; Pöhl, Christiana; Gogolin, Sina1; Fischer, Matthias2; Heinrich, Kai1; Ehemann, Volker3; Gillespie, Paul1; Schwab, Manfred1
1German Cancer Research Center, Tumour Genetics, Heidelberg, Germany; 2Department of Pediatric Oncology, Center for Molecular Medicine Cologne (CMMIC), University Children's Hospital of Cologne, Cologne, Germany; 3University of Heidelberg, Department of Pathology, Heidelberg, Germany; 4Hoffmann-La Roche Inc, Department of Discovery Chemistry, Nutley, United States
Background: The cell cycle regulator, SKP2, is overexpressed in various cancers, including neuroblastoma, and plays a key role in p27 degradation, which is involved in tumor cell dedifferentiation. Little is known about the mechanisms leading to impaired SKP2 transcriptional control in tumor cells.

Aim: To study SKP2 transcriptional regulation we used neuroblastoma as a model because SKP2 transcript levels gradually increase with aggressiveness of neuroblastoma subtypes.

Methods: We used quantitative realtime RT-PCR to assess the SKP2 transcript levels in neuroblastoma cell lines and tumors. SKP2 promoter deletion/mutation constructs were used to define the regulatory sites in the SKP2 promoter. Chromatin immunoprecipitation (ChIP) with pocket proteins (pRb, p103, p107), activating and repressing E2Fs was used to characterize regulatory protein complexes at the SKP2 promoter.

Results: Highest SKP2 mRNA levels are found in neuroblastomas with amplified MYCN. Accordingly, we found 5.5-fold (range 2.9-5.6) higher SKP2 core promoter activity in MYCN-amplified cells. Higher SKP2 core promoter activity in MYCN-amplified cells is mediated through a defined region at the transcriptional start site (TSSR). This region includes a specific E2F-binding site that makes SKP2 activation largely independent of mitogenic signals. We demonstrate by chromatin immunoprecipitation that SKP2 transcription through the TSSR in MYCN-amplified cells is associated with low abundance of pRB-E2F1 complexes bound to the SKP2 promoter. Transcriptional control of SKP2 via this regulatory mechanism can be re-established in MYCN-amplified cells by restoring pRB activity using selective small compound inhibitors of CDK4.

In contrast, doxorubicin or nutlin-3 treatment - both leading to p53-p21 induction - or CDK2 inhibition had no effect on SKP2 regulation in MYCN-amplified cells.

Conclusion: Together, these imply that deregulated MYCN protein levels in MYCN-amplified neuroblastoma cells activate SKP2 through CDK4 induction, abrogating repressive pRB-E2F1 complexes bound to the SKP2 promoter.

Email: f.westermann@dkfz.de

POT82
DHA is converted to hydroperoxides and potentiates the cytotoxic effect of chemotherapeutics in neuroblastoma
Wickström, Malin1; Gleissem, Helena1; Yang, Rong2; Martinod, Kimberly1; Serhan, Charles N.3; Johnsen, John Inge1; Kogner, Per1
1Karolinska Institutet, Childhood Cancer Research Unit, Department of Woman and Child Health, Stockholm, Sweden; 2Brigham and Women's Hospital and Harvard Medical School, Center for Experimental Therapeutics, Department of Neurosurgery, Boston, United States; 3Division of Woman and Child Health, Stockholm, Sweden

Background: Docosahexaenoic acid (DHA) is an omega-3 polyunsaturated fatty acid that protects neural cells from stress-induced apoptosis and at the same time exerts antiprion properties on neurodegenerative diseases (NB). Here, we examined the metabolic pathway of DHA in NB cells using LC-MS/MS based lipidomics, to elucidate the mechanisms behind its toxicity. In addition, we evaluated combination treatments between DHA and standard chemotherapeutics in NB cells for possible potentiating cytotoxic effects, as a possible clinical application.

Method/approach: NB cells were incubated with DHA, subjected to solid phase extraction, and analyzed with LC-MS/MS for downstream products of the DHA metabolic pathway such as 17-hydroperoxy-DHA (17HpDHA) and 17-hydroxy-DHA (17HDHA), and the newly discovered DHA-generated bioactive products resolvins and protectins, which have anti-inflammatory properties and protective properties. In addition, DHA was added in a fixed, low concentration to different concentrations of ten cytostatic drugs. Cell viability after 72h incubation with the drugs either alone or in combination was measured using a cell viability assay.

Results: NB cells converted DHA to 17HpDHA and further to 17HDHA. The toxicity of the omega-3 fatty acid DHA in neuroblastoma can partly be explained by intracellular conversion to 17HpDHA, an intermediate that can initiate radical reactions and lead to cell death. When combined with chemotherapeutics, DHA potentiated the toxic effects, especially of alkylating agents and the microtubule-stabilizing agent vincristine. The potentiating effects of DHA to several chemotherapeutics may involve increased ROS production and interactions with the glutathione system, DHA alone or in combination with cytostatics may constitute a novel option for neuroblastoma treatment.

Email: malin.wickström@ki.se

POT83
Exploiting cell cycle aberrations in neuroblastoma by targeting checkpoint kinase chk1 using AZD7762 in neuroblastoma with p14ARF/MDM2/p53 defects
Xu, Hong; Wei, Xiao; Cheung, Irene; Cheung, Nai-Kong
Memorial Sloan-Kettering Cancer Center, Pediatrics, New York, United States

Background: AZD7762 is a checkpoint kinase inhibitor currently in clinical trials. In this study, we assessed the potential synergy between AZD7762 and chemotherapeutic agents in the treatment of neuroblastoma (NB) cell lines which harbor cell cycle aberrations because of p14ARF/MDM2/p53 pathway defects.

Method/approach: AZD7762 was tested in vitro and in vivo with chemotherapeutic agents against a representative panel of p14ARF/MDM2/p53 pathway-defective NB cell lines encompassing p53 mutations, MDM2 amplification, or p14ARF deletion, p53/p21 functional assay, cell cytotoxicity, S/G0 checkpoint abrogation, and tumor growth in NB xenografts were monitored.

Results: All four p53 mutant lines failed to show endogenous p21 induction after DNA damage (defined as p53 pathway nonfunctional), two of the three MDM2 amplified lines and one of the three p14ARF deleted lines were p53 nonfunctional as well. While p53 pathway-nonfunctional lines failed to show G0 checkpoint arrest, the remaining S/G1 checkpoint arrests were abrogated by AZD7762 in vitro. In cytotoxicity assays, p53 nonfunctional lines were more resistant to the cytotoxic effects of DNA-damaging agents when compared to p53 functional lines, and synergy between AZD7762 and DNA-damaging agents was strong in all p53 non functional lines. Moreover, AZD7762 treatment abrogated DNA damage-induced S/G0 checkpoint arrest in NB xenografts and potentiated antitumor activity of DNA-damaging agents in these mouse models.

Conclusion: AZD7762 potentiated both the in vitro and in vivo antitumor effects of DNA-damaging agents against NB. These results suggest that the addition of AZD7762 may reverse or prevent drug resistance, especially if cell cycle checkpoint defect is present.

Email: cheungn@mskcc.org

POT84
Preoperative analysis of 11q loss of heterozygosity using circulating tumor-derived DNA in serum for a novel diagnostic tool for therapy stratification of neuroblastoma
Yagyu, Shigeki1; lehara, Tomoko2; Gotoh, Takahito3; Miyachi, Mitsuru3; Katsumi, Yoshiko1; Kikuchi, Ken1; Tsuchiya, Kunihiko3; Osone, Shin'ya4; Kusuda, Hiroshi5; Hossii, Hajime1
1Kyoto Prefectural University of Medicine, Department of Pediatrics, Kyoto, Japan; 2Kyoto City Hospital, Department of Pediatrics, Kyoto, Japan

Background: Because the loss of heterozygosity in the long arm of chromosome 11 (11q LOH) is independently associated with the prognosis of neuroblastoma (NB), routine assessment of 11q LOH status is required for therapy stratification of NB. Here we examined the use of serum DNA, which predominately originates from tumor-derived DNA, for a preoperative, non-invasive assessment of 11q LOH.

Method/approach: We screened serum, primary tumor and nontumor DNA, and primary culture samples from 24 NB patients. The allelic intensity score was calculated with a panel of polymorphic markers located on 11q23 and 11p15.1 to determine the unbalanced 11q LOH, using capillary electrophoresis and PCR with fluorescence-labeled primers. The existence of 11q LOH was confirmed by two-color FISH analysis using primary culture samples.

Results: The allelic intensity score of polymorphic markers in 11q23 in serum DNA was significantly different between 11q LOH-positive group and -negative group. The 11q LOH-positive and -negative group did not overlap when a cut-off value of 0.5 or 2.0 was chosen for the allelic intensity score of the STS marker on 11q23. With these cut-off values, the sensitivity and specificity of the serum-based 11q LOH analysis as a diagnostic test to distinguish 11q LOH-positive and -negative patients were both 100% for our limited number of patients.

Conclusion: Our serum-based 11q LOH analysis could predict 11q LOH in tumors. The analysis is a surgery-free, rapid, sensitive, and specific genetic assessment tool that should help choose risk-adopted therapy preoperatively.

Email: shigeyuki@koto.kpu-m.ac.jp
Neuroblastoma tumor initiating cells express CD22 making them susceptible to HA22 anti-CD22 immunotoxin induced cell death

Methods: NB-TICs and NB cell lines were incubated with different concentrations (0.1-1000ng/ml) of HA22, a recombinant anti-CD22 immunotoxin comprised of a truncated derivative of pseudomonas exotoxin A (PE) engineered to an anti-CD22 Fv fragment. ERB38, a PE immunotoxin targeting ErbB2 served as a negative control and HB21, a PE immunotoxin targeting the transferrin receptor served as a positive control.

Results: After 7 days HA22 induced cell death in NB-TICs to the same extent or better than the positive control HB21: NB78 (HA22IC50= 0.3ng/ml vs. HB21 IC50=0.1ng/ml), NB67 (HA22IC50= 10ng/ml vs. HB21IC50=30ng/ml), NB61 (HA22IC50= 30ng/ml vs. HB21IC50=30ng/ml), NB61 cells express membrane CD22 while less than 1% AS (0.88%) and NB61 cells express membrane CD22 while less than 1% AS (0.88%) and CNKR (0.25%) cells express membrance CD22.

Conclusion: These data indicate that NB-TICs are sensitive to the anti-CD22 immunotoxin HA22. HA22 is in clinical trials for adult and pediatric patients with CD22+ hematologic malignancies. Our study raises the possibility that strategies targeting CD22 may prove to be active against NB.

Email: ct47d@nih.gov

Genotype-guided neuroblastoma therapy, CP751,871 or Rapamycin

Background/Aims: Remarkable heterogeneity in neuroblastoma (NB) genotype regulates different prognosis and drug response. We aimed to develop new strategies targeting mTOR and IGF-1R pathways based on the diversity of NB genotypes. We also investigated the efficacy of CP751,871 and rapamycin on NB tumor initiating cells (TICs) since these cells are responsible for maintenance of the malignant phenotype in NB.

Methods: Both NB cell lines and TICs were used for drug evaluation. CP751,871 and rapamycin, as well as their combination with vinblastine were evaluated both in vitro and in vivo. IGF-1R/mTOR expression was measured by Western blot and RT-PCR. Tumor angiogenesis was evaluated by Von Willebrand Factor immunostaining, and angiogenic factors were screened with Angiogenesis CDNA Microarray.

Results: NB tumors overexpressing IGF-1R was responsive to CP751,871 due to the suppression of tumor angiogenesis instead of cell proliferation. In cells overexpressing IGF-1R, with addition of CP751,871, cytotoxicity of vinblastine was significantly enhanced with decreased IC50 values by 2 - 3.4 times. In xenograft models, both CP751,871 and vinblastine inhibited tumor growth as single agents, while the combined treatment significantly improved antitumor activity (p<0.05). Differential antitumor effect of rapamycin was observed among NB cell lines. NB cells with low mTOR expression are sensitive to mTOR inhibitors, while limited response was observed in mTOR overexpressing cells. Synergistic antitumor effects were obtained by combining rapamycin and vinblastine (p<0.05). Remarkably, we found that NB TICs, NB12 and NBB8R2, are responsive to mTOR inhibitors.

Conclusions: IGF-1R and mTOR expressions could be used as predictive biomarkers for drug response to CP751,871 or rapamycin (Tab. 1). mTOR inhibitors served as a promising antimtumor agent effectively targeting TICs. Adding CP751,871/rapamycin to traditional chemotherapy could result in significant advances in NB treatment.

<table>
<thead>
<tr>
<th>IGR-1R expression</th>
<th>mTOR expression</th>
<th>NB cells</th>
<th>Reactive drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>Low</td>
<td>NUB-7, SH-SY5Y</td>
<td>CP751,871 &amp; Rapamycin</td>
</tr>
<tr>
<td>High</td>
<td>High</td>
<td>SK-N-BE(2), LAN-5</td>
<td>CP751,871</td>
</tr>
<tr>
<td>Low</td>
<td>Low</td>
<td>NB12, NBB8R2</td>
<td>Rapamycin</td>
</tr>
<tr>
<td>Low</td>
<td>High</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

Email: libo.zhang@sickkids.ca
POC1

Neuroblastoma in children-experience in Croatia

Anicic, Mirna; Rajic, Ljubica; Femenic, Ranka; Blicl, Erist; Konja, Josip
University Hospital Center, University Department of Pediatrics, Zagreb, Croatia

In the past decade, considerable modifications in the management of neuroblastoma in children have resulted in continuously rising rate of recovery.

Purpose: To describe the experience in the management of children with neuroblastoma in Croatia, staged and treated at a single institution.

Patients and Methods: Since January 1998 till December 2009, 12 children with neuroblastoma (7 male and 5 female) were treated at Department of Hematology and Oncology, University Department of Pediatrics, University Hospital Center, Zagreb, Croatia. The patients were treated with protocol NB 97 and NB 2004.

Results: Patients were allocated to treatment groups by disease stage. Patients distribution by stage was as follows: stage I (63%), stage II (33%), stage III (25%), stage IV (50%) and stage IV S (50%). MYCN status was investigated in 8 patients (positive in 6). Median of follow up is 28 months.

All patients had surgery (biopsy or partial or complete resection), underwent 2, 4, 6 or 10 cycles of chemotherapy, in high risk also autologous stem cell/bone marrow transplantation, radiotherapy, and in all maintenance therapy (consolidation with cyclophosphamide or 13-cis-retinoic acid).

Results: Remission was achieved in all patients; in 6 (50%) HR) with relapse of disease, highly aggressive cytostatic therapy and radiotherapy were introduced. Four patients (33.33%) died. 8 patients (66.66%) are still alive without disease remission. Three were alive with disease remission but there was no case of secondary malignancies in any of patients.

Conclusion: Better treatment results were achieved in patients with stage I, III and IV S. Combined modality therapy using surgery, chemotherapy, ASCT, radiotherapy and consolidation is optimal treatment for the majority of children with high risk neuroblastoma. New treatment approaches should be promoted for high risk patients.

Email: minaranicc@gmail.com

POC2

Phase I study of single agent perifosine for recurrent pediatric solid tumors

Becker, Oren; Khakoo, Yassin; Modak, Shashikant; Lyden, David; Gilheeney, Stephen; Kolesar, Jeff; Trittany, Yatana; Holland, Eric; Kushner, Brian; Cheung, Nai-Kong; Kramer, Kim; Haque, Sofia; Sima, Camelia; Dunkel, Igor
1MSKCC, Pediatrics, New York, United States; 2University of Wisconsin, Genetics, New York, United States; 3University of Wisconsin, Pharmacology, Madison, United States; 4MSKCC, Cancer Biology and Genetics, New York, United States; 5MSKCC, Radiology, New York, United States; 6China Medical University, Medicine, New York, United States

Background: Perifosine, a synthetic alkylphospholipid, inhibits Akt while also affecting JNK and MAPK signaling pathways. Perifosine is cytotoxic to neuroblastoma and gloma cell lines at uM concentrations. Phase I trials of perifosine in adults have demonstrated responses in patients (pts) with renal cell carcinoma, brain tumors, sarcomas, hepatocellular carcinoma, and hematologic malignancies (e.g. multiple myeloma and Waldenstrom’s macroglobulinemia).

Methods: Pediatric pts less than 18 years of age with recurrent solid tumors were enrolled in a phase I, open-label, dose-escalating study to assess pharmacokinetics (PK) and maximally tolerated dose. Cohorts of 3 pts were treated at three dose levels: (1) 25 mg/m2/day, (2) 50mg/m2/day and (3) 75 mg/m2/day using 50mg tablets, all after a loading dose of 100-200mg/m2/day on D1.

Results: 9 pts (4 male, 5 female) with high-grade glioma (n=5), medulloblastoma (n=2) and stage 4 neuroblastoma (n=2) were enrolled, at a median age of 13 years (range 5-18). Most were heavily pretreated, with a median of 3 prior treatment regimens (range 1-10). No dose limiting toxicities, or ≥ grade 3 toxicities have been encountered to date (CTCAE v3.0). Grade 2 toxicities that were possibly related to perifosine included asthenia (22%), transaminase elevations (22%), neutropenia (33%), leukopenia (11%), hyperglycemia (22%), hypermagnesemia (22%), hypophosphatemia (11%), and coiffs which resolved despite drug continuation (11%). Preliminary PK analyses revealed steady state levels of 1.4 µM at dose level #1, 32.8±8.1 µM at dose level #2, and 31.6±7.8µM at dose level #3. Two patients with stage 4 neuroblastoma were high risk (ages of 4 y and 5 y at diagnosis, bone and marrow metastases, both with recurrent widespread disease, persistent despite salvage chemotherapy and autologous BMT +/- immunotherapy). Both are now clinically well at 9+ and 11+ months, with improved and stable 123I-metaiodobenzylguanidine scans, respectively.

Conclusion: Perifosine is well tolerated in children with advanced solid tumors. Perifosine may have antitumor activity as a single agent in neuroblastoma.

Email: bechero@mskcc.org

POC3

Does the amount of bone marrow disease determinate the outcome of patients with stage 4 neuroblastoma?

Berthold, Frank; Schumacher-Kuckelkorn, Roswitha; Her, Barbara; Simon, Thorsten
University Hospital of Cologne, Pediatric Oncology and Hematology, Cologne, Germany

Background: The methods for detecting bone marrow disease in neuroblastoma patients have been considerably improved during the recent two decades. This study investigates the clinical relevance of the amount of bone marrow infiltration on the outcome of the patients.

Methods: Bone marrow samples of 192 stage 4 neuroblastoma patients (trial NB97, age 0.27 - 21.5 years) were investigated by conventional cytology and by GD2 immunocytoLOGY in one central lab according to international standards. The cytological/ immunocytological amount of bone marrow disease at diagnosis and during treatment was correlated with outcome. For categorization, the results with the highest percentage of inflammation were used for the calculations.

Results: Cytology and immunocytoLOGY results were congruent in 89.8 %. Discrepancies (+/- or -/) were mainly observed in patients with marrow infiltration < 1 %. At diagnosis 15.1 % of patients had 0 % bone marrow infiltration, 15.7 % < 1 %, 23.3 % between 1 and 10 %, 12.3 % between 10 and 30 % and 33.7 % between 30 and 100 % tumor cells in bone marrow. A higher infiltration grade was associated with gradually worsed 5 year event free survival (EFS) and overall survival (OS) (p < 0.001).

Follow-2 cycles of chemotherapy the incidence of patients without residual bone marrow disease increased to 82.4 %. 13.3 % of patients still had < 1 % bone marrow infiltration, and 4.2 % of children between 1 - < 10 %. None had > 10 % residual neuroblastoma cells. The cellular bone marrow response to chemotherapy after 2, 4, 6 cycles had no impact on event free and overall survival. Similarly, neither mIBG response at metastatic sites nor tumor marker response were prognostically informative. Limitation of the current study was the small sample size (n=192) and that the follow up was > 12 and > 18 months of ages at diagnosis yielded the same results.

Conclusion: This study suggests that the outcome of patients with stage 4 neuroblastoma is influenced by the amount of bone marrow disease at diagnosis, but not by the bone marrow disease progression to chemotherapy. It challenges the clinical use of MRD investigations.

Email: frank.berthold@uk-koeln.de

POC4

Outcome of metastatic neuroblastoma treated with multi-modality approach including murine antiangioglsid-2 monoclonal antibody (3F8)

Chen, Godfrey Chi-Fung1; Shing, Matthew Ming-Kong; Li, Rever Chak-Ho2; Luk, Chun-Wing; Ling, Siu-Cheng3; Li, Chi-Kong; Ha, Shau-Yee; Tam, Paul Kong-Hang4
1The University of Hong Kong, Paediatrics & Adolescent Medicine, Hong Kong, China; 2The Chinese University of Hong Kong, Paediatrics & Adolescent Medicine, Hong Kong, China; 3Tuen Mun Hospital, Paediatrics, Hong Kong, China; 4Queen Elizabeth Hospital, Paediatrics, Hong Kong, China; 5Princess Margaret Hospital, Paediatrics, Hong Kong, China; 6The Hong Kong University, Paediatrics & Adolescent Medicine, Hong Kong, China; 7The University of Hong Kong, Surgery, Hong Kong, China

Background: The prognosis of metastatic neuroblastoma remains to be poor despite surgery, intensive chemotherapy and immunotherapy. Immunotherapy with monoclonal antibody appears to provide an additional advantage to the current multi-modality treatment strategy. We previously reported our preliminary results with this approach and would like to present our updated information with longer follow-up.

Methods: This is a single arm prospective study from Jan 1996 to Dec 2007 and the results were compared to our historical control from 1990 when a common data registry was started. Uniform treatment protocol was adopted by 5 public hospitals since 1996. Our treatment was based on a modified N6 & N7 protocol adopted from MSKCC which included intensive chemotherapy + autologous BMT +/- immunotherapy with murine monoclonal anti-angioglsid-2 antibody (3F8, anti-GD2 provided by MSKCC after 1999) for patients with stage 4 neuroblastoma. The analysis of outcome was by Kaplan-Meier analysis.

Results: 61 children (all >12 months) with metastatic neuroblastoma were diagnosed and treated in our centers. The 5yrs OS were 12.5±6.1% and 49.2±10.1% after treated with chemotherapy +/- auto-BMT (n=34) and chemotherapy + auto-BMT + 3F8 (n=27) respectively (p=0.016). The 5 yrs EFS were 10±12% versus 43.5±14% of the 2 groups respectively (p=0.014). Their median survival were 1.8yrs vs. 3.5yrs respectively (p=0.015). The most frequent encountered side effect of 3F8 was transient but severe pain and allergic reaction. The minimal follow-up period was 2.3yrs.

Conclusion: Patients with stage 4 neuroblastoma treated with 3F8 antibody containing regimen seems to have a higher and longer survival as compared to the historical control in using similar regimen without 3F8. Side effects including pain and allergic reactions were manageable in our cohort.

*The project was funded by the Children’s Cancer Foundation Research Fund. We would like to thank Dr. Cheung-NK of MSKCC in assisting us to obtain the 3F8.

Email: gcflchan@hkucc.hku.hk

Abstract Book
Posters

Delebinski, Catharina1; Kemnitz-Hassanin, Kristin1; Schmidt, Ulrike1; Crosazzo Fransconi, Laura1; Faouzi, Mohamed2; Beck-Popovic, Maja1

1University Hospital CHUV, Pediatric Hematology-Oncology Unit, Lausanne, Switzerland; 2University Hospital CHUV, Department of Clinical Pharmacology and Toxicology, Lausanne, Switzerland; 3University Hospital CHUV, Institute of preventive and social medicine, Lausanne, Switzerland.

Background: Urine catecholamines and their metabolites are used for diagnosis and follow-up of patients with neuroblastoma. O-methylated metabolites of catecholamines (plasma free or total metabolites) are the most sensitive tests for diagnosis of phaeochromocytoma. We postulate that neuroblastoma transforms catecholamines into metanephrines which may be more sensitive and specific than urine catecholamines for diagnosis and follow-up of the disease. The aim of the study was to establish reference values for plasma fractionated metanephrines in children and provide preliminary results on their diagnostic values.

Methods: We included into the study 191 healthy children aged 0-17 years needing a venous puncture. Two ml of heparinized Blood were drawn to measure plasma total metanephrines. Additionally, we included ten patients with neuroblastoma to evaluate the diagnostic utility of the test.

Results: Upper reference limit of total plasma normetanephrine (NMN), metanephrine (MN) and methoxytyramine (MT) were: 9.67 nmol/l (95% CI: 8.32-10.1) for NMN, 4.95 nmol/l (95% IC: 4.19-6.05) for MN, 5.8 nmol/l (95% IC: 4.85-7.37) for MT. There was no significant difference between boys and girls. A linear trend gradually decreasing from birth to 17 years was observed for NMN and MT which was statistically significant for both. Plasma concentration of MN showed a normal relationship to age. In patients with neuroblastoma, plasma total MT and NMN measurements were highly sensitive and specific at diagnosis. The highest sensitivity and specificity were observed at 9.43 nmol/l (ODds Ratio 1.8, CI 1.32-2.46) for MT. There was no significant difference between patients with different histologic subtypes. The highest sensitivity and specificity (100% and 99.48%, respectively) were achieved for plasma total NMN with a cutoff at 19.64 nmol/l (ODds Ratio 1.18, CI 1.06-1.31) and for plasma total MT (sensitivity 100% and specificity 98.95%) with a cutoff at 9.43 nmol/l (ODds Ratio 1.8, CI 1.32-3.48).

Conclusions: The preliminary results provide diagnostic reference limits for total plasma metanephrines and suggest a promising role in diagnosis and follow-up of neuroblastoma patients. Further data are necessary to confirm the validity.

Email: Maja.Beck-Popovic@chuv.ch

POC6

Apopotic and adjuvant effects of triterpene-containing Viscum album L. (mistletoe) extract on neuroblastoma (NB) cells

Delebinski, Catharina1; Kemnitz-Hassanin, Kristin1; Schmidt, Ulinka1; Jäger, Sebastian1; Lode, Holger2; Seifert, Georg2

1Charité, Universitätmedizin Berlin, Department of Pediatric Oncology/ Hematology, Otto-Heubner-Center for Pediatric and Adolescent Medicine (OHC), Berlin, Germany; 2Carl Gustav Carus Institut, Department of Cancer Therapy, Niemann-Oschilbrom, Germany; 3University of Greifswald, Department of Pediatric Hematology and Oncology, Greifswald, Germany; 4Universitätsmedizin Charité Berlin, Department of Pediatric Oncology/ Hematology, Otto-Heubner-Center for Pediatric and Adolescent Medicine (OHC), Berlin, Germany.

Viscum album L. (mistletoe) is one of the most widely used complementary cancer therapies. Differential effects of defined lectin and triterpene containing mistletoe extracts on neuroblastoma (NB) are largely unknown.

In the present study, we determined for the first time the effect of clearly defined mistletoe extracts, containing either lectins (aqueous extract) or triterpenes (STE) such as oleanolic - and betulinic acid and combinations thereof (viscum TT) against NB in vitro and in vivo.

For this purpose, we used the well established syngeneic NX2 mouse model and tested efficacy and mechanisms of the treatment with these preparations in vitro and in vivo. NX2 neuroblastoma cells were incubated with increasing concentrations of mistletoe preparations and tested for their cytotoxicity in vitro. Apoptosis was determined using mitochondrial potential, DNA fragmentation and Annexin/V assays. In vivo, we used the NXS2 neuroblastoma mouse model. For this purpose, 1x106 murine NXS2 cells were injected s.c. into groups of syngeneic A/J mice (n=6) and STE extracts were administered three times per week for 4 days by intraperitoneal injection. After removal of primary tumors and continuing treatment for two weeks, the level of spontaneous liver and lymph node metastasis was analyzed by measuring the weights of affected organs. We could demonstrate that Viscum album L. extracts inhibited cell proliferation and show cytotoxic properties in vitro. The highest level of apoptosis with a decrease of the mitochondrial potential was observed with STE preparation at a concentration of 60µg/ml (IC50), whereas we detected only a moderate effect in lectin-treated cells (IC50).

Based on these data, we investigated the effect of STE extract on the level of spontaneous NB metastases in vivo. For this purpose 60mg/kg oleanolic acid were administered. Intraperitoneally treated mice treated with triterpenes showed a significant decrease of spontaneous metastases in contrast to control groups. In conclusion, we believe that triterpene-containing preparations may have a potential to provide a promising approach for the adjuvant treatment of neuroblastoma.

Email: catharina.delebinski@charite.de

POC7

Phase I study of vincristine, irinotecan, and 131I-MIBG for patients with relapsed or refractory neuroblastoma: A new approach to neuroblastoma therapy consortium study

DuBois, Steven1; Chester, Louis1; Grouseh, Susan2; Hawkins, Randall2; Jackson, Hollie2; Daldrup-Link, Heike2; Yanik, Greg2; Stewart, Clinton2; Mosse, Yael1; Mari5; John5; Jäger2; Lode3; Seifert, Georg4

1University of Southern California, Department of Pediatrics, Los Angeles, United States; 2University of California, Davis Medical Center, Section of Paediatric Oncology, London, United Kingdom; 3University of Southern California, Department of Biostatistics, Los Angeles, United States; 4UCSF School of Medicine, Department of Pediatric, San Francisco, United States; 5Institute of Cancer Research, Section of Paediatric Oncology, London, United Kingdom.

Background: Urine catecholamines and their metabolites are used for diagnosis and follow-up of patients with neuroblastoma. O-methylated metabolites of catecholamines (plasma free or total metabolites) are the most sensitive tests for diagnosis of phaeochromocytoma. We postulate that neuroblastoma transforms catecholamines into metanephrines which may be more sensitive and specific than urine catecholamines for diagnosis and follow-up of the disease. The aim of the study was to establish reference values for plasma fractionated metanephrines in children and provide preliminary results on their diagnostic values.

Methods: We included into the study 191 healthy children aged 0-17 years needing a venous puncture. Two ml of heparinized Blood were drawn to measure plasma total metanephrines. Additionally, we included ten patients with neuroblastoma to evaluate the diagnostic utility of the test.

Results: Upper reference limit of total plasma normetanephrine (NMN), metanephrine (MN) and methoxytyramine (MT) were: 9.67 nmol/l (95% CI: 8.32-10.1) for NMN, 4.95 nmol/l (95% IC: 4.19-6.05) for MN, 5.8 nmol/l (95% IC: 4.85-7.37) for MT. There was no significant difference between boys and girls. A linear trend gradually decreasing from birth to 17 years was observed for NMN and MT which was statistically significant for both. Plasma concentration of MN showed a normal relationship to age. In patients with neuroblastoma, plasma total MT and NMN measurements were highly sensitive and specific at diagnosis. The highest sensitivity and specificity were observed at 9.43 nmol/l (ODds Ratio 1.8, CI 1.32-2.46) for MT. There was no significant difference between patients with different histologic subtypes. The highest sensitivity and specificity (100% and 99.48%, respectively) were achieved for plasma total NMN with a cutoff at 19.64 nmol/l (ODds Ratio 1.18, CI 1.06-1.31) and for plasma total MT (sensitivity 100% and specificity 98.95%) with a cutoff at 9.43 nmol/l (ODds Ratio 1.8, CI 1.32-3.48).

Conclusions: The preliminary results provide diagnostic reference limits for total plasma metanephrines and suggest a promising role in diagnosis and follow-up of neuroblastoma patients. Further data are necessary to confirm the validity.

Email: Maja.Beck-Popovic@chuv.ch
POC9
Geftinib (GFB) and Irinotecan (IRN) for children with high-risk (HR) neuroblastoma
Furman, Wayne1; McGregor, Lisa1; Stewart, Clinton1; Oncu, Mihaiela1; Kovach, Sandy2; Davidoff, Andrew3; Santana, Victor1
1St. Jude Children’s Research Hospital, Oncology, Memphis, United States; 2St. Jude Children’s Research Hospital, Pharmacological Sciences, Memphis, United States; 3St. Jude Children’s Research Hospital, Pathology, Memphis, United States; 4St. Jude Children’s Research Hospital, Surgery, Memphis, United States

GFB, an oral EGFR and ABC transport receptor inhibitor has been shown to be a potent inhibitor of NB cell proliferation in vitro (Brodeur et al Ca Res, 2005) and in combination with IRN have greater than additive activity against NB xenografts (Stewart et al, Ca Res, 2004). For these reasons we evaluated the combination of GFB and IRN in newly diagnosed children with HR NB.

Aims: The primary objective was to estimate the response rate to two courses of intravenous IRN (daily low dose) at a dose of 15mg/m2/day combined with 112.5mg/m2/day of oral GFB for 10 days in untreated children with HR NB.

Patients and Methods: 23 children were enrolled and 20 were evaluable for the primary endpoint. Pharmacokinetic studies for IRN and metabolites were done in consenting patients.

Results: The median age at study enrollment was 3.15 years (range, 18 days - 12.67 years). Most were < 24 months (n=20; 87%), male (n=18; 78%), while (n=16; 70%), had INSS 4 disease (n=19; 83%), had adrenal primaries (n=18; 78%) and 9/23 had amplified MYCN tumors. Three patients did not receive the IRN/GFB therapy: one had the primary tumor resected prior to enrollment and thus had no primary disease evaluable, one had spinal cord compression at study enrollment and one was too young (age 18 days old at study enrollment). Toxicity associated with IRN/GFB was evaluable in all 20 patients who received this therapy. Common non-dose limiting toxicities ascribed to the combination included nausea (5/20), diarreal (4/20) and vomiting (3/20). F ineligible patients had partial responses and 9 others had between 11-60% decrease in primary tumor volume and/or improved MIBG, improved tumor status in the bone marrow, decreased pain and improvement in performance activities. Median (range) IRN and SN-38 AUC values were 282 ng/m2/hr (162 to 889 ng/m2/hr) and 28 ng/ml/hr (3.6 to 297 ng/ml/hr), respectively. Expression of EGFFR, MRPR-2, BCRP and Pgp, in tumor samples did not correlate with antitumor activity.

Conclusions: Although the combination of IRN/GFB was very tolerable in these children and there were clinical responses, sufficient additive activity was not observed to warrant further investigation.

Email: wayne.furman@stjude.org

POC10
Does tumor histology after induction therapy predict outcome in patients with high-risk neuroblastoma?
George, Ran E1; Perez-Alayde, Antoni02; Yao, Xiaopan3; London, Wendy B.1; Shimberger, Robert C.3; Diller, Lisa1
1Dana-Farber Cancer Institute, Harvard Medical School, Department of Pediatric Oncology, Boston, MA, United States; 2Children’s Hospital Boston, Harvard Medical School, Department of Pathology, Boston, MA, United States; 3Children’s Hospital Boston/Dana-Farber Cancer Institute, Division of Hematology/Oncology, Boston, MA, United States; 4Children’s Hospital Boston, Harvard Medical School, Department of Surgery, Boston, MA, United States

Background: Histopathology at diagnosis predicts outcome in patients with neuroblastoma (NB). The aim of our study was to determine whether histopathological response to induction chemotherapy is useful in predicting outcome in patients with high-risk NB.

Method: Newly diagnosed high-risk NB patients treated at our institution between 1994 and 2002 for whom tumor material was available were characterized as follows: stroma-poor tumors: 94% (D), 75% (R); minimal neuropil: 6% (D), 19% (R); differentiating tumors: 6% (D), 81% (R); >10% tumor necrosis: 37% (D), 56% (R). Histological features in 32 resection specimens were: stroma poor: 23/32 (73%); intermediate/high MKI: 56% (D), 6% (R); minimal neuropil: 69% (D), 19% (R); differentiating tumors: 6% (D), 81% (R); >10% tumor necrosis: 37% (D), 56% (R). Functional studies.

Results: Specimens from 43 patients were analyzed. Eleven patients had available specimens at both diagnosis (D) and resection (R) which were characterized as follows: stroma-poor tumors: 94% (D), 75% (R); intermediate/high MKI: 56% (D), 6% (R); minimal neuropil: 69% (D), 19% (R); differentiating tumors: 6% (D), 81% (R); >10% tumor necrosis: 37% (D), 56% (R). Histological features in 32 resection specimens were: stroma poor: 23/32 (72%); high/intermediate MKI: 2/32 (6%); differentiating tumors: 21/32 (66%); minimal neuropil: 10/32 (31%); >10% necrosis: 23/32 (72%) and 14/32 (44%) with ganglioneuromatous elements. At resection, intermediate/high MKI and >90% viable tumor (compared to <10%) were significantly predictive of poor OS and DFS (both p<0.05). At resection, there was a trend towards a lower survival for patients with <10% tumor necrosis (OS p=0.09, DFS p=0.08).

Conclusion: High proliferative tumor activity following induction therapy portends a poor outcome in patients with high-risk NB. If confirmed in a larger cohort, these patients may benefit from further stratification to more intensive therapy.

Email: rani_george@dfci.harvard.edu

POC11
Aromatic hydrocarbon receptor down-regulates MYCN expression and promotes neuronal differentiation of neuroblastoma
Hsu, Wen-Ming1; Wu, Pei-Yu1; Juan, Hsueh-Fen1; Lee, HsinYu2
1National Taiwan University Hospital and National Taiwan University College of Medicine, Department of Surgery, Taipei, Taiwan; 2Institute of Zoology, National Taiwan University, Department of Life Science, Taipei, Taiwan

Background/Aims: MYCN amplification is an adverse prognostic factor of neuroblastoma (NB). However, how MYCN expression is regulated in NB cells remains unclear. This study aims at defining the machineries to regulate MYCN expression in NB cells.

Methods: Ten MYCN amplified and 10 MYCN non-amplified NB tumors were subjected to oligonucleotide microarray analysis. Signaling pathways related to MYCN expression were analyzed by IPA. The relationship between genes with the highest score in the pathway analysis and MYCN expression were further evaluated and verified in 85 NB tumor samples by quantitative PCR and immunohistochemistry as well as in NB cell lines by functional studies.

Results: IPA analysis revealed that aromatic hydrocarbon receptor (AHR) had the highest score to be reversely related to MYCN expression. AHR expression in NB tumor cells was well with histological grade of differentiation but reversely correlated with advanced disease stages and MYCN amplification. Positive AHR immunostaining predicted a favorable prognosis in NB patients independent of other prognostic factors. Ecotop expression of AHR in SK-N-DZ cells promoted neuronal differentiation by directly inhibiting of MYCN promoter activity with the cooperation of E2F1 transcription factor.

Conclusion: AHR may negatively regulate MYCN expression and promotes NB cell differentiation in vivo and in vitro. Further study of the role of AHR expression in NB may not only shed light to the tumorigenesis but also the novel targeted therapy of NB.

Email: d96b41003@ntu.edu.tw

POC12
Results of treatment strategy of stage 4 infantile neuroblastoma based on born metastasis and MYCN amplification
Iehara, Tomoko1; Hamazaki, Minoru2; Tanaka, Takero1; Hosoi, Hajime1; Tajiri, Tatsuro4; Kaneko, Michio5; Sugimoto, Tohru1; Sawada, Tadashi1
1Kyoto Prefectural University of Medicine, Pediatrics, Kyoto, Japan; 2Hiroshima-nishi Medical Center, Pediatrics, Hiroshima, Japan; 3Kyuushu University, Pediatric Surgery, Fukuoka, Japan; 4University of Tsukuba, Pediatric Surgery, Tsukuba, Japan

Background: Subgroups of infants in stage 4 neuroblastoma showed heterogeneous. The purpose is to investigate the validity of treatment of the infants in stage 4 neuroblastoma based on born metastasis and MYCN amplification (MNA).

Patients and Methods: Fifty seven stage 4 infants aged <1 year were treated in the Japanese prospective Study from 1994 to 2004. Patients with MNA, classified into a high risk group, were taken most intensive treatment. Patients without MNA were assigned chemotherapy by the presence of the bone metastasis.

Results: The number of stage 4 neuroblastoma patients with MNA is 12. Over all survival rate was poor in cases with MNA (5-year-OS 40.0%) than in cases without MNA (5-year-OS 95.0%, p<0.001). In cases without MNA, the number of patients with bone metastases is 23. In cases without MNA, over all survival rate was better in cases without bone metastases (5-year-OS 100%) than in cases with bone metastases (5-year-OS 85.1%, p<0.05). On the other hand, the number of patients with and without bone marrow metastases was 19 and 22. The overall survival rates between the patients with and without bone marrow metastases were almost equal (5-year-OS 93.8% and 95.7% respectively, p=0.94).

Conclusion: The infants without MNA and bone metastases have excellent prognosis. Therapy reduction for these patients is possible. For patients with MNA or bone metastases, new therapeutic approaches are needed.

Email: iehara@koto.kpu-m.ac.jp
POC13
Irwin, Meredith S.1; Naranjo, Arlene1; Altijeh, Edward P2; Seeger, Robert3; Asgharzadeh, Shahar; Spisot, Richard4; Jr., Lingyun; Yanik, Gregory5; Moss, Yael P1; Marx, John M1; Park, Julia R2; London, Wendy5; Kreisman, Susan5; Hogathy, Michael D.3
1Hospital for Sick Children, Pediatrics/ Hematology-Oncology, Toronto, Canada; 2Children's Oncology Group, University of Florida, Gainesville, United States; 3Children's Hospital of Philadelphia, Pediatric Oncology, Philadelphia, United States; 4Children's Hospital Los Angeles, Hematology-Oncology, Los Angeles, United States; 5University of Michigan, Pediatric Cancer, Ann Arbor, Michigan, United States; 6Children's Hospital of Philadelphia, Oncology, Philadelphia, United States; 7Children's Hospital of Philadelphia, Oncology, Seattle, United States; 8Children's Oncology Group, Dana-Farber Cancer Institute, Boston, United States; 9Duke University Medical Center, Hematology-Oncology, Durham, United States

Background: Current prognostic factors do not predict treatment failure for high-risk (HR) NB patients. The goal of the COG CBRF Task Force was to identify factors at diagnosis or during therapy (response-based) that define an Ultra-High-Risk (UHR) group for whom upfront novel therapies may be justified.

Method/approach: We defined UHR as including >10% of HR patients with an EFS<15%. We performed univariate analyses to identify an HR UHR group, using COG A3973 HR study population (N=487) to control for therapy received. Candidate factors analyzed were: stage, age, MYCN status, specific genomic alterations (whole-genome SNP), RT-PCR based expression profile, and MIBG scores; and (2) response-based: bone marrow (BM) clearance by histoogy, PBSC MIBG score, and quantitative MIBG scores. Radiotherapy end-induction predicted an UHR group (13% of A3973 patients with a 3-y EFS<3.4±6%; Yanik, ANR 2010). A 14-gene signature applied to non-amplified NBs predicted an HR UHR group of >40% of patients enrolled on CCG, POH and COG trials with EFS<14±4%, 14±8% and 22±8%, respectively (Asgharzadeh, ANR 2010). Detection of MYCN on PBSC was also predictive of poor outcome, but did not define an EFS<20% (Seeger, ANR 2010). As single variables neither age, stage, initial MIBG score, MYCN status or BM clearance defined an UHR group.

Results: Detection of MYCN on PBSC was also predictive of poor outcome, but did not define an EFS<20% (Seeger, ANR 2010). As single variables neither age, stage, initial MIBG score, MYCN status or BM clearance defined an UHR group.

Conclusion: In addition to progressive disease, MIBG score at end-induction and a multi-gene tumor signature at diagnosis predict an UHR group. We plan to identify UHR patients prospectively within COG HR trials using MIBG response and perhaps other biomarkers and test novel therapies in this group with predicted dismal outcome.

Email: meredith.irwin@sickkids.ca

POC14
False negative studies of neuroblastoma metastatic to the central nervous system (CNS) from initial surgery
Kramer, Kim1; Kushner, Brian H2; Modak, Shakeel3; Khakoo, Yasmin1; Pandit-Taskar, Neeta1; Stambuk, Hilda4; Souweidane, Mark M5; Cheung, Nai-Kong1
1Memorial Sloan-Kettering Cancer Center, Pediatrics, New York, United States; 2Memorial Sloan-Kettering Cancer Center, Radiology, New York, United States; 3Memorial Sloan-Kettering Cancer Center, Neurosurgery, New York, United States

Purpose: CNS NB was once considered rare and lethal. Although the outlook for these patients (pts) has improved with a salvage regimen incorporating intrathecal targeted radioimmunotherapy (RIT) (J Neuro Oncol 2009), early detection may reduce mortality. We present an analysis of the largest series of pts with CNS NB.

Patients and Methods: As a referral center for RIT, we assessed the clinical, biologic and radiographic features in 61 pts evaluated for CNS NB at MSKCC since 2000. 34 pts had MYCN amplified disease, 9 had a lumbar puncture at initial diagnosis, known risk factors.

Results: 59 of 61 pts had confirmed CNS NB, 47 by pathology and 12 radiographically (multiple enhancing parenchymal lesions and/or nodular leptomeningeal spread). 2 pts had neurosurgery for isolated enhancing lesions on routine MR found to be gliosis and necrosis, but not NB. 2 pts had CNS disease at initial NB presentation, both with headaches and high intracranial pressure requiring shunts. The other 57 pts had CNS NB at 5-61 months (median 21.7) from diagnosis, median 18.5 months in the MYCN amplified cohort. 23 pts (41%) were asymptomatic, with CNS NB detected on routine MIBG (6), CT head (8), MR brain (8) or MR spine (3). CNS NB was undetectable in 31 of 37 (84%) CSF analyses. Presenting symptoms in 38 pts included headache (+/- vomiting in 15 (25%), seizures in 6 (10%), gait disturbance in 6 (10%), altered mental status in 5 (8.5%), visual disturbance in 2 (3%), facial palsy in 1 (<2%) and depression in 1 (<2%). 40 pts (68%) had isolated CNS relapse including 26 (44%) with a single parenchymal focus. Leptomeningeal spread occurred in 19 (32%), the remainder having multifocal disease.

Conclusions: As the natural history of CNS NB has changed, so too has the clinical evaluation and management. Isolated CNS relapse remains a complication for pts with high-risk NB. Given the false negative rates of routine studies, periodic CNS MR imaging in high-risk patients is indicated. Routine screening detects >40% of asymptomatic lesions. Biopsy to confirm disease is mandatory for isolated non-MIBG avid lesions.

Email: kramerk@mskcc.org

POC15
Evolution of treatment strategies and risk stratification in management of neuroblastoma over two decades at tertiary cancer centre in India
Kurkure, Purna1; Vora, Tushar2; Arora, Bhijeesh3; Banavali, Sripad4; Qureshi, saiji5; Laskar, Siddharth1; Mukkaden, Maryann5; Seethalaxmi, V6; Ramadwar, Mukta1; Desai, Sangeeta7; Medhi, Seema8; Rajan, MGR9; Nahar, Akash10; Bahi, Gaurav11; Gulla, Seema12; 1Tata Memorial hospital, Pediatric Oncology, Mumbai, India; 2Tata Memorial hospital, Pediatric Surgery, Mumbai, India; 3Tata Memorial Hospital, Cancer, Mumbai, India; 4Tata Memorial hospital, Pediatric Surgery, Mumbai, India; 5Tata Memorial hospital, Molecular Pathology, Mumbai, India; 6Tata Memorial hospital, Radiation Oncology, Mumbai, India; 7Radiation Medicine centre, Nuclear Medicine, Mumbai, India

Background: With increasing amenities of risk stratification and treatment optimisation of NB over three distinct era, we present our observations and lessons learnt in the evolution of treatment strategies over two decades at Tata Memorial Hospital(TMH), Mumbai, India.

Methods: Retrospective analysis of patients with NB presenting at TMH between 1987-2008 was performed and has been reported.


In 128 children of neuroblastoma in first era(1987-2000); only age(<1y r v/s >1yr ) and stage were considered for risk stratification.

Access to optimal surgery for gross total resection (GTR) , ABMT & 13 Cis retinoic Acid (13 Cis RA) was limited.I131 MIBG imaging was introduced in diagnostic armamentarium in second half.DFS for 1 yr was 19%.In second era (2000-2004) 51 children were evaluable.Besides age(18months) & stage,histopathology was incorporated in appropriate cases for risk stratification.

Access to optimal surgery (GTR),ABMT & 13 Cis RA was still limited. However induction chemotherapy was more dose intense and MIBG therapy was introduced. DFS for 18 months is 20%.In third era (2004-2008) 62 children were evaluable. Optimal surgery (GTR) & 13 Cis RA were routinely employed in treatment strategy ; however ABMT was done occasionally due to cost constrains.MIBG therapy was more used. New techniques employed by FISH has been introduced in the risk stratification.OS for this group is 65% with 18 months median follow up.

Conclusion: As risk stratification improves treatment gets optimised. Improved survival can be obtained in low and intermediate risk group accounting for approximately 60% of children with NB.Management of High risk NB remains a challenge in cost constrained environment.

Email: purna.kurkure@gmail.com
**POC16**

**High-dose cyclophosphamide (Cy)-irinotecan (CPT-11)-vincristine (VCR) (HD-CCV) for primary refractory neuroblastoma child.**

Kushner, Brian; Kramer, Kim; Modak, Shakeel; Yataghene, Karima; Cheung, Nai-Kong

Memorial Sloan-Kettering Cancer Center, Pediatrics, New York, United States

**Background:** HD-CCV is a novel regimen for NB resistant to standard chemotherapy which now includes irinotecan in either induction or salvage therapy.

**Patients and methods:** We retrospectively studied 37 unselected patients (pts) treated with HD-CCV for high-risk stage 4 NB that responded incompletely to induction, but had never progressed. Their prior therapy included topotecan and 32 pts (86%) had already received 2nd-line chemotherapy. Treatment was outpatient and comprised high-dose Cy (140 mg/kg)-CPT-11 (250 mg/m2)-VCR (0.067 mg/kg). Pts received a 2nd course if the 1st showed anti-NB activity but assessable disease remained. Response was scored by international criteria, including extensive bone marrow evaluations and MIBG scan. The overall response rate was 49%.

**Results:** 21 pts (57%) had a CR and 11 pts (30%) had a PR. The remaining 5 pts (14%) had NR. 20 pts remain progression-free at 2+-to-40+ (median, 11+) months, including 9 in 1st CR/VGPR at 6+ to 32+ (median, 14+) months after HD-CCV therapy. The latter depended on response to HD-CCV and included 3F8, 13-cis-RA, targeted radiotherapy using 131I-3F8, and/or chemotherapy. PFS was 59% (SE=10%) at 24 months and 25% (SE=19%) at 36 months.

**Conclusion:** HD-CCV is an active treatment option against topotecan-resistant NB. The results suggest that CPT-11 may synergize with high-dose alkylators.

Email: kushnetb@mskcc.org

**POC18**

**Illness experience and factors that constitute resilience in families with a neuroblastoma child.**

Lee, Ya-Ling; Chen, Tsu-Chun1; Hsu, Wen-Ming2

1National Taiwan University, Department of Nursing, Taipei, Taiwan; 2Cardinal Tien College of Health & Management, Department of Nursing, Taipei, Taiwan

**Background:** Neuroblastoma (NBL) is a cancer of the sympathetic nervous system. It manifests as a malignant tumor that originates in the adrenal glands, neck, chest, abdomen, or pelvis. Neuroblastoma affects very young children, and survival rate in the highest-risk group is about 30%. Aim: The purpose of this qualitative study was to explore the illness experience and treatment process of families with a high-risk group NBL child.

**Methods:** A purposive sample of eight caregivers was selected for in-depth interviews and participant observation. Data were analyzed and integrated into seven themes using the Giorgi (1997) phenomenological analysis method.

**Results:** 1. The results of this study show that the experiences of families of children undergoing NBL treatment can be classified by 2 categories: sequence of treatment course and utility of resources. The former includes shock and impact experienced when the illness is first discovered, the uncertainty of the characterization of this disease, the experiences related to the treatment course and the learning process, and the responses after treatment courses. The later includes support derived from family cohesion, family resilience, and assistance from the health care team and social resources. 2. Ten resilience factors were derived from the results to support family function: courage, life style adjustment, learning, positive outlook, spirituality, comparison with other families, family communication, support, expectation adjustment, and collaboration in family members. The results show that family resilience exists in every family, that is, every family has potential ability to ask for assistance and adjust itself to function as well as it can. 3. Resilience factors can be derived as a guide to family centered care and the development of family resilience to aid families cope with medical treatment processes.

Email: yll.always@gmail.com

**POC17**

**A proposal for antibody based immunotherapy combined with haploidentical stem cell transplantation for high risk neuroblastoma.**

Lang, Patricia; Pfliber, Matthew; Ingo1; Heiko Manuel, Teltzschik; Feuchtinger, Tobias; Schwarz, Philipp; Handgretinger, Rupert

1Children's University Hospital, Hematology/Oncology, Tuebingen, Germany; 2University Children's Hospital, Hematology/Oncology, Vienna, Austria; 3University Children's Hospital, Hematology/Oncology, Greifswald, Germany; 4University Children's Hospital, Hematology/Oncology, Tuebingen, Tuebingen, Germany

**Background:** Pediatric patients with relapsed solid tumors have a poor prognosis and additional therapeutic strategies are warranted. We present preliminary results of our institution with haploidentical stem cell transplantation (SCT) in high risk neuroblastoma (NB) patients.

**Patients and methods:** Pediatric patients with NB who were treated with CPT-11-containing induction therapy in 5 pts, achieving 1 CR, 1 MR, 3 stable disease (ND). All 5 pts previously treated with CPT-11-temozolomide had responses (2 PR, 3 MR) to HD-CCV. In contrast, all 6 pts with incomplete responders to HD-CCV had NR. 20 pts remain progression-free at 2–40+ (median, 11+) months, including 9 in 1st CR/VGPR at 6+ to 32+ months (median, 14+) months after HD-CCV therapy. The latter depended on response to HD-CCV and included 3F8, 13-cis-RA, targeted radiotherapy using 131I-3F8, and/or chemotherapy. FFS was 59% (SE=10%) at 24 months and 25% (SE=19%) at 36 months.

**Conclusion:** HD-CCV is an active treatment option against topotecan-resistant NB. The results suggest that CPT-11 may synergize with high-dose alkylators.

Email: kushnetb@mskcc.org

**POC19**

**The impact of a multidisciplinary team approach in the case management of neuroblastoma.**

Liu, Yan-Lin; Hsu, Wen-Ming; Chang, Hsiu-Hao; Lin, Dong-Tsamin; Lin, Kai-Hsin1; Jou, Shiang-Tarng; Lu, Meng-Yao; Yang, Yung-Li; Tzen, Kai-Yuan; Peng, Steven Hsin-Feng; Huang, Shiu-Feng; Lee, Ya-Ling1

1National Taiwan University Hospital, Department of Pediatrics, Taipei, Taiwan; 2National Taiwan University Hospital, Department of Surgery, Taipei, Taiwan; 3National Taiwan University Hospital, Department of Laboratory Medicine, Taipei, Taiwan; 4National Taiwan University Hospital, Department of Nuclear Medicine, Taipei, Taiwan; 5National Taiwan University Hospital, Department of Radiology, Taipei, Taiwan; 6National Health Research Institutes, Division of Molecular and Genomic Medicine, MiaoL, Taiwan; 7National Taiwan University College of Medicine, Department of Nursing, Taipei, Taiwan

**Background:** Protocol-based therapy of neuroblastoma had been adopted in our hospital, a 2000-bed tertiary medical center, for decades. In 2002, we incorporated a new protocol, TPOG-N2002, modified from the United States Children’s Oncology Group (COG) protocol with high-risk chemotherapy, radiotherapy, and autologous stem cell transplantation or continuing intensive chemotherapy per parental decision.

**Method:** To improve the quality of care in children with neuroblastoma, a multidisciplinary team consisting of specialists in pediatric oncology, pediatric surgery, radiation oncology, pediatric nursing, radiology, and nuclear medicine was organized in our institution in 2007. The demographic data, treatment adherence, surgical resectability, complications, and treatment outcome of patients before and after this approach were analyzed.

**Results:** Since 2002, total 54 patients (38 boys and 16 girls) with neuroblastoma have been treated at our institution, with 46 (85%) of them received the N2002 protocol as frontline therapy. Among them, 35 (65%) were diagnosed during 2002-2007 (group 1), and the other 19 (35%) were diagnosed during 2008-2009 (group 2). The mean age (3.52 vs. 3.01), sex, staging, and MYCN amplification rate at diagnosis between the two groups were not significantly different. In group 1, 17 (55.6%) of the 19 children to recommended treatment courses was almost 100% after diagnosis at our institution; the gross total response rate was not different from that in group 1 (44% vs. 52%; p=0.694); and there was a trend toward lower rate of nonresponders (17% vs. 30%) in group 2. The 5-year overall survival and progression-free survival approach reached 76.9% and 70.3% in group 2, in comparison to those of 68.6% and 45.4% in group 1 (p=0.550 and 0.9161, respectively).

**Conclusion:** A case management program using a multidisciplinary team approach might have improved quality of care and provided a management model for children with neuroblastoma. The findings of outcome survey need further follow-up to confirm benefits.
POC20 Second stem cell transplantation for relapsed high-risk neuroblastoma in Japan
Matsumoto, Kimikazu1; Kato, Koji2; The Stem Cell Transplantation Committee
1Japanese Red Cross Nagoya Daiichi Hospital, Children’s Medical Center, Division of Hematology and Oncology, Nagoya, Japan; 2Japanese Society of Pediatric Hematology, Tokyo, Japan
Background: We have previously presented that total body irradiation (TBI) based preparative regimen for autologous stem cell transplantation (SCT) of advanced neuroblastoma has significant superiority in event-free survival (EFS). There are few reports concerning about preparative regimen and transplantation source for second SCT after relapse of advanced neuroblastoma.
Patients/Method: 116 patients who achieved second SCT between 1995 and 2005 were analyzed retrospectively based on the transplantation registry of Japanese Society of Pediatric Hematology. We excluded 66 patients with planned tandem transplantation, 3 transplanted for graft failure and 5 without detailed information. Finally 42 patients with relapsed neuroblastoma were analyzed.
Results: EFS after second transplantation at 3 and 5 years were 16.7±6.0% and 5.64±7.7% respectively. Three (one with autologous SCT and two with autologous SCT) out of 42 patients survived without disease after second SCT for 1492, 555 and 449 days. 3-year-EFS of 11 patients with TBI containing regimen was 18.2±11.6%, and EFS of 25 without TBI was 19.7±6.6% (p=0.896). Allogeneic transplantation for the second SCT was performed for 15 patients with 3-year-OS of 0.0±0.0%, which is significantly lower survival rate compared with 24.4±18.0% for autologous SCT (p=0.003). Fourteen out of 15 allogeneic transplantation died of disease relapse (n=12), respiratory failure (n=1; non TBI) and multi-organ failure (n=1; TBI). Twenty-four out of 27 autologous SCT died of relapse (n=21), respiratory failure (n=1; non TBI) and unknown cause (n=2; non TBI). We compared 42 secondary transplanted patients with one hundred one patients who did not perform second SCT after relapse (CHEMO). Five-year-over-all survival (OS) was 8.4±7.4% for SCT and 16.0±4.5% for CHEMO. 8 year-OS was 0.0±0.0% and 12.0±4.1% respectively (p=0.006).
Conclusion: TBI based preparative regimen does not have significant superiority in EFS for second SCT of relapsed neuroblastoma. For second SCT, allogeneic SCT does not seem to have better survival than autologous SCT, so it might be possible that autologous-Tumor effect would not work properly after second SCT for relapsed neuroblastoma.
Email: kmatsumo@nagoya-1st.jrc.or.jp

POC21 Role of minimal access surgery in children affected by neuroblastoma
Mattioni, Girolamo1; Avanzini, Stefano1; Buffa, Piero1; Michelazzi, Alberto1; Garaventa, Alberto1; Conte, Massimo1; Jazollo, Bruno1; Viale, Leonardo1; 1Gianna Gaslini Children Hospital, Pediatric Oncology, Genoa, Italy
Background: Minimal Access Surgery (MAS) is one of the cornerstones in the fast-track organization model and is nowadays applied to almost all pediatric surgical fields. This study presents possible MAS approaches in a series of pediatric patients affected by neuroblastoma.
Method/approach: This 2-year (2008-2009) prospective on-going study includes all patients suspected to be affected by neuroblastoma who were diagnosed and/or treated through a MAS approach. Attention has been specifically focused on surgical risk factors (SRFs) and complications.
Results: A total of 44 MAS procedures were performed in children affected by cancer during the study period, 16 of whom presenting with neuroblastoma. Biopsies were performed in 7 out of 16 patients, in 4 patients a tru-cut biopsy being the procedure adopted, whereas an incisional biopsy was performed in the remaining 3 patients. Nine out of 16 procedures consisted in a complete resection of the mass associated with lymph-nodes sampling when indicated. Intraoperatively, 2 episodes of bleeding occurred and were successfully managed without conversion. One case required a conversion to traditional open surgical technique to achieve complete excision of the mass. On discharge, after a median hospital stay of 3 days (range 1-7 days), all patients were judged eligible to proceed to further treatments.
Conclusion: As far as oncological criteria are respected in terms of SRFs and risk of tumor spreading, MAS can represent the mean to obtain an accurate diagnosis, staging, complete resection, palliation or management of oncological complications in children affected by cancer.
Email: stefano.avanzini@libero.it

POC22 Role of nursing in the implementation of chimeric anti-GD2 antibody with immunotherapy (ANBL0032) into clinical practice
Mills, Denise1; Maloney, Anne Marie1; Chang, Ann1; 1The Hospital for Sick Children, Haematology/Oncology Program, Neuroblastoma Program, Toronto, Canada; 2The Hospital for Sick Children, Haematology/Oncology Program, Toronto, Canada
Background/Aims: Interim analysis of the Children’s Oncology Group (COG) (ANBL0032) phase III randomized trial of the chimeric anti-GD2 antibody ch14.18 with GM-CSF and IL2 for high-risk neuroblastoma demonstrated superiority of the experimental immunotherapy arm. After ANBL0032 was reopened as a single arm clinical trial, enrolment at our institution was significantly increased. As we had not used this study in more than two years, a comprehensive implementation plan was developed to facilitate the reintroduction of this complex and nursing care intensive therapy. The planning process required the collaboration of Nurse Practitioners (NP), Nurse Educators, Nurse Managers and the inpatient nursing staff as well as development of specialized order sets, educational materials and patient management guidelines.
Methods: The need for extensive education was identified as a high priority. Consequently, a detailed knowledge transfer plan was developed and implemented using COG education modules as well as additional tools developed specifically for this protocol. The required observations and the delivery of care on this protocol necessitated precise timing of admissions and planning of resources necessary to deliver care as well provide accurate documentation of therapy. Clinic visits and detailed coordination of required testing and clinical assessments was organized by the education component of the knowledge transfer plan. The NP continues to serve as a resource regarding this protocol.
Conclusion: The implementation of this plan has resulted in meeting the ongoing learning needs of the nursing staff. Patient care has been enhanced by the coordination of inpatient stays and outpatient evaluations. Our education program has been adapted and modified for other caregivers in our institution. An analysis is underway to assess the success of our educational knowledge transfer plan and determine the frequency for training updates.
Email: denise.mills@sickkids.ca

POC23 Phase I trial of Lestaurtinib for children with refractory neuroblastoma: A new approaches to neuroblastoma therapy (NANT) study
Minturn, Jane E1; Evans, Audrey E1; Villablanca, Judith G1; Yanik, Gregory A2; Park, Julie R1; Groshen, Susan1; Heilriegel, Edward T1; Benson, Kennedy D1; Brodeur, Garrett M1; Marks, John H1; 1The Children’s Hospital of Philadelphia and the University of Pennsylvania, Pediatrics, Philadelphia PA, United States; 2Keck School of Medicine and Children’s Hospital Los Angeles, Pediatrics, Los Angeles CA, United States; 3University of Michigan and C.S. Mott Children’s Hospital, Pediatrics, Ann Arbor MI, United States; 4Seattle Children’s Hospital, Pediatrics, Seattle WA, United States; 5Keck School of Medicine and Children’s Hospital Los Angeles, Biostatistics, Los Angeles, United States; 6Cephalon, Inc., Drug Safety and Disposition, Frazer PA, United States; 7Cephalon, Inc., Clinical Research, Oncology, Frazer PA, United States; 8University of California San Francisco School of Medicine, Pediatrics, San Francisco CA, United States
Background: TrkB acts as an oncogenic kinase in a subset of human NBs. Lestaurtinib, a multi-kinase inhibitor with potent activity against Trk kinases, has demonstrated anti-tumor activity in preclinical models of human NB.
Method/approach: We performed a Phase I trial of Lestaurtinib in subjects with recurrent or refractory high-risk neuroblastoma starting at 80% of the recommended Phase 2 dose. Subjects received lestaurtinib twice daily for 5 days out of 7 in 28-day cycles. Lestaurtinib dose was escalated using a 3+3 design. Pharmacokinetics and plasma phospho-TrkB inhibition during the first cycle were evaluated in 10 subjects. Response data were obtained after the first and then every other cycle.
Results: Forty-seven subjects were enrolled, and 10 dose levels were explored starting at 25 mg/m²/dose BID. All subjects were evaluable for response, and 42 subjects were evaluable for dose escalation. Asymptomatic and reversible grade 3-4 transaminase elevation was dose limiting in 4 subjects. Reversible pancreatitis (grade 2) was observed in 3 subjects after prolonged treatment at higher dose levels. Other toxicities were mild and reversible. Pharmacokinetic analyses revealed rapid drug absorption, however inter-patient variability was large. Plasma inhibition of phospho-TrkB activity was observed 1 hour post-dose at 85 mg/m² with uniform inhibition at 120 mg/m². There were two objective responses and 9 subjects had prolonged stable control of disease at dose levels ≥5 (median: 12 cycles) before disease progression. We established a biologically effective and recommended phase II dose of 120 mg/m²/dose BID.
Conclusion: Lestaurtinib was well tolerated in subjects with refractory NB, and a dose level sufficient to inhibit TrkB activity was established. Safety and signs of activity at the higher dose levels warrants further evaluation of this drug or other Trk-directed therapies in NB. The tolerability of this drug without hematologic toxicity makes it suitable for combination therapy with conventional cytotoxic agents.
Email: minturn@email.chop.edu
POC24

Arsenic trioxide as radiosensitizer for 131-I-MIBG therapy: Results of a pilot phase II study

Modak, Shakteel1; Pandit-Taskar, Neeta2; Carrasquillo, Jorge3; Kushner, Brian H.1; Kramer, Kim1; Zanzonico, Pat2; Smith-Jones, Peter1; Larson, Steven1; Cheung, Nai-Kong V.1

Memorial Sloan-Kettering Cancer Center, Pediatrics, New York, United States; 2Memorial Sloan-Kettering Cancer Center, Radiology, New York, United States; 3Memorial Sloan-Kettering Cancer Center, Radiology and Medical Physics, New York, United States

Background: Arsenic trioxide (ATO) has in vitro and in vivo radiosensitizing effects. Given its non-overlapping toxicity profile with the known anti-NB radiotherapeutic agent 131-I-MIBG, we hypothesized that ATO would enhance the efficacy of the latter and tested the combination in a pilot study.

Method/approach: Patients with heavily pretreated recurrent or refractory stage 4 NB were treated on an IRB-approved pilot phase II study (NCI-CTCAE version 2.0). A total of 12 patients (11 with stage 4S, 1 with stage 4) were enrolled, 11 of whom were evaluable. ATO was added to 131-I-MIBG therapy 12 or 18mcg/kg intravenously (IV) on day 1 plus ATO 0.15 or 0.25mg/kg IV days 6-10 and 11-15. Toxicities were measured using NCI CTCAE version 2.0 and responses were assessed using International NB response criteria (INRC).

Results: Nineteen patients were enrolled: 14 received 131-I-MIBG and ATO at maximal tolerated doses, 2 received 12mcg/kg 131-I-MIBG plus 0.15mg/kg dose ATO. 1 (at 131-I-MIBG dose of 12mcg/kg) did not receive ATO due to transient central line-induced cardiac arrhythmia, while another (at 131-I-MIBG dose of 18mcg/kg) received only 6/10 doses of AT due to significant diarrhea. All patients experienced grade 4 myelosuppression, though none required autologous stem cell rescue. Other >grade 2 adverse events were transient and included: hyperamylasemia from transient sialoadenitis (12/13 evaluable patients), hypokalemia (3), hyperbilirubinemia and hepatic transaminits (1), and hyponatremia (1). By INRC, 14 patients had no response, 5 had 9% partial response, 3 had stable disease, 5/5 patients who entered the study with prior PD. However, objective improvements in one or more NB markers were observed in 12 patients. 18/19 patients were continued on further chemotherapy and/or immunotherapy. Three-year progression free survival (PFS) was 37±11% with a median PFS of 9.5 months.

Conclusion: The combination of 131-I-MIBG plus ATO was well tolerated with adverse event profile similar to that of 131-I-MIBG therapy alone. Objective responses were observed in most patients. However, the addition of ATO to 131-I-MIBG did not significantly improve response rates when compared to historical data with single agent 131I-MIBG therapy alone.

Email: modaks@mskcc.org

POC25

Comparison of I-123 and I-131 mIBG scans in predicting survival in patients with stage 4 neuroblastoma

Narang, Arina1; Parisi, Marguerite T.1; Shuikin, Barry L.1; London, Wendy B.1; Matthay, Katherine K.1; Kreissman, Susan G.1; Yanik, Gregory A.1;1Children’s Oncology Group, University of Florida, Gainesville, FL, United States; 2Seattle Children’s Hospital, Radiology, Seattle, WA, United States; 3St. Jude Children’s Research Hospital Memphis, Radiological Sciences, Memphis, TN, United States; 4Children’s Oncology Group, Children’s Hospital and Dana-Farber Cancer Center, Boston, MA, United States; 5University of California San Francisco Children’s Hospital, Pediatrics, San Francisco, CA, United States; 6Duke University Medical Center, Pediatric Hematology-Oncology, Durham, NC, United States; 7C.S. Mott Children’s Hospital, University of Michigan, Pediatrics, Ann Arbor, MI, United States

Background: Historically, I-123 mIBG scans are preferred to I-131 for neuroblastoma (NB) imaging as they deliver less patient radiation yet have greater sensitivity in disease detection. Both I-123 and I-131 mIBG scans were used for disease assessments of NB patients (pts) treated on Children’s Oncology Group (COG) front-line high-risk study A3973. We tested for differences in survival prediction by Curie score for I-123 vs. I-131. The hypothesis was that I-123 and I-131 mIBG scans were sufficiently similar for clinical purposes in terms of sensitivity, specificity, and ability to predict survival.

Method/approach: Pts enrolled on COG A3973 with INSS stage 4 disease who completed I-123 or I-131 mIBG scans at any of the following time points - diagnosis, post-induction, post-transplant, or post-biotherapy - were analyzed. The performance of the Curie score for each mIBG scan type in predicting an event was evaluated using receiver operating characteristic (ROC) curve and area under the curve (AUC) analyses. At each time point, AUCs and survival curves for I-123 vs. I-131 were compared and responses were assessed using International NB response criteria (INRC).

Results: Of the 413 pts on A3973 with at least one mIBG scan, 350 were stage 4. Median age was 3.1 years (range 6.8 months–29 years) at diagnosis. At final patient-follow up, overall survival was 33.4 ± 3.6% and 45.6 ± 4.0% (n=350). No statistically significant differences in EFS were found with respect to age, MYCN amplification, chromosome alterations (NCA), without any segmental alterations detected by array-CGH, is associated with an excellent overall survival. For some of these patients, treatment reduction might be possible. The aim of this study was to analyse if the knowledge of a favourable genomic profile, characterised by NCA, only, influenced the treatment decision for individual children < 18 months.

Methods: This is a retrospective study of children aged less than 18 months with a neuroblastoma without MYCN amplification and a numerical genomic profile.

Normand, Charline1; Schiesermacher, Gudrun1; Pierron, Gaelle1; Ribeiro, Agnès1; Janoueix-Lerosey, Isabelle1; Philippe-Chomette, Pascale1; Van den Abbeele, Thierry1; Samnack, Sabine1; Manach, V.1; Delattre, Olivier1; Michon, Jean1

1Institut Curie, Département d’Oncologie Pédiatrique, Paris, France; 2Institut Curie, Unité de Génétique Somatique, Paris, France; 3Institut Curie, U830 INSERM, Paris, France; 4Hôpital Robert Debré, Pôle de Chirurgie Pédiatrique, Paris, France

Background: In neuroblastoma (NB), children aged less than 18 months have a good outcome in the absence of MYCN amplification. It has recently been shown that a genomic profile characterized by numerical chromosome alterations (NCA), without any segmental alterations detected by array-CGH, is associated with an excellent overall survival. For some of these patients, treatment reduction might be possible. The aim of this study was to analyse if the knowledge of a favourable genomic profile, characterised by NCA, only, influenced the treatment decision for individual children < 18 months.

Conclusion: The knowledge of a favourable genomic profile was taken into account for treatment decisions in 14 children, enabling a reduction of chemotherapy while maintaining the excellent overall survival. Prospective clinical trials are urgently required to confirm these results.

Email: charline.normand@curie.net
POC28
Retinoids (RA) relieve EZH2-mediated epigenetic suppression of neuroblastoma differentiation

Samuel L. Younglin, Shiraishi, Junji, Doi, Kunio, Cohn, Susan L, Volchenboum, Carol J

1University of Chicago, Section of Pediatric Hematology, Oncology and Pinto, Navin; Abe, Hiroyuki; Appelbaum, Daniel; Hara, Takeshi; Pu, neuroblastoma

Metaiodobenzylguanidine (mIBG) scans in patients with neuroblastoma. mIBG scans are typically interpreted qualitatively or semi-quantitatively by radiologists, often with poor inter-reader reliability. We have developed an automated quantitative method for scoring mIBG uptake in patients with neuroblastoma treated at the University of Chicago Medical Center for over 25 years. This method allows accurate and consistent analysis of mIBG uptake by radiologists.

Design/Methods: A retrospective analysis of mIBG scans from 30 patients with neuroblastoma was performed. The scans were scored by two experienced radiologists, according to the currently accepted standard of assigning each of 9 body segments a segmentation score of 0-3, depending on uptake. An automated, computerized segmentation algorithm was developed to divide the scan image into 9 segments and assign an extension score by relative mIBG signal intensity.

Results: Of a total of 250 events (25 scans with 9 segments each) for each patient, our algorithm agreed with one or both of the radiologists in 93% of cases, leading to a pairwise agreement of 92.5% (p<0.001). The median number of lymph node dissected was 8 and the median number of positive lymph node was 5. The median duration of surgery was 4 hours and 30 minutes; the median intraoperative blood loss was 205 ml. There were no major intraoperative complications leading to visceral insufficiency or perioperative deaths. The median duration of hospital stay was 7.5 days. Postoperative complications included intestinal obstruction (2 patients), chyle leak (9 patients) and infection (1 patient). Nineteen patients are alive without any disease; four patients are alive with disease. 14 patients died due to disease progression and one patient died due to chemotherapy related toxicity. There were no local recurrences in patients who died of disease except in one patient who had extensive local recurrence. Conclusion: There is high incidence of lymph node metastases in high risk neuroblastoma.

POC29
Development of an automated quantitative method for scoring Metabolobenzoguanine (mBGG) scans in patients with neuroblastoma

Shafique Sajjad, Shafique Sajjad, Abhishek, Khosla, Suraj, Jitendra, Desai, Ankit, Shukla, Dheeraj

1University of Chicago, Section of Pediatric Hematology, Oncology and Stem Cell Transplantation, Chicago, United States; 2University of Chicago, Department of Radiology, Chicago, United States; 3University of Chicago, Department of Intelligent Image Information, Gifu, Japan; 4University of Chicago, Kurt Rossman Laboratories for Radiologic Image Research, Chicago, United States; 5University of Chicago, Section of Pediatric Hematology, Oncology and Stem Cell Transplantation, Computation Institute, Chicago, United States

Objective: Treatment decisions for children with neuroblastoma are often made based on results of mBGG scanning, and current methods are semi-quantitative at best. We sought to develop a computerized, automated segmentation and scoring algorithm for mBGG scan analysis, thus enhancing therapeutic decision-making.

Design/Methods: With IRB approval, data from 70 mBGG scans from 17 patients with neuroblastoma treated at the University of Chicago Medical Center were collected for evaluation, and of these, raw data needed for further analysis was available for 25 scans from 11 patients. Images were scored by two experienced radiologists, according to the currently accepted standard of assigning each of 9 body segments a segmentation score of 0-3, depending on uptake. An automated, computerized segmentation algorithm was developed to divide the scan image into 9 segments and assign an extension score by relative mBGG signal intensity.

Results: Of a total of 250 events (25 scans with 9 segments each) for each patient, our algorithm agreed with one or both of the radiologists in 93% of cases, leading to a pairwise agreement of 92.5% (p<0.001). The median number of lymph node dissected was 8 and the median number of positive lymph node was 5. The median duration of surgery was 4 hours and 30 minutes; the median intraoperative blood loss was 205 ml. There were no major intraoperative complications leading to visceral insufficiency or perioperative deaths. The median duration of hospital stay was 7.5 days. Postoperative complications included intestinal obstruction (2 patients), chyle leak (9 patients) and infection (1 patient). Nineteen patients are alive without any disease; four patients are alive with disease. 14 patients died due to disease progression and one patient died due to chemotherapy related toxicity. There were no local recurrences in patients who died of disease except in one patient who had extensive local recurrence. Conclusion: There is high incidence of lymph node metastases in high risk neuroblastoma.

POC30
Is retroperitoneal lymphadenectomy for high risk abdominal neuroblastoma relevant

Oureshi, Said; Kurkure, Purna; Vishwanathan, Seethalakshmi; Ramadwar, Mukti; Laskar, Sidharth

1Tata Memorial Hospital, Pediatric Oncology, Ernest Borges Road, Parel, Mumbai, India; 2Tata Memorial Hospital, Pathology, Ernest Borges Road, Parel, Mumbai, India; 3Tata Memorial Hospital, Radiation Oncology, Ernest Borges Road, Parel, Mumbai, India

Objectives: Complete tumor removal in cancer surgery includes organ specific regional nodal dissection. However the extent of resection of primary tumor in treatment of high-risk neuroblastoma itself is controversial, therefore the efficacy of retroperitoneal lymphadenectomy has not been assessed. We reviewed our experience of retroperitoneal lymphadenectomy in high risk abdominal neuroblastoma in terms of its relevance, safety, local control and overall survival. Method: Thirty eight patients with high risk abdominal neuroblastoma with primary site restricted to the adrenal gland or the adjoining sympathetic ganglia operated between October 2004 and November 2009 are included in this prospective analysis. There are 26 males and 12 females, with a median age of 4 years (1 to 14 years). Retroperitoneal lymphadenectomy was performed in 48 patients and lymph node sampling in 3 patients. Results: The median number of lymph node dissected was 8 and the median number of positive lymph node was 5. The median duration of surgery was 4 hours and 30 minutes; the median intraoperative blood loss was 205 ml. There were no major intraoperative complications leading to visceral insufficiency or perioperative deaths. The median duration of hospital stay was 7.5 days. Postoperative complications included intestinal obstruction (2 patients), chyle leak (9 patients) and infection (1 patient). Nineteen patients are alive without any disease; four patients are alive with disease. 14 patients died due to disease progression and one patient died due to chemotherapy related toxicity. There was no local recurrence in patients who died of disease except in one patient who had extensive local recurrence. Conclusion: There is high incidence of lymph node metastases in high risk neuroblastoma. Complete excision of abdominal neuroblastoma and retroperitoneal lymphadenectomy can be performed safely with acceptable morbidity and is associated with excellent local control and better survival.

Email: sajidshafique@gmail.com
POC31
Concurrent ipsilateral nephrectomy versus kidney-sparing surgery in high-risk, intra-abdominal neuroblastoma
Roberts, Amanda1; Nasr, Ahmed2; Inwir, Meredith3; Gerstle, J. Ted4
1Hospital for Sick Children, Surgery, Toronto, Canada; 2Hospital for Sick Children, Hematology/Oncology, Toronto, Canada

Background: Surgical resection of the primary tumour is important in the management of high-risk neuroblastoma (NB). However, it is not clear what the effect is of a concurrent ipsilateral nephrectomy at the time of resection of the primary intra-abdominal NB. The purpose of this study was to determine if concurrent unilateral nephrectomy in high-risk NB has an impact on recurrence rate, renal function, morbidity and overall survival.

Methods/approach: A retrospective cohort review was performed on all patients with high-risk NB at one institution between 1998 and 2008. Research Ethics Board approval was obtained. Analysis was done using t-test for continuous variables and Chi-square/Fischer’s exact test for categorical ones.

Results: A total of 62 patients with high risk NB with intra-abdominal tumors were eligible: 56 had nephrectomy surgery and 6 had nephrectomy. Patient characteristics (gender and age) and tumor characteristics (side and size) were not significantly different between the two groups. The following table summarizes the results:

<table>
<thead>
<tr>
<th>Table</th>
<th>Nephrectomy (N=6, ± SD)</th>
<th>Kidney-sparing (N=56, ± SD)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBL (ml)</td>
<td>820 ± 480</td>
<td>110 ± 174</td>
<td>0.001</td>
</tr>
<tr>
<td>Operating time (hrs)</td>
<td>9.2 ± 4.0</td>
<td>5.9 ± 2.4</td>
<td>0.005</td>
</tr>
<tr>
<td>GFR (ml/min) post-operative</td>
<td>104 ± 30</td>
<td>148 ± 36</td>
<td>0.006</td>
</tr>
<tr>
<td>Complications: intra-operative</td>
<td>0/6 (0%)</td>
<td>13/58 (23%)</td>
<td>0.1</td>
</tr>
<tr>
<td>Local recurrence at time of BMT</td>
<td>0/6 (0%)</td>
<td>13/52 (25%)*</td>
<td>0.5</td>
</tr>
<tr>
<td>Metastatic recurrence</td>
<td>4/6 (67%)</td>
<td>32/56 (57%)</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Conclusion: Concurrent nephrectomy did not result in improved survival. The side and size of the tumor were not significantly different between the two groups. This is in contrast to the results of our previous study where there was a significant difference in recurrence rate and overall survival.

POC32
Pilot study of high-dose chemotherapy using a novel preparative regimen with Busulfan, Melphalan, and Topotecan (TBM) followed by autologous hematopoietic stem cell transplant in high-risk neuroblastoma and other advanced stage solid tumors
Rosenthal, Joseph; Pawlowska, Anna; Bolotin, Ellen; Naem, Hossameldin; Dagis, Andrew; Qian, Dajun; Anderson, Clarke
City of Hope National Medical Center, Duarte, CA, United States

Background/aims: High-dose chemotherapy followed by autologous hematopoietic stem cell transplant (AHSTC) has been used a 6% consolidation therapy for pediatric solid tumors, including neuroblastoma (NB). Relapse from resistant disease, traditional acute (veno-occlusive disease (VOD), mucositis, and 6% toxic death) and late (hearing loss) toxicities following AHSTC suggest that novel regimens are needed to improve outcome and minimize morbidity.

Methods: 19 patients (4 Ewing’s, 7 NB, 3 Wilms, 1 PTEN, 3 Rhabdomyosarcoma, 1 undifferentiated sarcoma) were treated with Topotecan (continuous infusion days -8 to -4 ) plus Melphalan (q 6 hours x 16 doses during Topotecan) and Busulfan (q 6 hours x 16 doses during Topotecan) and Melphalan (days -3 and -2). Outcome: 10 patients experienced toxic death, 1 patient suffered liver failure, 6 patients had GFR below normal, 4 patients developed organ failure due to VOD, 9 pts had sepsis and 8 pts had grade 3 mucositis. Only 1 patient experienced grade 3/4 VOD. Neutrophils (>0.5 x 10^9) recovery occurred at day 14 (8-18).

Conclusion: TBM conditioning regimen has acceptable toxic death rate and marrow recovery time. Large phase 1-2 studies are indicated to determine if TBM could be used as a consolidation regimen in high-risk NB.

POC33
Immunocytological GD2 negativity in treated and untreated neuroblastoma patients with bone marrow metastases
Schumacher-Kuckeiorn, Roswilla; Hero, Barbara; Gradenhart, Anke; Simon, Thorsten; Berthold, Frank
University Hospital of Cologne, Pediatric Oncology and Hematology, Cologne, Germany

Background: The expression of the GD2 ganglioside on the surface of neuroblastoma cells has important implications for bone marrow assessment and for treatment of minimal residual disease. Earlier detection of three cases prompted us in 2006 to study GD2 negativity prospectively.

Methods: Paired bone marrow samples of neuroblastoma patients with cytological bone marrow involvement were investigated by routine light microscopy and by GD2 immunocytochemistry according to published standards. Only cytologically unquestionable tumor cell clumps with complete loss of GD2 staining were considered GD2 negative. Cytosin immunostaining results were controlled by additional immunostaining directly on bone marrow smears in most cases.

Results: During 42 months, 493 bone marrow samples of 366 patients were investigated. In 28 cases lacking immunocytological GD2 expression of cytological unequivocal neuroblastoma tumor cell clumps was detected. Of the 28 patients, 14 were at initial diagnosis, and 8 at recurrence. In 10 cases all tumor cells and in 18 a varying number of cells were GD2 negative. The frequency of patients with GD2 negative cells at diagnosis was 7.2% (in stage 4 (12/166) and 3.4% in stage 4S (2/59). No association to other diagnostic criteria like mIBG uptake, urinary catecholamine excretion, NSE levels, LDH, MYCN amplification, 1p aberration, and NPCR histochemistry was detected. GD2 negative stage 4 patients (at diagnosis) had a worse outcome (EFS logrank p = 0.019, OS p < 0.001).

Conclusion: GD2 negativity is a more frequent phenomenon in neuroblastoma than currently known and has important implications for diagnosis, monitoring disease and for treatment.

POC34
Hematopoietic stem cell transplantation for high risk neuroblastoma in children
Shelkova, Larisa
Russian State children Hospital and Institute of pediatric hematology, Onchemotherapy, Moscow, Russian Federation

Neuroblastoma is a malignancy of neural crest cells which usually give rise to the sympathetic nervous system. Patient with high risk NB have a very poor result after standard treatment. High-dose chemotherapy followed by HSC may improve their prognosis.

Patients and methods: From October 1995 until December 2009 30 patients (17 11/3 m) with high risk NB were referred to Russian State children hospital. The median age at diagnosis was 3.39 y.o (range 6m-15y.). The primary sites were adrenal glands, followed by retropertoneum in 24pts, and thoracic cavity in 6pts. All patients had stage IV of the disease, 19pts had bone lesions. Their were 12 pts with urinary catecholamine excretion, NSE levels, LDH, MYCN amplification, 1p aberration, and NPCR histochemistry was detected. GD2 negative stage 4 patients (at diagnosis) had a worse outcome (EFS logrank p = 0.019, OS p < 0.001).

Conclusion: GD2 negativity is a more frequent phenomenon in neuroblastoma than currently known and has important implications for diagnosis, monitoring disease and for treatment.

Conclusion: VOD, milder mucositis and absence of hearing loss requiring hearing aids) than classic CEM regimens. Study continues accrual in a phase 2 setting.

Email: canderson@coh.org
POC35 Follow-up study of survivors of childhood neuroblastoma - Report from a single institute in Japan
Shichino, Hiroki1; Chinn, Motoaki; Okuma, Hirotsugu; Nishikawa, Eri; Hirai, Maiko; Kato, Maiko; Yagasaki, Hiroshi; Urakami, Tatsuhiko; Sumitomo, Naokata; Inamo, Yasui; Mugishima, Hideo
Itabashi Hospital/Nihon University School of Medicine, Pediatrics and Child Health, Tokyo, Japan
Background: Neuroblastoma is the most common malignant solid tumor of childhood, but the prognosis of high-risk neuroblastoma (HRNB) is still very poor. Furthermore, children who survive HRNB are also at risk for treatment-related complications. We analyzed these complications in survivors of HRNB in our institute.

Methods: From 1992 to 2004, at Nihon University Itabashi Hospital, we treated 25 newly diagnosed HRNB patients with multimodal treatment which consisted of induction chemotherapy (CDDP+THP+VCR+CPA or IFM-CBDDA+VP-16), high-dose chemotherapy (CBDDA+VP-16+LPAM with low-dose TBI), primary delayed surgery and local radiotherapy. We investigated many long-term problems, including second neoplasia, physiologic disturbance, and organ system dysfunction (especially for endocrine, musculoskeletal, neurologic, sensory, cardiac, and pulmonary impairments).

Results: Thirteen patients were still surviving and 10 of these survivors were examined with regard to long-term problems. Five were male and 5 were female. The median age at diagnosis was 4 years old (range, 1y7m to 9y6m) and the median follow-up time was 9 years 10 months (range, 9y6m to 18y5m). None of the patients had a second neoplasia patient and only one showed physiologic disturbance. Many organ system dysfunctions were noted: 10 high-frequency (>4kHz) hearing loss, 2 cataract, 5 small height (4 growth hormone therapy needed), 2 goiter, 6 hypothyroidism, 2 hypogonadism, and 6 chronic-sinusitis. None of the patients showed cardiac disturbance or pulmonary complication.

Conclusion: There were many long-term problems, especially regarding patients showed cardiac disturbance or pulmonary complication. Itabashi Hospital/Nihon University School of Medicine, Pediatrics and Child Health, Tokyo, Japan

POC36 Retrospective analysis of treatment results of high risk neuroblastoma
Shorshor, Sabella1; Lemesheva, Olga1; Popova, Tatiana1; Vyatkin, Igor2; Tsaur, Grigory1; Popov, Alexander1; Saveliev, Leonid3; Fechina, Larisa1
1Regional Children’s Hospital # 1, Pediatric Oncology and Hematology Center, Ekaterinburg, Russian Federation; 2Ural State Medical Academy, Chair of Clinical Laboratory Medicine, Ekaterinburg, Russian Federation
Background: The most important risk factors in neuroblastoma are well known: age more than 1 year at the time of diagnosis, advanced stage of disease and MYCN amplification.

Aim: To determine the efficacy of high risk neuroblastoma treatment a retrospective analysis in Pediatric Oncology and Hematology Center, Regional Children’s Hospital, Ekaterinburg, Russia.

Methods: Among 140 children with primary neuroblastoma admitted to our clinic since January 1991 18 November 2009 aged from 10 days to 15 years (median 18 mo.) were 91 patients (45 girls and 46 boys) with known MYCN status. 41 (43,9%) patients older than 1 year with stage IV or MYCN amplification were assigned to high risk group (HRG) and have been treated according to NB 92 protocol, 56 (60,4%) patients - according to NB 2004 protocol: 9(22%), 19(46,3%) and 13(31,7%) correspondingly. Only 4(9,7%) children underwent high dose chemotherapy and autologous peripheral blood stem cells transplantation (PBSCT). Median of follow up is 48 months.

Results: Patients distribution by stage was as follow: stage I -2(4,9%) patients; stage II -21(4,9%); stage III -9(18,8%); stage IV -33(80,5%).

MYCN amplification has been detected in 21(51,2%) of 41 HRG patients. Complete remission and very good partial remission have been achieved in 16 (39%); partial remission in 17 (41,5%) children. Favorable events (relapses, stable disease, progressive disease and therapy related death) were detected in 31 (75,6%) cases. 11 (26,6%) patients are alive; 10(24,4%) are alive without progression; 2(4,9%) patients were lost to follow up. 12-years event free survival (EFS) is 18%±2% and overall survival is 33%±8%. EFS in patients with MYCN amplification (20%±10%) did not differ significantly in comparison with other HRG patients (19%±8%) (p>0.08). Among 4 children after high dose chemotherapy consolidation consolidation therapy was performed. Tissue diagnosis was not performed. Local relapse was registered in 11 case and 2 patients are alive in complete remission.

Conclusion: Treatment results of HR neuroblastoma remain unsatisfactory. The preliminary results suggest auto-PBSCT is a curative therapeutic approach in patients with HR neuroblastoma.

Email: cohch@bk.ru

POC37 Comparison of anti-GD2-antibody ch14.18 and 13-cis-retinoic acid as consolidation therapy for high-risk neuroblastoma. Results of the German NB97 trial
Simon, Torsten1; Hero, Barbara1; Handgretinger, Rupert1; Schraper, Martin1; Kingebiel, Thomas1; Freuhwald, Michael C1; Henze, Guenter1; Breitland, Frank1
1University of Cologne, Pediatric Oncology and Hematology, Cologne, Germany; 2University of Tubingen, Pediatric Oncology and Hematology; Tubingen, Germany; 3University of Schleswig Holstein Campus Kiel, Pediatric Oncology and Hematology, Kiel, Germany; 4University of Frankfurt, Pediatric Oncology and Hematology, Frankfurt am Main, Germany; 5University of Munster, Pediatric Oncology and Hematology, Munster, Germany; 6Charite University Hospital, Pediatric Oncology and Hematology, Berlin, Germany

Background: High risk neuroblastoma patients require intensive treatment consisting of induction chemotherapy, high dose chemotherapy (HDCT) followed by autologous stem cell transplant (ASCT) as consolidation therapy. Consolidation therapy has been studied in several trials, however, information on the efficacy of single agents are limited. We thus retrospectively analyzed the patients of trial NB97.

Method/approach: Patients were included in the analysis when they fulfilled all of the following criteria: stage 4 neuroblastoma, >=1 year at diagnosis, successful induction chemotherapy, HDCT, and ASCT, no combination of antibody ch14.18 and retinoic acid, and at least one cycle of consolidation therapy. Between 1997 and 2002, all trial patients were scheduled for consolidation therapy with the anti-GD2-antibody ch14.18 (six courses consisting of 20 mg/m²/day for 5 days every 2 months, group ch14.18). Between 2002 and 2004, all patients received 13-cis-retinoic acid (nine courses 150 mg/m²/day for 14 days every 28 days with a three-months rest between the 6th and the 7th course; group RA).

Results: A total of 149 consecutive neuroblastoma patients were included; 74 patients received ch14.18, and 75 patients received RA. Both groups were balanced in all parameters, except for MYCN amplification (p=0.018), and remission status prior to ch14.18/RA (p= 0.51). The median observation time was 7.72 years. The 3-year-event free survival rate from diagnosis was 52.7±5.8% in the ch14.18 group and 50.5±5.8% in the RA group (p=0.209). The 3-year overall survival rates were 68.3±5.4 and 65.0±5.5% (p=0.262) for ch14.18 treated and RA treated patients, respectively. Multivariate analysis including the variables age, MYCN status, remission status after ASCT, and type of consolidation also demonstrated no independent impact of consolidation therapy on event free survival and overall survival.

Conclusion: This retrospective analysis of a very homogenous cohort of high-risk neuroblastoma patients demonstrated no difference between 13-cis-RA and ch14.18 as consolidation therapy after high-intensive induction chemotherapy and ASCT.

Email: torsten.simon@uk-koeln.de

POC38 Metachronous neuroblastoma in an infant with constitutional unbalanced translocation t(12;16)(p23.113.3): involving ALK
Sohn, Shu Yer1; Slavopoulous, Dimitri1; Bowdin, Sarah1; Thorner, Paul1; Baruchel, Sylvain1; Malvin, David1; Meyn, Stephen2; Irwin, Meredith1
1The Hospital for Sick Children, Division of Haematology/Oncology, Toronto, Canada; 2The Hospital for Sick Children, Division of Molecular Genetics, Toronto, Canada; 3The Hospital for Sick Children, Division of Clinical and Metabolic Genetics, Toronto, Canada; 4The Hospital for Sick Children, Division of Pathology, Toronto, Canada

Background and Results: The patient, a full-term male born to non-consanguineous parents, had dysmorphic features, hypospadias, inguinal hernia, malrotation, feeding difficulties, left partial anotia, microcytic anemia, and seizures. Chromosomal analysis by G-bandning and array comparative genomic hybridization (aCGH) revealed an unbalanced translocation t(12;16)(p23.13;p11.3), resulting in partial monosity for 16p13.3-pter and partial trisomy for 2p23-ppter. At 3 months, he developed a right adrenal stage I neuroblastoma (NB) and had a complete surgical resection. Six months later, he developed a NB in the contralateral adrenal, which was again treated by resection. Both tumors exhibited favorable histology. Cytogenetics from the first tumor demonstrated the constitutional t(12;16) translocation as well as trisomy 17 and (14)(q12)p(12)11. Analyses by FISH did not detect rearrangement of ALK however, in keeping with the constitutional karyotype, 3 copies of MYCN and 3 copies of ALK gene were detected. After six months follow-up since the second surgery there is no evidence of recurrence or additional tumors.

Discussion: The ALK gene (2p23.2), located ~13 Mb proximal to the MYCN gene (2p23.4), has been identified as a major familial NB predisposition gene. Somatic ALK mutations and copy number gains have also been reported in sporadic NB. Partial trisomy of 2p has been previously reported to be associated with NB in the context of multiple aneuploidies (5 case reports, one with bilateral NB). Most of the cases with germline partial trisomy 2p were expected to harbor either 3 copies of ALK and MYCN. We propose that ALK copy number gain, which drives cellular proliferation in culture, may be one factor that predisposes patients with partial trisomy 2p to develop NB. Given the role of other high-resolution genetic platforms, we have recently identified two patients with duplications of 2p (one included ALK and MYCN, the other only ALK), Long-term prospective studies of patients with increased 2p (and ALK) copy number will help to determine potential risk for tumors in these patients and need for surveillance screening.

Email: sofshulyen@hotmail.com
POC39
Neuroblastomas with non-avid 18F-MIBG scan and negative urinary catecholamine analysis: A single institution experience
Soh, Shu; Yeo, Banuchel; Sylvain, Inrin; Meredith
The Hospital for Sick Children, Division of Haematology/Oncology, Toronto, Canada
Background: Small studies have reported that non-avid MIBG scans at diagnosis are associated with favorable prognosis. We retrospectively examined clinical characteristics and outcomes for neuroblastoma patients with non-avid MIBG scans and/or negative urinary catecholamines at diagnosis.
Methods: We reviewed chart records for all neuroblastoma patients diagnosed from September 1999 to August 2009.
Results: There were a total of 148 patients - 29 INSS stage 1 (20%), 24 diagnosed from September 1999 to August 2009.

POC40
Efficacy of tandem high-dose chemotherapy and autologous stem cell rescue in high-risk neuroblastoma: a preliminary report of NB 2004 study at Samsung Medical Center
Sung, Ki Woong; Cheuh, Heewon; Lee, Soo Hyun; Yoo, Keon Hee; Hoo, Hong Hoe; Jeon, Choo; Eun Joo Lee; Koo, Hong Hoe
1Samsung Medical Center, Sungkyunkwan University School of Medicine, Department of Pediatrics, Seoul, Republic of Korea; 2Kyungpook National University Hospital, Kyungpook National University School of Medicine, Department of Pediatrics, Daegu, Republic of Korea
Background: Although the strategy using high-dose chemotherapy and autologous stem cell rescue (HDCT/ASCRTT) has improved the survival of patients with high-risk neuroblastoma, the survival rate after single HDCT/ASCRT was unsatisfactory. In the present study, the efficacy of tandem HDCT/ASCRT was investigated to further improve the outcome of patients with high-risk neuroblastoma.
Methods: Patients who were newly diagnosed with high-risk neuroblastoma (stage 4 tumors over 1 year of age or N-myc amplified disease) and is well with no relapse after 9 years. For the MIBG-negative, overall and relapse-free survivals were 100% and 82%, respectively, compared to 73% and 66% for the entire cohort. In addition, there were 2 patients who relapsed with MIBG- non-avid disease (avoid at first diagnosis). Urine homovanillic acid (HVA) and vanillylmandelic acid (VMA) results were available for 145 patients. The levels were negative for 17 (12%), all of whom had low stage disease (1 or 2) and are alive without relapse. Only 2 patients had both normal catecholamines and non-avid MIBG scan at diagnosis. We also identified 9 patients who failed urine catecholaminergic at diagnosis who later had normal levels at relapse. All underwent further therapy and are alive (median follow-up 4 years).
Conclusions: Negative MIBG scans and negative urinary catecholamines are more commonly associated with low stage disease and favorable outcome. Our findings are consistent with two other reports from similar size cohorts. Larger population studies are needed to verify and understand potential mechanisms for this association.
Email: soshshuiyen@hotmail.com

POC41
Measurement of tyrosine hydroxylase transcripts in bone marrow using biopsied tissue instead of aspirate for neuroblastoma
Sung, Ki Woong; Lee, Seung-Taere; Suh, Yeon Lim; Ko, Young-Hye; Ki, Chang-Seok; Kim, Hae-Jin; Kim, Jong-Won; Kim, Sun-Hee; Cheuh, Heewon; Lee, Soo Hyun; Yoo, Keon Hee; Koo, Hong Hoe
1Samsung Medical Center, Sungkyunkwan University School of Medicine, Department of Pediatrics, Seoul, Republic of Korea; 2Samsung Medical Center, Sungkyunkwan University School of Medicine, Department of Laboratory Medicine and Genetics, Seoul. 3Samsung Medical Center, Sungkyunkwan University School of Medicine, Department of Pathology, Seoul, Republic of Korea
Background: Molecular detection of tyrosine hydroxylase (TH) transcripts by quantitative RT-PCR (qRT-PCR) is a sensitive method to detect neuroblastoma (NB) cells in the bone marrow (BM). However, its clinical utility following chemotherapy has not been thoroughly investigated.
Methods: TH transcripts in the BM were measured by qRT-PCR both at diagnosis and during the course of chemotherapy. The results were analyzed with respect to assay timing, tumor volume and histological findings.
Results: A total of 475 BM specimens from 105 patients were analyzed (66 at diagnosis, 48 after three cycles, 48 after nine or more cycles of chemotherapy, 35 at or after relapse/progression). Of the 475 BM specimens, tumor cells were detected in 63 specimens by conventional histological examination (22 at diagnosis, 9 after three cycles, 6 after six cycles, 15 after nine or more cycles of chemotherapy, 11 at or after relapse/progression). TH transcripts were detected in 100% of BM aspirates at diagnosis in cases with concurrent tumor involvement in the BM section; however, the proportion of TH transcript positive BM aspirates in cases with concurrent tumor involvement in the BM section gradually decreased following chemotherapy (55.5% after three cycles, 28.6% after six cycles and 0% after nine or more cycles of chemotherapy). Decreased proportion of TH transcript positive BM aspirates was associated with reduced tumor volume in the BM and differentiation of tumors into mature forms during chemotherapy. When qRT-PCR was performed with both aspirated and biopsied tissue during chemotherapy, TH transcripts were detected in BM tissue not only in all of the histology-positive cases but also in some of the histology-negative cases, while the proportion of TH transcript positive BM aspirates was low, even in histology-positive cases.
Conclusions: Measurement of TH transcripts in BM aspirate does not appear to be clinically useful during or after chemotherapy. Therefore, molecular monitoring of NB cells during or after chemotherapy using BM tissue is more optimal than testing on BM aspirates.
Email: kwsped@skku.edu

POC42
Reduced-intensity allogeneic stem cell transplantation in children with neuroblastoma who have failed a prior tandem autologous stem cell transplantation
Sung, Ki Woong; Cheuh, Heewon; Lee, Soo Hyun; Yoo, Keon Hee; Koo, Hong Hoe; Kim, Juyoun
1Samsung Medical Center, Sungkyunkwan University School of Medicine, Department of Pediatrics, Seoul, Republic of Korea
Background: The prognosis of children with neuroblastoma who have failed a prior tandem autologous stem cell transplantation (SCT) is dismal because they can not tolerate additional intensive therapy. In this context, investigators evaluated the feasibility and efficacy of reduced-intensity allogeneic stem cell transplantation (RIST) in children who have failed a prior autologous SCT.
Methods: Four to eight cycles of conventional chemotherapy were administered prior to RIST. Surgery and local radiotherapy were done whenever possible prior to RIST. CyFlu (cyclophosphamide 120 mg/kg + fludarabine 150 mg/m2 regimen) and CyFlu + ATG (7.5 mg/kg) regimen were used as conditioning regimen for HLA matched SCT and for mismatched related SCT, respectively. Peripheral blood stem cells were transplanted. Cyclosporine (CSA) alone for matched related SCT, and CSA + short-course methotrexate for unrelated or mismatched SCT were used for GVHD prophylaxis. CSA was rapidly tapered from day 30 (matched related SCT), day 60 (matched unrelated SCT) or day 90 (mismatched related SCT). GVHD was absent or complete donor chimerism was not achieved. Results: A total of 5 patients were enrolled (1 matched related, 2 matched unrelated and 2 haplo-identical related). Tumor status prior to RIST was VGPR in 1, PR in 3 and SD in 1. Conditioning regimen-related toxicities were mild. Hematologic recovery was rapid and complete donor chimerism was achieved in all patients until day 90. Grade 1-2 acute GVHD developed in all patients and extensive chronic GVHD developed in all patients who had survived for more than 100 days after RIST. Although CR was achieved on day 90 in 1 patient (VGPR prior to RIST), tumor progressed for the remaining 4 patients in PR (1 patient), PR or SD (1 patient) and SD (2 patients). The only patient who achieved CR after RIST died from viral myocarditis later.
Conclusions: Although conditioning regimen-related toxicities were mild and complete donor chimerism was achieved, tumor progression during the early period after RIST in patients who were in PR or SD prior to RIST. More effective treatment to reduce tumor burden prior to RIST might be needed to improve the outcome after RIST.
Email: kwsped@skku.edu
POC45
Identification of a therapy-sensitive subtype or stratification of aggressive risk in advanced neuroblastoma
Tanaka, Takeo1; Kyo, Yoko2; Hayashi, Kuniko2; Iehara, Tomoko1; Hosoi, Hajime3; Sugimoto, Tohru2; Hamasaki, Minoru1; Kaneko, Michio1; Sawada, Tasdahi1
1National Hospital Organization Hiroshimashi Medical Center, Pediatrics, Ootake, Hiroshima, Japan; 2Gunma University School of Health Science, Epidemiology and Biostatistics, Maebashi, Gunma, Japan; 3The Japanese Infantile Neuroblastoma Cooperative Study, Pediatrics, Kyoto, Japan
*The Japanese Infantile Neuroblastoma Cooperative Study, Pathology, Shizuoka, Japan; 4The Japanese Advanced Neuroblastoma Cooperative Study, Pediatric Surgery, Tsukuba, Japan

Background: The aim of this study is to propose a new risk stratification in advanced neuroblastomas (NBs).
Methods: 196 non-mass NBs (56 in st III, 117 in st IV, 23 in st IVs) were assessed with ‘MYCN status, INPC finding’ and Ha-ras/TRK A expression. Statistics: Kaplan-Meier method (patient’s survival) and Cox proportional hazard model (independence of each factor) were used.
Results: (1) Predictors: Each of MYCN Amplification, INPC Unfavorable histology and Low Ha-ras/TRK A expression associated significantly with poor patient’s outcome. The multivariate analysis could show an independence of each predictors for the outcome. However, when the risk was assessed by a single predictor, a half of the high risk NBs were missed. (2) Stepwise stratification was done in 103 advanced NBs (59 were event-free survivors and 44 died), in which the all three predictors were examined. Total 69 NBs were enrolled stepwise into high risk group (1st: 28 with MYCN Amplification, 2nd: 24 with INPC Unfavorable histology and 3rd: 17 with Low Ha-ras/TRK A expression). The 38 patients died in this high risk group and occupied 86% of total 44 patients died in this study. Moreover, 21NBs with High Ha-ras/TRK A expression, Unamplified MYCN and INPC favorable histology were classified as a subgroup (therapy sensitive NB) with 90% survival rate. (3) Stratification by the pile of the risk: The 69 high risk NBs could be classified into three subgroups according to number of the risk factors. NBs with triple risk were most aggressive and the survival rate was only 10%. Those in the double and single risk subgroups were 26% and 66%, respectively. Including the therapy sensitive group, survivals of the four groups showed significant difference (p<0.0001).
Conclusion: We provided a new means for risk stratification and presented a subgroup: therapy sensitive NBs in the presence of high risk subcategories. This is reliable grounds to select an therapeutic intensity at diagnosis for respective patients, which might improve the efficacy of the therapies in high risk NBs and decrease sequence in the lesser risk NBs.
Email: tanaka@hiroshimashi-nh.hosp.go.jp

POC46
18F-FDOPA PET scan is still useful in the presence of 18F-MIBG and 123I-MIBG for neuroblastoma imaging
Tzen, Kai-Wu1; Lu, Meng-Hsi1; Chang, Hsiu-Hao2; Hsu, Wen-Ming1; Luo, Tsai-Yueh4; Shen, Lie-Hang4
1National Taiwan University Hospital, Department of Nuclear Medicine, Taipei, Taiwan; 2National Taiwan University Hospital, Department of Pediatrics, Pediatric Surgery, Taipei, Taiwan; 3National Taiwan University Hospital, Department of Surgery, Taipei, Taiwan; 4Institute of Nuclear Energy Research, Department of Radiosotope Application, Sun Yat-Sen College, Taiwan

Background: In our previous studies using 18F-FDOPA and 18F-FDG PET scans for neuroblastoma imaging, we proved that 18F-FDOPA is very helpful when 123I-MIBG is not available. In this study we added 18F-MIBG for imaging study to re-evaluate the value of 18F-FDOPA in detecting neuroblastoma lesions.
Method/approach: In all 15 cases of neuroblastoma, 18F-FDOPA PET, 18F-FDG PET and 18F-MIBG scans were done in random sequence with each other within 2 weeks. Both 18F-FDOPA and 18F-FDG were produced in our PET Center and 18F-MIBG was produced in INER, Taiwan. 18F-FDOPA PET was done after carbipad premedication; 18F-FDG PET was done with fasting for 4 hours and 18F-MIBG scan was done with Lugol’s solution for 3 days. All scans were correlated with pathological findings, other imaging studies and clinical follow up for at least 6 months.
Results: The imaging findings can be classified in the following 4 categories. In 3 cases after carbipad premedication, all three scans were negative. In a case of multifocal systemic recurrence all three scans were positive and the uptake patterns for 3 agents were similar. In these 4 cases (27%) 18F-FDOPA PET scan showed no advantage over 18F-MIBG scan. In 9 cases (60%) with abnormal 18F-FDOPA uptake, 4 of them were negative on both 18F-FDG and 18F-MIBG scans, 3 showed abnormal 18F-FDG and 18F-MIBG uptake in different sites and 2 showed negative 18F-FDG scan but positive 18F-MIBG scan (less well demonstrated by 18F-FDOPA). The last category included 2 cases (13%) of poorly differentiated tumor at the primary site where 18F-FDOPA showed no uptake while 18F-FDG and 18F-MIBG were positive.
Conclusion: In conclusion, 18F-FDOPA PET was clinically useful in 73% of our cases in: (a) detecting more or different lesions than 18F-FDG/18F-MIBG scans (60%); (b) characterizing a poorly differentiated primary lesion (13%). Only 27% of the cases, 18F-FDOPA scan did not show advantage over 18F-MIBG scans. In a malignancy with variable biological characteristics like neuroblastoma, we recommended adding 18F-FDOPA in the diagnosis and follow up of the tumor after therapy.
Email: tzenky@tnh.tuei.gov.tw
POC47
Molecular imaging with 18F-FDOPA PET in the early detection of new metastatic lesions in bone marrow aspirate

Tzen, Kai-Yuan1; Lu, Meng-Yao; Chang, Hsiu-Hsue; Hsu, Wen-Ming1
1National Taiwan University Hospital, Department of Nuclear Medicine, 7, Chung-Shain S. Rd., Taipei, Taiwan; 2National Taiwan University Hospital, Department of Pediatrics, Taipei, Taiwan; 3National Taiwan University Hospital, Department of Surgery, Taipei, Taiwan

Background: The biosynthesis of catecholamine in neuroblastomas needs tyrosine hydroxylase (TH) and dopa decarboxylase (DDC). The TH mRNA and DDC mRNA detected by RT-PCR (reverse transcription-polymerase chain reaction) amplification have been used for detecting minimal residual disease in marrow of neuroblastoma patients since 1991 and 1999 respectively. In this study, we tried to use the 18F-FDOPA PET scan to detect and localize new marrow metastasis of neuroblastoma with DDC as a target.

Method/approach: From 8/1/06 to 7/31/09, we collected 17 cases of childhood neuroblastoma. The mean age of onset was 2y 10m. There were 2 of stage 3, 12 of stage 4 and 3 of stage 4S of disease. All patients were treated with chemotherapy/retinoic acid after surgery. We used 18F-Tc MDP bone scans, 18F-FDG and 18F-FDOPA PET scan every 3-6 months (ms) to monitor the progress of the disease and for new bone/ bone marrow metastases. Neither 18F-MIBG nor 18F-MIBG was used in this study because governmental regulation did not approve for import or production locally. The final diagnosis is based on tissue proof, correlation with other imaging studies and/or clinical confirmation.

Results: In these 17 cases we detected 8 new bone/marrow metastases, including one of each at skull base, rib, sacrum, radius, femur, calcaneus, and 2 at mandible. In all these cases 18F-FDOPA PET scan detected metastasis earlier than other imaging modalities. They can be detected 0-15 ms, 0-27 ms and 0-20 ms earlier than bone scan, 18F-FDG PET scan and MRI respectively. Four of them are ganglioneuroblastoma and 3 are neuroblastoma.

Conclusion: Uptake of 18F-FDOPA is indicative of the presence of neuroblastoma with DDC. If 18F-MIBG or 18F-MIBG was used in this study, the detection of minimal residual disease was impossible. 18F-FDOPA PET scan is highly recommended for early detection of bony metastasis in the follow up of neuroblastoma patients, especially those treated with retinoic acid. We plan to do further study comparing 18F-FDOPA PET scan with MIBG when it is ready.

Email: tzenky@nuth.gov.tw

POC48
Efficacy of Treosulan as a single agent in newly diagnosed neuroblastoma stage IV patients

Vasily Boyarchov1; Igor, Dolgopolov; Roman, Pimenov; George, Mentchew1
Pediatric Oncology and Hematology Institute, Bone Marrow Transplantation, Moscow, Russian Federation

Treosulan (Treo) is a structural analogue of Busulfan currently used for high-dose chemotherapy of advanced Ewing sarcomas, neuroblastomas (NB) and high-risk leukemias. There is a clinical evidence supporting the hypothesis that Treo is active as a single agent in pediatric malignancies. There for, from March 2009 to January 2010, 9 pts (M/F 5/4) with NB st IV, > then 2 years of age at the time of diagnosis were included in our window study. The median age was 8.2 (3-15). Seven pts had stage IV newly diagnosed, and two - relapse of NB (one 3 yrs after haploidentical PBSCT). Treo was applied two times in a dose 10g/m2 on days 1 and 7. After evaluation of the response high-risk protocol including 4 courses of CT, surgery, and High Dose CT with Auto PBSC rescue, followed by biological phase was delivered. PBSC harvest was performed after the 4 course. MIBG 1123 positive lesions retained after transplant were irradiated.

Efficacy of Treo was as follow: bone marrow was morphologically clean after 2 courses in 4 out of 9 pts (in 1 pt with relapse). Four pts achieved PR and 4 SD (3 of them without tumor clearance in BM) after 2 courses of Treo. Toxicity was minor with hematological toxicity of stage 1 in 2 pts. Another toxicity was not registered. Four out of 7 newly diagnosed pts completed protocol of 2 courses and 7 pts, correspondingly - 1 of them in CR and 3 in VGPR before biotherapy. Three pts are still on treatment. One relapsed pt is in VGPR 5 mo. after treatment and one pt died from PD.

In conclusion Treo is an effective agent in newly diagnosed NB pts and further evaluation of the doses, place and schedule are warranted.

Email: indolg@rambler.ru

POC49
Irinotecan/Temodal therapy as salvage treatment for children with neuroblastoma - single centre experience

Wieczorek, Aleksandra; Balwierz, Walentyna
Polish-American Institute of Pediatrics, Jagiellonian University, Medical College, Oncology/Hematology Department, Krakow, Poland

Aims: In spite of intensive multimodal first line therapy, still most of high risk Neuroblastoma patients have the disease. Unfortunately, so far there is no curative therapy for this group of patients, especially in the case of disseminated relapse. The aim of the study was evaluation of Irinotecan/Temodal therapy as salvage treatment in children with therapy resistant high risk neuroblastoma.

Methods: This is observational study in children with NBL progression/ relapse. From 2008-2009, 7 patients with relapse or progression of NBL treated in Hematology/Oncology Department in Krakow received Irinotecan/Temodal therapy (Kushner 2006). The group was heterogenous (1st - 4th relapse, different first line and previous relapse therapy). The end point of the study was survival analysis as well as evaluation of toxicities, quality of life and patient’s compliance. Observation was finished on 31.12.2009 r.

Results: In all 7 patients who received Irinotecan/Temodal therapy at last partial response was observed, including 2 with VGPR (residual tumor, no metastases). Median survival time was 31 (24-70.5) months from the first relapse and 61 (37-129) months from diagnosis. Patients received 6-20 chemotherapy cycles. Generally, therapy was well tolerated. The main toxicities were thrombocytopenia and anemia, requiring transfusions after almost every cycle in 47 pts as well as elevated transaminase (ALT up to 1500 IU). In 17 patients persistent and recurring transaminase increase led to need of decreasing drug dosage and necessity of less frequent chemotherapy employment. Diarrhea was not very severe and was manageable. Between chemotherapy cycles, as a rule children did not require hospitalization. The quality of life and compliance was satisfactory both for parents and their patients.

Conclusion: Irinotecan/Temodal chemotherapy seems to be reasonable choice for heavy pre-treated children with neuroblastoma, allowing for long-lasting therapy control without unacceptable toxicities, ensuring relatively good quality-of-life.

Email: wieczorek.aleksandra@wp.pl
POC51
Clinical report on the treatment of children in the late stage of neuroblastoma using chemotherapy combined with Zhongluo 3
Zhang, Jinhu1; Chen, Suning1; Yu, Fei1
1The 4th Affiliated Hospital, China Medical University, Pediatric Caner, Shenyang, China; 1The 2nd Affiliated Hospital, China Medical University, Traditional Chinese Medicine, Shenyang, China
Objective: To evaluate the effect, toxicity and lifecycle of small-dose chemotherapy combined with traditional Chinese decoction-Zhongluo3(L3) in treating children in the late stage of neuroblastoma (NB).
Methods: Forty-four children with NB in the 3rd or 4th stage were treated with VP, VCP, or VAP chemotherapy combined with L3.
Results: Twenty-five children got complete remission (CR), 7 got partial remission (PR). The total efficiency was 72.72% in this group, and no side effect related to death was found. With follow-up of 3-24 years, free-survival rate was found to be 51.3% within 3 years, 41.0% within 5 years, and 30.8% within 10 years.
Conclusion: The combination of small-dose chemotherapy with L3 is safe and effective in the treatment of children with NB in the 3rd or 4th stage, and the survival rate was found to increase greatly.
Email: ywang68@gmail.com

POC52
Effect of retinoic acid and chemotherapeutic agents on ultrastructural localization of Myc-N in neuroblastoma
Aktas, Safiye1; Altun, Zekiyi1; Ozgul, Candarr1; Olgun, Nur1; Gunes, Dilak1
1Dokuz Eylul University, Institute of Oncology, Dept. of Basic Oncology, Izmir, Turkey; 2Dokuz Eylul University, Institute of Oncology, Dept. of Pediatric Oncology, Izmir, Turkey
Purpose: Neuroblastoma is an important pediatric tumor that myc-n amplification is a well known poor prognostic indicator. The effect mechanism of pharmacological agents used in neuroblastoma treatment on myc-N expression is still unclear. Myc-N amplification does not change with any agent. The aim of this study is to investigate the effect of chemotherapeutic agents and cisplatin on ultrastructural localization of myc-N in neuroblastoma.
Method: We analyzed ultrastructural localization changes of n-myc by immunoelectron microscopy in n-myc positive, Kelly human neuroblastoma cell line using retinoic acid and cytotoxic drugs (cisplatin, vincristine, cyclophosphamide, etoposide, doxorubicin) and their combinations incubated for 24 hours in preoptimised LD50 doses in cell culture compared with control conditions. Cells were fixed in gluteraldehyde fixative and n-myc was applied by immunoelectron microscopy method using colloidal gold for visualization. Results were scored semiquantitatively as negative, mild, moderate, or high positive in nucleus, ribosome and cell membrane.
Results: Immunogold particles labeling myc-N was high in nucleus, ribosomes and low in cell membrane in control without any drug. It was moderate in nucleus in retinoic acid, cyclophosphamide, etoposide, cisplatin and their combinations groups. The nuclear expression was mild in, vincristine, doxorubicin and their combinations groups. N-myc expression was negative in cell membrane in all drugs. It was negative in ribosomes in all combination groups and doxorubicin and retinoic acid combined with vincristine group. Immunoelectron microscopic results showed that chemotherapeutic agents and their combinations caused a prominent decrease in myc-N expression in cell membrane, a medium level decrease in ribosomal level and a low decrease in nuclear ultrastructural localization.
Email: safiyeaktas@yahoo.com

POLB1–POLB13
POLB1
Effect of retinoic acid and chemotherapeutic agents on ultrastructural localization of Myc-N in neuroblastoma
Aktas, Safiye1; Altun, Zekiyi1; Ozgul, Candarr1; Olgun, Nur1; Gunes, Dilak1
1Dokuz Eylul University, Institute of Oncology, Dept. of Basic Oncology, Izmir, Turkey; 2Dokuz Eylul University, Institute of Oncology, Dept. of Pediatric Oncology, Izmir, Turkey
Purpose: Neuroblastoma is an important pediatric tumor that myc-n amplification is a well known poor prognostic indicator. The effect mechanism of pharmacological agents used in neuroblastoma treatment on myc-N expression is still unclear. Myc-N amplification does not change with any agent. The aim of this study is to investigate the effect of chemotherapeutic agents and cisplatin on ultrastructural localization of myc-N in neuroblastoma.
Method: We analyzed ultrastructural localization changes of n-myc by immunoelectron microscopy in n-myc positive, Kelly human neuroblastoma cell line using retinoic acid and cytotoxic drugs (cisplatin, vincristine, cyclophosphamide, etoposide, doxorubicin) and their combinations incubated for 24 hours in preoptimised LD50 doses in cell culture compared with control conditions. Cells were fixed in gluteraldehyde fixative and n-myc was applied by immunoelectron microscopy method using colloidal gold for visualization. Results were scored semiquantitatively as negative, mild, moderate, or high positive in nucleus, ribosome and cell membrane.
Results: Immunogold particles labeling myc-N was high in nucleus, ribosomes and low in cell membrane in control without any drug. It was moderate in nucleus in retinoic acid, cyclophosphamide, etoposide, cisplatin and their combinations groups. The nuclear expression was mild in, vincristine, doxorubicin and their combinations groups. N-myc expression was negative in cell membrane in all drugs. It was negative in ribosomes in all combination groups and doxorubicin and retinoic acid combined with vincristine group. Immunoelectron microscopic results showed that chemotherapeutic agents and their combinations caused a prominent decrease in myc-N expression in cell membrane, a medium level decrease in ribosomal level and a low decrease in nuclear ultrastructural localization.
Email: safiyeaktas@yahoo.com

POLB2
Betulinic acid affects metastasis related genes in neuroblastoma cells
Altun, Zekiyi1; Aktas, Safiye1; Gunes, Dilek1; Olgun, Nur1
1Dokuz Eylul University, Institute of Oncology, Dept. of Basic Oncology, Izmir, Turkey; 2Dokuz Eylul University, Institute of Oncology, Dept. of Pediatric Oncology, Izmir, Turkey; 3Dokuz Eylul University, Institute of Oncology, Dept. of Pediatric Oncology, Izmir, Turkey
Purpose: Betulinic acid is a pentacyclic triterpene found in many plant species. BA was reported to display a wide range of biological effects, including antibacterial and anti-inflammatory activities, and in particular to inhibit growth of cancer cells. The aim of this study is to explore effect of cisplatin, betulinic acid and their combination on metastasis related genes in neuroblastoma.
Methods: Kelly (N-Myc positive) and SHSY5Y (N-Myc negative) neuroblastoma cell lines were cultured and the agents and their combinations were applied for 24 hour in pre-optimized doses. After RNA isolation and cDNA converting, expression of 84 custom array genes of tumor metastasis (SABiosciences, PATS028A) was determined by Real Time PCR for each condition. Kelly and SHSHY neuroblastoma cell lines without any agents were used as control. Fold changes according to control group of each three condition were calculated at manufacturer's online free data PCR expression analysis page.
Results: High expressed genes after betulinic acid application are only PNN in SHSY5Y cells and most of the metastatic genes in Kelly cells. Betulinic acid, cisplatin or the combinations showed in low expression most of the metastatic genes in SHSY5Y cells. Cisplatin and betulinic acid combination was showed same as betulinic acid metastatic gene expression pattern in each cell line. Cisplatin caused increases only in adhesion associated FXYDS in Kelly while DENR, SMAD and PNN in SHSY5Y cells. In Kelly cells, cisplatin decreased some metastatic gene expressions but betulinic acid or betulinic acid-cisplatin combination reduced only in DENR and RB1 gene expressions.
Conclusion: Betulinic acid showed prominent effect of gene expression related with tumor metastasis after application to neuroblastoma cells alone and combination with cisplatin in particularly N-myc positive Kelly neuroblastoma cells. Contrarily, betulinic acid and combinations decreased the metastatic gene expressions like as cisplatin in N-myc negative SHSY5Y neuroblastoma cells. These results suggesting that cisplatin is the most relevant agent in especially aggressive neuroblastoma treatment.
Email: dilek.gunes@deu.edu.tr
POLB3 Human neuroblastoma microenvironment supports T-cell activation in tumors associated lymphocytes

Carlo DiMaio, Partners in Medicine, Department of Radiation Oncology, Massachusetts General Hospital, Boston, MA; 2Karolinska Institutet, Karolinska University Hospital, Department of Oncology-Pathology, Stockholm, Stockholm, Sweden; 3Karolinska Institutet, Department of Oncology-Pathology, Stockholm, Stockholm, Sweden; 4Göteborg University, Sahlgrenska University Hospital, Department of Clinical Genetics, Göteborg, Sweden; 5Karolinska Institutet, Karolinska University Hospital, Department of Women’s and Children’s Health, Stockholm, Stockholm, Sweden; 6Göteborg University, Sahlgrenska University Hospital, Department of Clinical Genetics, Göteborg, Sweden; 7Johns Hopkins University, School of Public Health, Baltimore, Baltimore, Maryland.

Background: Although lymphocytic infiltration has been previously linked to increased survival of patients with neuroblastoma (NB), the development of these tumors was not prevented by the presence of T cells in their microenvironment, suggesting that either the tumor milieu is not permissive for T cell activation, or lymphocytic NB infiltrates are devoid of T-cells, able to efficiently control tumor progression. Here, we report systematic analysis of T-cell subsets present in the peripheral blood and tumor samples from NB patients, representing all clinical and genetic forms of the tumor. We also characterize the pattern of cytokine production by T-cells in both compartments.

Methods: Analysis of T-cell phenotype was done by immunostaining and flow cytometry. Neuroblastoma-infiltrating T-cells were visualised by immunohistochemistry. In vitro cytokine production was measured using multiplex analysis and ELISA. All patients followed for >3 years.

Results: This study shows that tumor-associated lymphocytes (TALs) from NB show different peripheral blood lymphocyte (PBL) profiles and TNF-β levels in comparison to other malignancies. The analysis of T-cell phenotype revealed that TALs expressed higher levels of regulatory T-cells.

Conclusion: The results obtained with ongoing specific activation of CD4+ T-cells in NB microenvironments, with a view to be permissive for effector memory T-cell differentiation and suppress the development of regulatory T-cells.

Email: jelevits@jhsphs.edu

POLB5 Alllicin increases metastasis related genes in neuroblastoma

Allicin: Dilek Gunes1; Aktaş, Safiyə2; Altim, ZeiKate; Oğuz, Nur3

1Dokuz Eylül University Institute of Oncology, Dept. of Pediatric Oncology, İzmir, Turkey; 2Dokuz Eylül University Institute of Oncology, Dept. of Basic Oncology, İzmir, Turkey.

Purpose: Overcoming of toxic effects of cisplatin in neuroblastoma treatment is a current issue. The try of discovering new agents to decrease toxicity of chemotherapeutic agents needs very much careful assessment not to cause tumor progression. The aim of this study is to explore effect of cisplatin, alllicin and their combination on metastasis related genes in neuroblastoma.

Method: Kelly cell line was cultured and the agents and their combinations were applied for 24 hour in pre-optimized doses. After RNA isolation and cDNA converting, expression of 84 custom array genes of tumor metastasis (SABiosciences, PAT021A) was determined by Real Time PCR for each condition. Kelly neuroblastoma cell line without any agent was used as control. Fold changes according to control group of each three condition were calculated at manufacturer’s online free data PCR expression analysis page.

Results: High expressed genes after alllicin application are ITGB3, TNFSF10, HGDCC, CCL7, CTSL1, ET14, KISS1R, HTATIP2, IL1B, IL8, IL2, ITGAV, KISS1, MMP10, MMP3, MMP7, MYC, MYCL1, SYK, TIMP4, MMP13, TRPM1, CDH11, and FXYD5. The high expressed genes are related with cell growth and proliferation, extracellular matrix proteins, transcription factors. This high expression was not observed alone with cisplatin, but also in cisplatin+alllicin combination.

Conclusion: Allicin showed prominent effect of gene expression related with tumor metastasis after application to neuroblastoma cells alone and in combination with cisplatin application. Our data showing increase effect of alllicin on metastasis related genes indicates that alllicin additive treatment as a protective agent of chemotherapy toxicity should be very well questioned in neuroblastoma.

Email: dilek.gunes@deu.edu.tr
**POLB7**

**Modeling the p53-Mdm2 core module in neuroblastoma**

Lamprecht, Elke1; Drexler, Dan1; Gogolin, Sonja2; Pöthker, Christina2; Schwab, Manfred1; Westermann, Frank1; Höfer, Thomas1

1DKFZ Heidelberg, Modeling of Biological Systems, Heidelberg, Germany; 2DKFZ Heidelberg, Tumor Genetics, Heidelberg, Germany.

**Background:** The p53-MDM2 regulatory unit controls the cellular decision to undergo apoptosis or cell-cycle arrest upon DNA damage. This p53-MDM2 core module is frequently altered in neuroblastoma leading to impaired cell-cycle arrest and DNA-damage response. Unlike in other tumor entities, the aberrant function is rarely attributed to genetic mutations. It appears to be due to an imbalance in the expression and function of p53 and MDM2, which is influenced by deregulated N-MYC.

**Aims:** To rationalize how deregulated H-MYC perturbs the p53-MDM2 core module (upon DNA-damage) using a systems biology approach.

**Method:** To describe N-MYC-dependent perturbations of the p53-MDM2 module, we used cell systems that allow targeted overexpression or knock-down of N-MYC and developed a data-based kinetic model. By means of ordinary differential equations the dynamics of p53, MDM2 as well as cell-cycle (e.g. p21) and apoptosis-related (e.g. PUMA) p53 targets are simulated.

**Results:** Our model simulations qualitatively reproduce the observed changes of the mRNA and protein measurements. First results indicate that the interplay of p53, MDM2, E2F1 and N-MYC can account for the experimentally observed dynamics upon induction of DNA-damage in N-MYC single-copy versus amplified tumor cells. Simulations suggest a dominant role of MDM2 in disabling the DNA-damage response by directly inhibiting both p53 and p21.

**Conclusion:** The mathematical model is capable of qualitatively reproducing the observed protein kinetics. Although in N-MYC amplified cells both p53 and p21 expression and subcellular localization, MDM2 appears to be dominant for the p21 response upon DNA-damage.

Email: ilamprecht@dkfz.de

**POLB8**

**Biological characteristics of neuroblastoma in children of Belarus.**

Proleskovskaya, Inna1; Valochnik, Alena1; Savva, Natalia1

1Belarusian Research Center for Pediatric Oncology and Hematology, Scientific, Minsk reg, Belarus; 2Belarusian Research Center for Pediatric Oncology and Hematology, Scientific, Minsk reg, Belarus.

**Background:** To evaluate the biological characteristics of neuroblastoma in Belarusian children and to estimate their prognostic value.

**Method/approach:** 101 patients (pts) received treatment from October 1997 till October 2009 were included into the study. N-MYC amplification, 1p deletion and ploidy of tumor cells were assessed by FISH method.

**Results:** N-MYC amplification was evaluated in 101 pts: negative n=70(69.3%); less than 10 copies n=8(7.9%); 10 copies and more n=23(22.7%). N-MYC amplification was detected in 2/18 pts with stage 1; 2/8 pts with stage 2; 5/32 pts with stage 3; 4/5 pts with stage 4S. N-MYC was detected in 101 pts. N-MYC expression and/or function was achieved by specific shRNAs, blocking intracellular signaling, which promotes tumor growth and/or survival and sensitizes neuroblastoma cells to TNF treatment.

**Conclusion:** In children of Belarus with neuroblastoma adverse prognostic tumor markers (N-MYC amplifications and 1p deletion) observed in 25.9% that correlate with the data in the world. Occurrence of N-MYC amplification associates with disease stages in the majority of established human NB lines and mouse short-term NB cell lines derived from TH N-MYC mice was monitored by immunostaining and flow cytometry. Inhibition of TNF expression and/or function was achieved by specific shRNAs, blocking antibody and soluble TNF-R2. Sensitivity of human neuroblastoma cells to TNF and natural killer (NK) cells was determined in cytokotoxicity assays. Viability and proliferation of human and mouse neuroblastoma cells were determined by flow cytometry-based methods.

**Results:** The majority of established human NB lines and spontaneous NB tumors from N-MYC transgenic mice express TNF at the cell surface. TNF is expressed on the surface of NB cells in the membrane-bound form and continuously occupies a large proportion of TNF-receptors expressed on the surface of NB cells. TNF blockade results in inhibition of proliferation, increased rate of apoptosis and increased sensitivity of NB cells to lysis by the cytoxic lymphocyes.

**Conclusion:** TNF alpha acting in an autotochthonous fashion initiates intracellular signaling, which promotes tumor growth and/or survival and results in decreased susceptibility of neuroblastoma cells for target cells by recognition by the immune system.

Email: jlejevts@zhsp.edu
An integrative bioinformatics approach in neuroblastoma identifies converging alterations in protein networks related to mitotic spindle assembly and splicing

Wen Fong, Olof; Re, Angela; Arseni, Natalia; Canella, Valentina; Guarguaglini, Giulia; Lavia, Patrizia; Scaruffi, Paola; Tonini, Gian Paolo; Quattrone, Alessandro

1University of Trento, Centre for Integrative Biology, Trento, Italy; 2National Cancer Research Institute (IST), Translational Paediatric Oncology, Genoa, Italy

Abstract Book 217

An integrative bioinformatics approach in neuroblastoma identifies converging alterations in protein networks related to mitotic spindle assembly and splicing.

Wen Fong, Olof; Re, Angela; Arseni, Natalia; Canella, Valentina; Guarguaglini, Giulia; Lavia, Patrizia; Scaruffi, Paola; Tonini, Gian Paolo; Quattrone, Alessandro

1University of Trento, Centre for Integrative Biology, Trento, Italy; 2National Cancer Research Institute (IST), Translational Paediatric Oncology, Genoa, Italy

Genome-wide studies, profiling either DNA copy number or gene expression, are importantly proposed for neuroblastoma prognosis and possible treatment choice. Nonetheless, it is still largely unclear how to integrate these systems-level molecular portrait types of tumor genetic instability in order to obtain the maximal informative power. We suggest a novel unbiased way to combine matching aCGH and microarray transcriptome analysis from single neuroblastoma tumor samples, initially applied to public domain data. At the core of our approach is the ability to use both data types in an unbiased and unsupervised fashion, identifying statistically significant high correlation modules. Each module associates copy number alterations and genes whose expression follows a common profile in different tumors, therefore representing putative cause-effect genome-transcriptome maps. Enrichment analysis through gene ontology categories was conducted on the significant modules and the derived results were used to isolate the maximally informative genes. Remarkably, increased expression of each of these genes significantly correlated with the clinical outcome of the profiled patients, and the corresponding proteins were all interacting in a functional network. A panel of parental neuroblastoma cell lines profiled at high resolution for copy number alterations allowed us to more precisely define the genomic lesions highly related to the informative genes, and to visualize the corresponding binary network. This integrative analysis led to the formulation of an unanticipated hypothesis on converging genomic alterations in neuroblastoma, polarized on the upregulation of several genes active in the spindle assembly process and in the spliceosome complex. We also report a phenotypic analysis in neuroblastoma cells confirming functionally impacting alterations in both cell machineries, therefore highlighting the power of an unbiased integration of genome and transcriptome data.

Email: alessandro.quattrone@unitn.it
<table>
<thead>
<tr>
<th>Author Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
</tr>
<tr>
<td>Abdelhady, Shaimaa OR14, PL11</td>
</tr>
<tr>
<td>Abe, Hiroyuki</td>
</tr>
<tr>
<td>Abel, Frida POB42, SEL4*</td>
</tr>
<tr>
<td>Abrahamsson, Jonas POB71, POB72, SEL8</td>
</tr>
<tr>
<td>Acipayam, Can POB58, POB59</td>
</tr>
<tr>
<td>Ackermann, Sandra OR5*, OR40</td>
</tr>
<tr>
<td>Acosta, Sandra POB1</td>
</tr>
<tr>
<td>Acquaviva, Massimo POB27, POT25</td>
</tr>
<tr>
<td>Adami, Valentina OR52</td>
</tr>
<tr>
<td>Admiralai, Pieter POT21, POT22</td>
</tr>
<tr>
<td>Aigner, Gerhard OR38</td>
</tr>
<tr>
<td>Akira, Nakagawara POB106, POB107</td>
</tr>
<tr>
<td>Akogul, Nurdan POB106, POB107, SEL20</td>
</tr>
<tr>
<td>Akter, Jesmin POB101, POT40, POT41, POT68</td>
</tr>
<tr>
<td>Al-awar, Rima OR14, PL15</td>
</tr>
<tr>
<td>Albert, David POB47</td>
</tr>
<tr>
<td>Albinh, Ami POT26</td>
</tr>
<tr>
<td>Albin, Sonia OR49, OR49</td>
</tr>
<tr>
<td>Alcock, Leah OR46, OR75, PL21, PL27, POB2*, POB18, POB29</td>
</tr>
<tr>
<td>Aleinikova, Olga POLB9</td>
</tr>
<tr>
<td>Alferiev, Ivan POT56</td>
</tr>
<tr>
<td>Ali, Rouknuddin SEL34</td>
</tr>
<tr>
<td>Allan, Balmain POB52</td>
</tr>
<tr>
<td>Altun, Zekiye POLB1, POLB2, POLB5, SEL20</td>
</tr>
<tr>
<td>Amann, Gabriele OR38, OR84, POB15</td>
</tr>
<tr>
<td>Ambrogio, Chiara OR71</td>
</tr>
<tr>
<td>Ambros, Inge OR23, OR38*, OR84, POB15, SEL40*, SEL41, SEL42, OR23*, OR38, OR60, OR64, OR84, POB15, SEL40, SEL41, SEL42, OR57</td>
</tr>
<tr>
<td>Ambros, Peter</td>
</tr>
<tr>
<td>Ames, Matthew M. POT11</td>
</tr>
<tr>
<td>Amiel, Jeanne PL3*</td>
</tr>
<tr>
<td>Amoroso, Loredana OR14, PL11*</td>
</tr>
<tr>
<td>Anders, Clarke POC32*</td>
</tr>
<tr>
<td>Anderson, Clarke POC44</td>
</tr>
<tr>
<td>Anderson, John POB21</td>
</tr>
<tr>
<td>Ando, Hiromi POT76</td>
</tr>
<tr>
<td>Ando, Kyojio OR10, OR11*</td>
</tr>
<tr>
<td>Ando, Koji OR74*, OR86</td>
</tr>
<tr>
<td>Angelini, Paola PL10, POB3*, SEL48*</td>
</tr>
<tr>
<td>Anicic, Mirna POC1*</td>
</tr>
<tr>
<td>Annis, James POT65</td>
</tr>
<tr>
<td>Antonio, Daga OR31, OR38, OR60, OR64, OR84, POB15, SEL40, SEL41, SEL42, OR57</td>
</tr>
<tr>
<td>Anuszkiwicz, Shelli POB4*</td>
</tr>
<tr>
<td>Appelbaum, Daniel PL9</td>
</tr>
<tr>
<td>Ara, Tasnim</td>
</tr>
<tr>
<td>Arakawa, Hirofumi</td>
</tr>
<tr>
<td>Arca, Alexandre OR26</td>
</tr>
<tr>
<td>Ardley, Kerry OR3</td>
</tr>
<tr>
<td>Arora, Brijesh POC15</td>
</tr>
<tr>
<td>Arseni, Natalia POB12</td>
</tr>
<tr>
<td>Asgharzadeh, Shahab OR36, PL8, POB5*, POC13, POB13, POT1*, POT2*, WS20</td>
</tr>
<tr>
<td>Ash, Shifra OR60, POB6, POT45</td>
</tr>
<tr>
<td>Ashley, David OR3</td>
</tr>
<tr>
<td>Ashton, Lesley OR31</td>
</tr>
<tr>
<td>Askenasy, Nadir POB66</td>
</tr>
<tr>
<td>Asmaa, Heggo POB68</td>
</tr>
<tr>
<td>Astrahantseff, Kathy POT19*</td>
</tr>
<tr>
<td>Atrash, Butrus PL16</td>
</tr>
<tr>
<td>Atisuko, Nakagawa POB61</td>
</tr>
<tr>
<td>Atsushi, Takatori POB61</td>
</tr>
<tr>
<td>Attiyeh, Edward OR61, OR76, PL5, PL8, PL18, PL19, POB5, POC13, POT2*, POT32, SEL23, WS20</td>
</tr>
<tr>
<td>Auger, Nathalie OR34</td>
</tr>
<tr>
<td>Ausserlechner, Michael POB81, SEL39*</td>
</tr>
<tr>
<td>Avanzini, Stefano OR36, POT45</td>
</tr>
<tr>
<td>Avigad, Smadar POT34</td>
</tr>
<tr>
<td>Axelson, Cecilia POB74, POB111</td>
</tr>
<tr>
<td>Axelsson, Hakan POT3*</td>
</tr>
<tr>
<td>Ayers, Duncan POT4</td>
</tr>
<tr>
<td>Azarova, Anna OR28</td>
</tr>
<tr>
<td>Azorsa, David</td>
</tr>
<tr>
<td>Babich, John OR59</td>
</tr>
<tr>
<td>Bachetti, Tiziana OR59</td>
</tr>
<tr>
<td>Bachmann, Andre OR66, OR72</td>
</tr>
<tr>
<td>Bachmann, Hagen PL24*</td>
</tr>
<tr>
<td>Badgett, Thomas OR28, OR37</td>
</tr>
<tr>
<td>Badgett, Tom POT15</td>
</tr>
<tr>
<td>Bahl, Gaurav OR61</td>
</tr>
<tr>
<td>Baker, David POB85</td>
</tr>
<tr>
<td>Bakkendorf, Wenche POT43, POT44</td>
</tr>
<tr>
<td>Balcerska, Anna POT49</td>
</tr>
<tr>
<td>Ball, LM SEL27</td>
</tr>
<tr>
<td>Balmas, Boullourd, Katia OR60, POB16, POC49</td>
</tr>
<tr>
<td>Balwierz, Walentyne POB15</td>
</tr>
<tr>
<td>Banavalli, Sripad OR24*</td>
</tr>
<tr>
<td>Barbieri, Eveline POB28</td>
</tr>
<tr>
<td>Barelli, Claire POB78, POB21, WS19</td>
</tr>
<tr>
<td>Barla, Annalisa PL16, SEL36</td>
</tr>
<tr>
<td>Barrett, John POB27, POT25</td>
</tr>
<tr>
<td>Bartolazzi, Armando OR59</td>
</tr>
<tr>
<td>Baruchel, Sylvain POB84, POB109</td>
</tr>
<tr>
<td>Barseghyan, Arman POB5, POB8, POC38, POC39, POT1*, POT5*, POT86, WS10*</td>
</tr>
<tr>
<td>Baryawno, Ninib POT23</td>
</tr>
<tr>
<td>Bates, David POT61</td>
</tr>
<tr>
<td>Battaglia, Florinda POB87</td>
</tr>
<tr>
<td>Bauer, Matthieu POB45</td>
</tr>
<tr>
<td>Bauer, Tobias PL7</td>
</tr>
<tr>
<td>Bawa, Olivia POT18</td>
</tr>
<tr>
<td>Bavetsias, Vassilios PL16</td>
</tr>
<tr>
<td>Bayat Mokhtari, Reza POB8*</td>
</tr>
<tr>
<td>Bayram, Ibrahim POC12</td>
</tr>
<tr>
<td>Beaudry Paul OR29</td>
</tr>
<tr>
<td>Becker, Oren OR60*, POC5*</td>
</tr>
<tr>
<td>Becker, Gabrielle POB48</td>
</tr>
<tr>
<td>Beckman, Siv OR8</td>
</tr>
<tr>
<td>Beckstead, Wesley WS16</td>
</tr>
<tr>
<td>Bedalov, Antonio OR47</td>
</tr>
<tr>
<td>Bedwell, C OR38, SEL41</td>
</tr>
<tr>
<td>Beiske, C OR38, OR60, OR65, SEL40</td>
</tr>
<tr>
<td>Beiske, Klaus OR26</td>
</tr>
<tr>
<td>Beissbarth, Tim SEL30</td>
</tr>
<tr>
<td>Bekassy, Albert N. SEL11</td>
</tr>
<tr>
<td>Bell, Emma POB70</td>
</tr>
<tr>
<td>Bell, Jessica POB764</td>
</tr>
<tr>
<td>Bell, John POT66</td>
</tr>
<tr>
<td>Bellotti, Marta POT67</td>
</tr>
<tr>
<td>Benard, J OR36, OR38, POB45, SEL40, SEL41</td>
</tr>
<tr>
<td>Bender, Ariane OR4</td>
</tr>
<tr>
<td>Benelli, Roberto POC30</td>
</tr>
<tr>
<td>Benetkiewicz, Magdalena OR1</td>
</tr>
<tr>
<td>Benhamou, Ellen SEL15</td>
</tr>
<tr>
<td>Benso-Kennedy, Debora POC23</td>
</tr>
<tr>
<td>Berbegall, Ana P. POT77, POT8, POT67</td>
</tr>
<tr>
<td>Berger, Elisa OR17, OR20*</td>
</tr>
<tr>
<td>Bergeron, Christophe OR34, OR87</td>
</tr>
<tr>
<td>Bergmann, Eckhard OR39</td>
</tr>
<tr>
<td>Bernardi, Roberto SIEL</td>
</tr>
<tr>
<td>Bernas, Tytus WS12</td>
</tr>
</tbody>
</table>
Abstract Book 219

Author Index

* = Presenting author

Berthier, Arnaud POT8
Berthold, Frank OR82, OR36, OR40, OR62, OR64, OR66, OR79, OR81, PL23, PL35, POC3*, POC26, POC33*, POC37, POT1, POT77, SEL13, SS4*
Berthold, Jessica SEL2
Besançon, Odette POB9*
Bets, DR POT32
Bhatia, Smita OR81, PL23, PL35, POC3*, POC26, POT1, POT77, SEL13, SS4*
Bianchi, Giovanna OR56*, POB10*, POB11*, POT9*
Biankin, Andrew OR82, OR36, OR62, OR64, OR66, OR79, OR81, PL23, PL35, POC3*, POC26, POC33*, POC37, POT1, POT77, SEL13, SS4*
Bleck, Julian PL16
Blakeny, Kim OR9, PL10
Bleeke, Matthias SEL26
Blobel, Gerd A. POT47
Blumrich, Anne POB12*, POB13*, SEL3
Boa, Paola OR16, OR17*
Boccardo, Simona POB30
Boe, U POT78
Boehr, de, Judith POT22
Boe, Valentina OR78, POB21, WS19
Bogen, Dominik POB15*
Bohlert, Anna SEL38
Bölk-Marzec, Katarzyna POB16*
Bolotin, Ellen POB18*
Boni, Stefano OR79, POT77
Bonghi, Silvia POB7
Borkardt, Anrdt OR62
Bosse, Kristopher OR33, OR76, PL5, PL19
Bos, Anna-Karin POB74
Bourdeaut, Franck OR34, POT11
Bowdin, Sarah POT8
Bown, N OR38, SEL40, SEL41
Boyd, Marie OR38, SEL40, SEL41
Bray, Isabella OR38, SEL40, SEL41
Brejon, Stephanie OR38, SEL40, SEL41
Brenner, Malcolm OR19
Brichard, Bénédicte OR60
Bridges, Esther OR8
Brign,...
<table>
<thead>
<tr>
<th>Author</th>
<th>Indexes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chen, Zaowen</td>
<td>SEL17</td>
</tr>
<tr>
<td>Cheng, Ngan Ching</td>
<td>OR24</td>
</tr>
<tr>
<td>Chesler, Louis</td>
<td>C12*, OR2, OR12, PL16, PL17, POC7, POT17, SEL36, SEL46, WS9</td>
</tr>
<tr>
<td>Cheuh, Heewon</td>
<td>POC40, POC42</td>
</tr>
<tr>
<td>Cheuk, Adam</td>
<td>WS16</td>
</tr>
<tr>
<td>Cheung, Belamy</td>
<td>OR25, POC70</td>
</tr>
<tr>
<td>Cheung, Irene</td>
<td>OR77, OR89*, PL34, POT83</td>
</tr>
<tr>
<td>Cheung, Nai-Kong</td>
<td>OR15, OR63*, OR77, OR89, PL34, POC2, POC14, POC16, POC24, POT27, POT83*, SEL12</td>
</tr>
<tr>
<td>Chiarle, Roberto</td>
<td>OR71, OR24, OR12, PL16, PL17, POC7, OR89*, PL34, POC2, POC14, POC16, POC24, POT27, POT83*, SEL12</td>
</tr>
<tr>
<td>Children’s, Oncology Group, USA</td>
<td>C8</td>
</tr>
<tr>
<td>Chin, Motoaki</td>
<td>POC35</td>
</tr>
<tr>
<td>Chlenski, Alexandre</td>
<td>POC40</td>
</tr>
<tr>
<td>Cho, Eun Joo</td>
<td>OR25</td>
</tr>
<tr>
<td>Chorny, Michael</td>
<td>POC40</td>
</tr>
<tr>
<td>Christensen, James</td>
<td>POC7</td>
</tr>
<tr>
<td>Christiansen, Holger</td>
<td>POC11, OR97, OR100, PL4, PL17, POC7, POT17, SEL36, SEL46, WS9</td>
</tr>
<tr>
<td>Christiansen, Nina Merete</td>
<td>OR36, OR39, SEL13</td>
</tr>
<tr>
<td>Chueh, Heewon</td>
<td>OR39</td>
</tr>
<tr>
<td>Chybicka, Alicja</td>
<td>POC44</td>
</tr>
<tr>
<td>Cilli, Michele</td>
<td>OR16, OR29, OR71, POC11, POT9, SEL35</td>
</tr>
<tr>
<td>Cinatl Jr., Jindrich</td>
<td>SEL18</td>
</tr>
<tr>
<td>Cinzia, Bersani</td>
<td>SEL32</td>
</tr>
<tr>
<td>Clark, Owen</td>
<td>POC29, POC19</td>
</tr>
<tr>
<td>Clarke, Jan</td>
<td>OR30</td>
</tr>
<tr>
<td>Clerico, Anna</td>
<td>POLB4</td>
</tr>
<tr>
<td>Cleveland, Don</td>
<td>OR1</td>
</tr>
<tr>
<td>Clifford, Steven</td>
<td>OR11</td>
</tr>
<tr>
<td>Cobo, Claudia</td>
<td>SEL35</td>
</tr>
<tr>
<td>Coco, Simona</td>
<td>OR79*, OR80, OR85, POC11, POT17, SEL36, SEL46, WS9</td>
</tr>
<tr>
<td>Cohn, Susan</td>
<td>C3, OR51, OR61, OR64, OR82, PL32, POC110, POC29, SEL9</td>
</tr>
<tr>
<td>Cole, Kristina</td>
<td>OR34, OR38, OR60, OR78, OR87*, POC45, SEL40, SEL14, WS19</td>
</tr>
<tr>
<td>Conte, Massimo</td>
<td>OR10, POC21, POC45, SEL40, SEL14, WS19</td>
</tr>
<tr>
<td>Cools, Jan</td>
<td>OR65</td>
</tr>
<tr>
<td>Cornero, Andrea</td>
<td>OR88*, PL29, POC70</td>
</tr>
<tr>
<td>Corrias, Maria</td>
<td>OR88*, PL29, POC70</td>
</tr>
<tr>
<td>Corrias, Maria Valeria</td>
<td>OR88*, POC2, POC16*</td>
</tr>
<tr>
<td>Corrigan, Kelly-Anne</td>
<td>SEL17</td>
</tr>
<tr>
<td>Courvetta, Daisy</td>
<td>OR54*</td>
</tr>
<tr>
<td>Coulon, Aurelie</td>
<td>POC39, POC65, SEL25*, WS6*</td>
</tr>
<tr>
<td>Courranger, Sonia</td>
<td>OR28</td>
</tr>
<tr>
<td>Courtright, Joshua</td>
<td>PL18</td>
</tr>
<tr>
<td>Couturier, J</td>
<td>SEL41</td>
</tr>
<tr>
<td>Couturier, Jerome</td>
<td>OR34, OR38, SEL40</td>
</tr>
<tr>
<td>Cozzi, Dennis A</td>
<td>POLB4</td>
</tr>
<tr>
<td>Croce, Michela</td>
<td>POC30, POC16</td>
</tr>
<tr>
<td>Crosazzo Franscini, Laura</td>
<td>POC5</td>
</tr>
<tr>
<td>Crotty, Catherine</td>
<td>OR82</td>
</tr>
<tr>
<td>Cui, Hongqian</td>
<td>OR25</td>
</tr>
<tr>
<td>Cullinan, Carleen</td>
<td>OR3</td>
</tr>
<tr>
<td>Cullis, Elizabeth</td>
<td>PL16, POC17*, SEL36</td>
</tr>
<tr>
<td>Cunningham, Anne</td>
<td>POC7</td>
</tr>
<tr>
<td>Currier, Erika</td>
<td>POC7</td>
</tr>
<tr>
<td>Czaplicki, Dominik</td>
<td>EL4</td>
</tr>
<tr>
<td>D</td>
<td></td>
</tr>
<tr>
<td>D’Haene, Nicky</td>
<td>OR77</td>
</tr>
<tr>
<td>Dagis, Andrew</td>
<td>OR77</td>
</tr>
<tr>
<td>Daldrup-Link, Heike</td>
<td>OR77</td>
</tr>
<tr>
<td>Dalevi, Daniel</td>
<td>OR77</td>
</tr>
<tr>
<td>Dallorso, Sandro</td>
<td>OR88*, PL29</td>
</tr>
<tr>
<td>Danen, Erik H.J.</td>
<td>OR77</td>
</tr>
<tr>
<td>Das, Bikul</td>
<td>OR46, POC21, POC82, POC18, POC29*</td>
</tr>
<tr>
<td>Das, Sudipto</td>
<td>POLB11</td>
</tr>
<tr>
<td>Dassi, Erik</td>
<td>PL15</td>
</tr>
<tr>
<td>Dauthoos, Dubes, Estelle</td>
<td>OR69, POC18*, POC66*</td>
</tr>
<tr>
<td>Daveau, Romain</td>
<td>OR78</td>
</tr>
<tr>
<td>Davidoff, Andrew</td>
<td>WP19</td>
</tr>
<tr>
<td>Davies, Neil</td>
<td>OR25</td>
</tr>
<tr>
<td>Day, Philip J</td>
<td>OR14</td>
</tr>
<tr>
<td>de Alava, Enrique</td>
<td>OR35, OR41, OR65, OR65, POC26, POC85, POC87, POC90, POC98, POC99, POC100, POC101</td>
</tr>
<tr>
<td>de Antonelis, Pasqualino</td>
<td>OR77</td>
</tr>
<tr>
<td>De Bernardi, Bruno</td>
<td>OR76, OR64, SEL40, SEL41</td>
</tr>
<tr>
<td>De Bondt, Ann</td>
<td>OR65</td>
</tr>
<tr>
<td>De Brouwer, Sara</td>
<td>OR41*, OR65, WP5</td>
</tr>
<tr>
<td>De Carolis, Boris</td>
<td>OR62, OR81*, POC26, SEL13</td>
</tr>
<tr>
<td>De Cicerq, Sarah</td>
<td>POC79</td>
</tr>
<tr>
<td>De Gouveia, Paolo</td>
<td>OR14</td>
</tr>
<tr>
<td>De Mariano, Marilena</td>
<td>OR35, OR41, OR65, OR66, PL27, POC18, POC32*, POC34, POC38, POC55, POC73, SEL4, SEL8, WS4, WS5*</td>
</tr>
<tr>
<td>De Paep, Anne</td>
<td>OR60</td>
</tr>
<tr>
<td>De Preter, Katleen</td>
<td>OR60</td>
</tr>
<tr>
<td>De Schrijver, Joachim</td>
<td>OR36</td>
</tr>
<tr>
<td>de Torres, Carmen</td>
<td>OR36</td>
</tr>
<tr>
<td>De Vecchi, Carla</td>
<td>POC32, POC34*</td>
</tr>
<tr>
<td>De Wilde, Bram</td>
<td>SEL35</td>
</tr>
<tr>
<td>Deaglio, Silvia</td>
<td>OR4</td>
</tr>
<tr>
<td>Debatin, Klaus-Michael</td>
<td>OR35</td>
</tr>
<tr>
<td>DeCarolis, Boris</td>
<td>OR79, POC77</td>
</tr>
<tr>
<td>DeClerck, Yves</td>
<td>OR89</td>
</tr>
<tr>
<td>Défachelles, Anne-Sophie</td>
<td>OR60</td>
</tr>
<tr>
<td>De Feffera, Raffaella</td>
<td>OR38, OR68, OR57, SEL40, SEL41</td>
</tr>
<tr>
<td>DeGeer, Anna</td>
<td>POC6</td>
</tr>
<tr>
<td>Degen, Stephanie</td>
<td>OR1</td>
</tr>
<tr>
<td>Degrand, Olivier</td>
<td>OR13</td>
</tr>
<tr>
<td>Del Grosso, Federica</td>
<td>OR14</td>
</tr>
<tr>
<td>Delhaye, Nicolas F.</td>
<td>OR21</td>
</tr>
<tr>
<td>Delattre, Olivier</td>
<td>OR54</td>
</tr>
<tr>
<td>Delekins, Catharina</td>
<td>OR24</td>
</tr>
<tr>
<td>Delehouze, Claire</td>
<td>OR24</td>
</tr>
<tr>
<td>Della Valle, Giuliano</td>
<td>OR24</td>
</tr>
<tr>
<td>Dembowska, Bozena</td>
<td>OR24</td>
</tr>
<tr>
<td>Demirhan, Osman</td>
<td>OR24</td>
</tr>
<tr>
<td>den Boer, Monique</td>
<td>OR24</td>
</tr>
<tr>
<td>Desai, Sangeeta</td>
<td>OR24</td>
</tr>
<tr>
<td>Desban, Nathalie</td>
<td>OR24</td>
</tr>
<tr>
<td>Dessmond, Higgins</td>
<td>OR24</td>
</tr>
<tr>
<td>Dessen, Philippe</td>
<td>OR24</td>
</tr>
<tr>
<td>Deubzer, Beate</td>
<td>OR24</td>
</tr>
<tr>
<td>Deubzer, Hedwig</td>
<td>OR24</td>
</tr>
<tr>
<td>Deveneney, Irene</td>
<td>OR24</td>
</tr>
<tr>
<td>Devoto, Marcella</td>
<td>OR24</td>
</tr>
<tr>
<td>DhAene, Nicky</td>
<td>OR24</td>
</tr>
<tr>
<td>Di Lascio, Simona</td>
<td>OR24</td>
</tr>
<tr>
<td>Di Leo, Korine</td>
<td>OR24</td>
</tr>
<tr>
<td>Di Paolo, Daniela</td>
<td>OR24</td>
</tr>
<tr>
<td>Di Virgilio, Francesco</td>
<td>OR24</td>
</tr>
<tr>
<td>Diamant, Maura</td>
<td>OR24</td>
</tr>
<tr>
<td>Diaz deStahl, Teresita</td>
<td>OR24</td>
</tr>
<tr>
<td>Dick, John</td>
<td>OR24</td>
</tr>
<tr>
<td>* = Presenting author</td>
<td></td>
</tr>
</tbody>
</table>

**Author Index**
<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dieckmann, Karin</td>
<td>OR84</td>
</tr>
<tr>
<td>Diller, Lisa</td>
<td>C10*, POB10, SEL9</td>
</tr>
<tr>
<td>Diloo, Dagmar</td>
<td>PL35</td>
</tr>
<tr>
<td>Ding, Han-Fei</td>
<td>OR25, POB35*</td>
</tr>
<tr>
<td>Ding, Jane</td>
<td>OR25, POB35</td>
</tr>
<tr>
<td>Diolaiti, Daniel</td>
<td>SEL5</td>
</tr>
<tr>
<td>Dirks, Peter</td>
<td>OR30</td>
</tr>
<tr>
<td>Diskin, Sharon</td>
<td>OR33, OR76, PL5*, PL18, PL19, POT2, SEL23</td>
</tr>
<tr>
<td>Distel, Robert</td>
<td>POB112</td>
</tr>
<tr>
<td>Djos, Anna</td>
<td>POB19</td>
</tr>
<tr>
<td>Doi, Kunio</td>
<td>POC29</td>
</tr>
<tr>
<td>Dolopolov, Igor</td>
<td>POC48</td>
</tr>
<tr>
<td>Dominici, Carlo</td>
<td>POB84, POB19, POLB4</td>
</tr>
<tr>
<td>Doolin, Edward</td>
<td>POB88</td>
</tr>
<tr>
<td>Dorf, Lee</td>
<td>SEL21</td>
</tr>
<tr>
<td>Dotti, Gianpietro</td>
<td>OR19</td>
</tr>
<tr>
<td>Douc-Rasy, Sêtha</td>
<td>POC45</td>
</tr>
<tr>
<td>Douglas, Lena</td>
<td>SEL14</td>
</tr>
<tr>
<td>Dreidax, Daniel</td>
<td>POB48, POLB7, POT79, POT81, SEL2*</td>
</tr>
<tr>
<td>*Drobics, Mario</td>
<td>OR84</td>
</tr>
<tr>
<td>Drozynska, Elżbieta</td>
<td>POT43, POT44</td>
</tr>
<tr>
<td>Druy, Alexander</td>
<td>PL28</td>
</tr>
<tr>
<td>DuBois, Steven</td>
<td>OR59, POC7*</td>
</tr>
<tr>
<td>Dubray, Sarah</td>
<td>POB77</td>
</tr>
<tr>
<td>Dufour, Christelle</td>
<td>SEL15</td>
</tr>
<tr>
<td>Düjkers, Floor</td>
<td>POT21*, POT22*</td>
</tr>
<tr>
<td>Dumanski, Jan</td>
<td>POB53</td>
</tr>
<tr>
<td>Dungwa, Josiah</td>
<td>POT23*</td>
</tr>
<tr>
<td>Dunkel, Ira</td>
<td>POC2</td>
</tr>
<tr>
<td>Duval, Michel</td>
<td>POB28</td>
</tr>
<tr>
<td>Dyberg, Cecilia</td>
<td>POB36*</td>
</tr>
<tr>
<td>Dykes, Josefina</td>
<td>SEL11</td>
</tr>
<tr>
<td>E</td>
<td>OR84</td>
</tr>
<tr>
<td>Ebetsberger, Georg</td>
<td>PL6, SEL7, SEL47, WS4</td>
</tr>
<tr>
<td>Ebus, Marli</td>
<td>POB89</td>
</tr>
<tr>
<td>Edo, Robby</td>
<td>POT49</td>
</tr>
<tr>
<td>Egeler, RM</td>
<td>OR5, OR35, OR41, OR65, OR66, OR72, OR85, OR86, PL6, PL22, POB32, POB38, POB96, POT3, POT19, SEL7, SEL18, SEL38, WS14*, WS18</td>
</tr>
<tr>
<td>Eggert, Angelika</td>
<td>OR5, OR40, PL7, POB48, POT81</td>
</tr>
<tr>
<td>Ehrenberg, Georg</td>
<td>SEL14</td>
</tr>
<tr>
<td>Ehnhra, Bo</td>
<td>OR2</td>
</tr>
<tr>
<td>Elers, Martin</td>
<td>SEL5</td>
</tr>
<tr>
<td>Elers, Martini</td>
<td>OR36, PL23</td>
</tr>
<tr>
<td>Elns, Roland</td>
<td>SEL29*</td>
</tr>
<tr>
<td>Einvik, Christer</td>
<td>POB42, SEL28</td>
</tr>
<tr>
<td>Ejeskær, Katarina</td>
<td>POT72</td>
</tr>
<tr>
<td>Elfman, Lotta</td>
<td>PL36, SEL10</td>
</tr>
<tr>
<td>Ellershaw, Caroline</td>
<td>OR25</td>
</tr>
<tr>
<td>Ellis, Tammy</td>
<td>POB20</td>
</tr>
<tr>
<td>Elston, Rebecca</td>
<td>OR16, OR29, OR56, POB11, POT9, SEL33*</td>
</tr>
<tr>
<td>Emionite, Laura</td>
<td>SEL20</td>
</tr>
<tr>
<td>Enomoto, Hideki</td>
<td>POB58, POB59</td>
</tr>
<tr>
<td>Erbayraktar, Zübyde</td>
<td>OR15, OR67, SEL9</td>
</tr>
<tr>
<td>Erbey, Fatih</td>
<td>SEL19*</td>
</tr>
<tr>
<td>Erichsen, Jennie</td>
<td>POB42</td>
</tr>
<tr>
<td>Eriksson, Emma</td>
<td>OR68*, POT72</td>
</tr>
<tr>
<td>Eriksson, Helena</td>
<td>POT12</td>
</tr>
<tr>
<td>Eriksson, Therese</td>
<td>OR81</td>
</tr>
<tr>
<td>Erminio, Giuseppe</td>
<td>POB37*</td>
</tr>
<tr>
<td>Erttmann, Rudolf</td>
<td>POT73</td>
</tr>
<tr>
<td>Eschenburg, Georg</td>
<td>SEL17</td>
</tr>
<tr>
<td>Esser, Ruth</td>
<td>POC23, POT56</td>
</tr>
<tr>
<td>Etchevers, Heather</td>
<td>POT64</td>
</tr>
<tr>
<td>Evagelou, Nicholas F.</td>
<td>OR36, OR64</td>
</tr>
<tr>
<td>Evans, Audrey</td>
<td>Fan, Qiashi</td>
</tr>
<tr>
<td>Everhart, Lindsay</td>
<td>Fanti, Stefano</td>
</tr>
<tr>
<td>F</td>
<td>Faouzi, Mohamed</td>
</tr>
<tr>
<td>F</td>
<td>Farace, Françoise</td>
</tr>
<tr>
<td>Fardin, Paolo</td>
<td>POC5</td>
</tr>
<tr>
<td>Fasci, Antonio</td>
<td>POT66</td>
</tr>
<tr>
<td>Fechine, Larisa</td>
<td>POCB27, POB87, POT25*</td>
</tr>
<tr>
<td>Feinberg-Gorenshtein, Galina</td>
<td>POT14</td>
</tr>
<tr>
<td>Felt, David</td>
<td>POT17</td>
</tr>
<tr>
<td>Femenic, Ranka</td>
<td>OR26</td>
</tr>
<tr>
<td>Ferguson-Smith, Malcolm A.</td>
<td>OR27</td>
</tr>
<tr>
<td>Ferraris, Chiara</td>
<td>OR28</td>
</tr>
<tr>
<td>Ferrini, Silvano</td>
<td>OR30</td>
</tr>
<tr>
<td>Ferrone, Soldano</td>
<td>POT30, POT16</td>
</tr>
<tr>
<td>Fest, Stefan</td>
<td>POT17</td>
</tr>
<tr>
<td>Feuchtinger, Tobias</td>
<td>OR31</td>
</tr>
<tr>
<td>Fiaschetti, Giulio</td>
<td>OR26</td>
</tr>
<tr>
<td>Flew, Annelies</td>
<td>OR32, POB38*</td>
</tr>
<tr>
<td>Finnbogason, Throstur</td>
<td>SEL14*</td>
</tr>
<tr>
<td>Fiore, Michele</td>
<td>SEL28</td>
</tr>
<tr>
<td>Fischer, Matthias</td>
<td>OR36, OR36*, OR40, OR45, OR66, OR79, PL7, PL23, POT82, POT1, POT77*, POT81, SEL2</td>
</tr>
<tr>
<td>Fitzsimons, Carlos P.</td>
<td>OR7</td>
</tr>
<tr>
<td>Flægstad, Trond</td>
<td>SEL29</td>
</tr>
<tr>
<td>Flahaut, Marjorie</td>
<td>POCB39*, POB65, SEL25, WS6</td>
</tr>
<tr>
<td>Flemming, Claudia</td>
<td>OR31, OR43</td>
</tr>
<tr>
<td>Fletcher, Jamie</td>
<td>OR31</td>
</tr>
<tr>
<td>Foley, Niamh</td>
<td>OR26, PL21, PL27, POB29, POB113, WS12</td>
</tr>
<tr>
<td>Foltz, Dan</td>
<td>SEL28</td>
</tr>
<tr>
<td>Fong, Abraham</td>
<td>POB40*</td>
</tr>
<tr>
<td>Forloni, Matteo</td>
<td>POB41</td>
</tr>
<tr>
<td>Fornsarisi, Diego</td>
<td>POB7</td>
</tr>
<tr>
<td>Fox, Autumn</td>
<td>OR10, POB90</td>
</tr>
<tr>
<td>Fransson, Susanne</td>
<td>POCB42*</td>
</tr>
<tr>
<td>Frantz, Christopher N.</td>
<td>SEL9</td>
</tr>
<tr>
<td>Frati, Luigi</td>
<td>OR49, POB84, POB109</td>
</tr>
<tr>
<td>Frediani, Simon</td>
<td>POC4</td>
</tr>
<tr>
<td>Fredlund, Erik</td>
<td>OR35, OR51, POB73, POB74</td>
</tr>
<tr>
<td>Frenzel, Anna</td>
<td>POT26*, SEL44</td>
</tr>
<tr>
<td>Friedman, Lori</td>
<td>OR4</td>
</tr>
<tr>
<td>Frigyesi, Attila</td>
<td>POC5</td>
</tr>
<tr>
<td>Fruci, Doriania</td>
<td>OR49, POB41*</td>
</tr>
<tr>
<td>Fruehwald, Michael C</td>
<td>POC37</td>
</tr>
<tr>
<td>Fruhwald, M C</td>
<td>POC78</td>
</tr>
<tr>
<td>Fuchs, Dieter</td>
<td>POC16</td>
</tr>
<tr>
<td>Fujitani, Mayumi</td>
<td>OR14, OR30</td>
</tr>
<tr>
<td>Fukatsu, Hiosoishi</td>
<td>POC44</td>
</tr>
<tr>
<td>Fukaya, Yasushi</td>
<td>POB98</td>
</tr>
<tr>
<td>Fulda, Simone</td>
<td>OR4*, WS25*</td>
</tr>
<tr>
<td>Furman, Wayne</td>
<td>POC9*</td>
</tr>
<tr>
<td>Fuskevåg, Ole Martin</td>
<td>SEL22</td>
</tr>
<tr>
<td>Futami, Hitoyasu</td>
<td>POB76</td>
</tr>
<tr>
<td>Fühlhuber, Verena</td>
<td>POB10</td>
</tr>
<tr>
<td>G</td>
<td>Gaal, Jose</td>
</tr>
<tr>
<td>Gade, Stephan</td>
<td>OR26, POB12, SEL30</td>
</tr>
<tr>
<td>Gadner, Helmut</td>
<td>OR23</td>
</tr>
<tr>
<td>Gallo, Fabio</td>
<td>OR79, POT77</td>
</tr>
<tr>
<td>Galván, Patricia</td>
<td>POB33</td>
</tr>
<tr>
<td>Gambini, Claudio</td>
<td>POB10, POB87, POT12, POT57, SEL35</td>
</tr>
<tr>
<td>Gamble, Laura D.</td>
<td>POB20, POB43*, POB44*</td>
</tr>
<tr>
<td>Gao, Wei</td>
<td>SEL31</td>
</tr>
<tr>
<td>Garaventa, A</td>
<td>PL33, POB68, POC21, POT57, POT77</td>
</tr>
<tr>
<td>Garaventa, Alberto</td>
<td>OR60</td>
</tr>
<tr>
<td>Garcia, Emma</td>
<td>OR60</td>
</tr>
<tr>
<td>Garcia, Idoia</td>
<td>POB1, POB33, POT27</td>
</tr>
</tbody>
</table>
Garcia-Echeverria, Carlos
Garkavij, Michael
Gaspar, Nathalie
Gastier-Foster, Julie
Gatto, Pamela
Gattolliat, Charles-Henry
Gauthier-Villars, Marion
Ge, Kai
Gee, Adrian
Geerts, Dirk
Geiger, Kathrin
Geograer, Birgit
George, Mentkevich
George, Rani E.
Gerhard, Daniela
Gershon, Timothy
Gerstle, J. Ted
Gevaert, Kris
Gent, Matthew
Gherardi, Samuele
Ghesquiere, Bart
Gioia, Giuseppe
Gilheeney, Stephen
Gillespie, Paul
Gilman, Andrew
Gisseros, David
Gleissman, Helena
Glennie, Martin
Glessner, Joseph
Goeser, Felix
Gogolin, Sina
Gogvadze, Vladimir
Goldsmith, Kelly C.
Goma, Gisele
Goodarzian, Fariba
Gordijn, Eli
Gotoh, Takahi
Gradshandl, Anke
Graf, Rolf
Graham, Regina
Grajowska, Wieslaw
Grandoi, Carla
Grau, Elena
Gray, Juliet
Greenberger, Lee M
Gregorio, Andrea
Gregory, Walter
Griffiths, Rebecca
Grigull, Lorenz
Grimmer, Matt
Grinstein, Natalie
Groshen, Susan
Gross, Michelle
Gross, N
Gross, Nicole
Grouzmann, Eric
Grundy, Richard G.
Gröne, Hermann-Josef
Guadaldini, Francesco
Guaraguagli, Giulia
Guest, James
Guler, Linda
Guilino, Alberto
Gumy-Pause, F
Gunes, Dilek
Guo, Xiang
Gurcan, Metin
Gustafson, W. Clay
Gustafsson, Göran
Güneri, Enis Alpin

H
Ha, Shau-Yin
Haas-Kogan, Daphne
Haber, Michelle
Hackett, Christopher
Hadjudjian, Michael
Hagenbuchner, Judith
Haglund, Elisabeth
Hakem, Razqaallah
Hakonarson, Hakon
Haley-Berko, Gill
Hallberg, Bengt
Hama, Ashihito
Hamasaki, Minoru
Hamberg, Mats
Handgrenatinger, Rupert
Hansford, Loen
Hansson, Magnus
Haque, Sofia
Hara, Takeshi
Hara, Toshiro
Haraguchi, Seiki
Harley, Calvin
Hartmann, Olivier
Hartwich, Joseph
Hasan, Kamrul
Hasegawa, Mamoru
Hassenauer, Beth
Haug, Bjørn Helge
Haupts, R
Haupt, Riccardo
Hawkins, Randall
Hayashi, Kunihiko
Hayashi, Yasuhide
Hedborg, Fredrik
Hedstrom, Elisabeth
Heiko Manuel, Techtsch
Hellman, Per
Hellriegel, Edward T
Henderson, Michelle
Henderson, Tara
Hennich, Kai-Oliver
Henriksen, Jørn Remi
Henriksson Arsenian, Marie
Henze, Guenther
Herland, Anna
Herrmann, Martin
Hernandez, Miguel
Hero, Barbara

POLB1*, POLB2*, POLB5*, SEL20*
OR28, OR37, PL24, POB5
POT33*
SEL46*
OR83
SEL20

POC4
OR58
OR25, OR31*, OR43, OR47,
PL20, POB70, SEL17
PL17, POBS2*
POB5, POLB13*
POB81, SEL39
PL18
OR44
OR76, PL5, POT2
POT45
OR68, POB72, POB95,
POT34*
POC44
POC12, POC45
POT29
POC17, POC37, POT13
OR8, OR9, OR30, PL1*,
PL11, POT64, POT85,
SEL48, WS5
POB72
POC2
POC29
POC43, POT70
PL9, POB60
OR30
SEL15
POT35*, POT36*
POT40, POT68
POT70
OR58
SEL29
SEL41
POC12
OR59, POC7
POC45
OR70, POB83
OR83, POLB5*
SEL37
POC17
POT10*
POC23
OR31, OR43
OR82
PL7*, POT81
SEL29
OR51, PL14*, POB115,
POT36, SEL44, WS13*
POC37
POB54*
POB81
POB50
OR5, OR36, OR40, OR45,
OR62, OR64, OR68, OR79,
OR81, OR85, PL23, PL35*,
POB10, POC3, POC26,
POC33, POC37, POT78,
SEL13*, SS4*
OR19
POB119, POT47, POT48
PL27
SEL45
OR21
POC36
SEL1
PL8
Laskar, Siddharth
Lau, Cynthia
Laudenslager, Marc
Laureys, Genevieve
Lavarrino, Cinzia
Lavía, Patricia
Lazar, Vladimir
Lazooz, Paula
Le Blanc, Katarina
Lee, David
Lee, Hisinyu
Lee, Kun Soo
Lee, Sue-Hyun
Lee, Ya-Ling
Leen, René
Lefever, Steve
Legentil, Marion
Leguenney, Ingrid
Lemesheva, Olga
Lenhoff, Stig
Lescaut, Pamela
Leuchs, Barbara
Leuschner, Ivo
Lewinser, Adi
Lewis, Ian J
Levitskaya, Jelena
Levitsky, Victor
Levy, Robert
Li, Chi-Kong
Li, Hongzhe
Li, Junwei
Li, Lan Hua
Li, Zhijie
Liao, Yung-Feng
Liberman, Julie
Libous, Jennifer
Light, Jennifer E.
Limon, Janusz
Lin, Dong-Tsamn
Linardopoulos, Spiros
Lindgren, David
Lindner, Verner
Lindsøk, Magnus
Ling, Siu-Cheung
Lingwood, Clifford
Linke, Jan-Peter
Lipman, Tatiana
Lipska, Beata Stefania
Liu, Cathy
Liu, Daofeng
Liu, Enli
Liu, Hao
Liu, Pei
Liu, Tao
Liu, Xueyuan
Liu, Yin-Lin
Liu, Zhihui
Loaec, Nadège
Lode, Holger

Lodrini, Marco
Loecher, Ciara
Loi, Monica
London, Wendy

POC15, POC30
OR13
OR76, PL5, PL18
OR35, OR41, OR65, OR88,
PL29, PL33, POC73
POB1, POB33, POT27*
POLB12
POB45, POB78
POB94
SEL11
OR56
POB56, POC11, POT15
POC40
POC41
POC40, POC41, POC42
POC18*, POC19
POB9
POB34, POB38, POB55
POB78
POT18
POC36
SEL11
OR53
SEL43
OR81
OR83
PL36, SEL10
POLB3*, POLB10*
POLB10
POT56
POC4
OR76, PL5
POC4
PL9*
POB104, POT85, WS24
POB56, POT15
POB65*, SEL25, WS6
OR53
POB99, POT48, POT52
POT43, POT44
PL31, POC19
PL31, POC19, POT15
PL16
POB111
SEL38
POT29, POT60
POC4
OR14
OR45, POB82
OR30, PL10
POT44*
OR18, SEL24
OR22
OR19
OR19
OR47, PL20
OR47, PL20, POT70
POB66, POT30, SEL17
POC19*
OR32, OR73, POB67
POT20, POT59
OR20, POB37, POC6,
POC17, POT73, POT76,
SEL26*, WS22*
POB82, POT54*
POB97
OR29, OR71, POT12
C3, C8, CI1*, OR31, OR35,
OR61, OR64*, OR76, OR82,
PL5, PL18, PL27, PL30,
PL32, POC10, POC13,
POC25, POC1, POT2, POT48,
POT32, SEL9*

Longo, Luca
Longo, Valter
Loo, A. Thomas
Lopez, Teresita
Lorenzi, Silvia
Lusty, Paul
Louis, Chrystal
Lovat, Penny, E
Lovén, Jakob
Lu, Congyi
Lu, Meng-Yao
Luk, Chun-Wing
Luksch, Roberto
Lundberg, Gisela
Lundin, Vanessa
Lunec, John
Luo, Tsai-Yueh
Luria, Doriit
Luther II, William
Lyden, David
Länsberg, John-Kalle
Lekke, Ceciile

M
Ma, Jinxia
Ma, Lin-Jen
Ma, Ming
Machin, David
Maillet, P
Mais, Michel
Mairis, Robert J
Majdazari, Afsaneh
Makin, Guy
Malavasi, Fabio
Malis, Josef
Malkin, David
Maloney, Anne Marie
Malyukova, Alena
Mamai, Ahmed
Manach, Y.
Mangino, Jennifer
Mann, Shan
Mao, Ling
Marabelle, Aurelien
Marachelian, Araz
Mardouck, Jack
Marigo, Ilaria
Marimpietri, Danilo
Marine, Jean-Christophe
Maris, John
Marques, B
Marques, Barbara
Marquez, Victor
Marra, Marco
Marrano, Paula
Marrone, Tami
Marchall, Tobias
Marshall, Glenn
Martí, Elisa
Martin, Marcel
Martinez, Francisco
Martinod, Kimberly
Martinsson, Tommy

MPOB31*, MPOB68*
OR56
PL12, SEL45
OR19
POB41
OR12, WS9
OR19*
POT6
OR51*, POB115, WS13
POB64
PL31*, POC19, POC46,
POC47
POC4
OR88, PL29, PL33, POB68
POT55*
POB69*
OR38, POB20, POB43,
POB44, POT76, SEL40, SEL41
POC46
POT45
PL12, SEL45
POC2
POT55
SEL29
POLB10
POB24
POB23
OR64, PL36, SEL10
POT32
SEL36
POT58
OR42
PL33
SEL35
OR88, PL29
OR30, POB116, POC38
POC22
POB70
OR14
POT27
POB99, POT38, POT56*
PT51
POB35
OR87
OR57, OR58
POT46
POB11
POT12, SEL35
POB73, POT79
OR28, OR33, OR57, OR58,
OR59, OR61, OR76*, PL4*,
PL5, PL8, PL18, PL19, POB5,
POB63, POC7, POC23,
POC13, POT2, SEL4, SEL23,
SS2*, WS20*
SEL41
OR38
OR32
OR9, PL8, PL10
PL10, POB3, POT66
PL18
OR85, POB86
OR25, OR31, OR43, OR47*,
PL20*, POT70*, SEL17
POB33
OR85, POB96
POB50, POC50
POT82
OR38, OR65, OR83, POB15,
POB19, POB42, POC53,
POB71*, POB72*, POB94,
POB3, POT55, SEL8,
SEL28, SEL40, SEL41
<table>
<thead>
<tr>
<th>Author</th>
<th>Presenting author</th>
<th>Index</th>
<th>PoB</th>
<th>Sel</th>
<th>Ws</th>
</tr>
</thead>
<tbody>
<tr>
<td>Massimi, Isabella</td>
<td></td>
<td>OR49, POB84, POB109,</td>
<td>POB66</td>
<td>SEL6</td>
<td></td>
</tr>
<tr>
<td>Master, Stephen R.</td>
<td></td>
<td>POB37, SEL1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Masujima, Tsutomu</td>
<td></td>
<td>C9*, OR57, OR58, OR59*, OR61, OR64, PL17, PL30, PL32, POB63, POC7, POC23, POC25, SEL9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matsumoto, Daisuke</td>
<td></td>
<td>C9*, OR57, OR58, OR59*, OR61, OR64, PL17, PL30, PL32, POB63, POC7, POC23, POC25, SEL9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matsumoto, Kimikazu</td>
<td></td>
<td>C9*, OR57, OR58, OR59*, OR61, OR64, PL17, PL30, PL32, POB63, POC7, POC23, POC25, SEL9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matthay, Katherine</td>
<td></td>
<td>C9*, OR57, OR58, OR59*, OR61, OR64, PL17, PL30, PL32, POB63, POC7, POC23, POC25, SEL9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mattioli, Girolamo</td>
<td></td>
<td>OR42, POB84, POB109,</td>
<td>POB66</td>
<td>SEL6</td>
<td></td>
</tr>
<tr>
<td>Maurer, Barry J.</td>
<td></td>
<td>OR57</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maurer, Jochen</td>
<td></td>
<td>SEL38</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mayer, Florian</td>
<td></td>
<td>POT13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mayes, Patrick</td>
<td></td>
<td>PL5, PL19, SEL23*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mayol, Gemma</td>
<td></td>
<td>POC1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mazucco, K</td>
<td></td>
<td>OR58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mazucco, Katia</td>
<td></td>
<td>OR3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>McArthur, Grant</td>
<td></td>
<td>OR5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>McCluskey, Anthony G</td>
<td></td>
<td>OR12, POB84, POB109, WS9</td>
<td>POT64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>McDowell, Heather</td>
<td></td>
<td>OR82, PL18, PL27, PL30,</td>
<td>POT52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>McFadden, Grant</td>
<td></td>
<td>OR82, PL18, PL27, PL30,</td>
<td>POT52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>McGrady, Patrick</td>
<td></td>
<td>OR82, PL18, PL27, PL30,</td>
<td>POT52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>McGregor, Lisa</td>
<td></td>
<td>OR82</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>McEwen, Amy</td>
<td></td>
<td>OR3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meijer, Laurent</td>
<td></td>
<td>OR7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meijerink, Jules</td>
<td></td>
<td>OR7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meier, Laurent</td>
<td></td>
<td>OR84, SEL39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meister, Bernhard</td>
<td></td>
<td>OR84, SEL39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mele, Ermelinda</td>
<td></td>
<td>OR84, SEL39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mellone, Massimiliano</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menezes, de Renee</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menten, Bjorn</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mestdagh, Pieter</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meteletsa, Leonid</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meurice, Guillaume</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meyer, Helmut</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meyn, M. Stephen</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mezzanotte, Laura</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Michaelis, Martin</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Michelazzi, Alberto</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Michon, Jean</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miguel, Solange</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mikan, Kelly</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milde, Till</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mills, Denise</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minard-Colin, Véronique</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minturn, Jane</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mir, M. Luis</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mirisola, Valentina</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitani, Yasuyuki</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miyachi, Mitsuru</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miyake, Izumi</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mizgalska, Danuta</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modak, Shakeel</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modritz, Ditha</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mody, Rajen</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moens, Ugo</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moffat, Jason</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mokhtari, Reza</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molenaar, Jan</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mollet, Julie</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monnet, Yann</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moore, Richard</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mora, Jaume</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moran, Mike</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morandi, Fabio</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moreno, Lucas</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moretti, Stefano</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morik, Katharina</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morin, Ryan D.</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moroz, Veronica</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morozova, Olena</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morton, Christopher</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mosci, Sofia</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mosconcari, Manuela</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moss, Diana</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mosse, Yael</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mosseri, Veronique</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muckaden, Marryann</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muckenthaler, Martina</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mueller, Tania</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mugishima, Hideo</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muhlethaler-Mottet, Annick</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mukai, Akira</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mullissery, Dhanya</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Munell, Francina</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Munier, Fabienne</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Munoz, Marcia</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Murakami-Tonami, Yuko</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muramatsu, Hideki</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Murphy, Derek</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutafodulu, Kamer</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muth, Daniel</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myers, Adrianne</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myers, G Doug</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muhlethaler-Mottet, Annick</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Müller, Inga</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mylvaganam, Murugesapillai</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N N</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naeem, Hossameldin</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nagashimada, Mayumi</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nahar, Akaash</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nakagawa, Atsuko</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nakagawa, Akira</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nakai, Hiroshi</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nakamura, Yohko</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nakao, Kazuki</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nakata, Rie</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nakayama, Masahiro</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nanni, Cristina</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naranjo, Arlene</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naraparaju, Koumudi</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nardou, Katya</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasr, Ahmed</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natali, Pier Giorgio</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nathraith, M</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naumann, Ivonne</td>
<td></td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Institution(s)</td>
<td>Author Index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-------------------------</td>
<td>--------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Navarro, Samuel</td>
<td>OR8, POC50, POT7, POT8, POT55, POT62, POT67</td>
<td>* = Presenting author</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neiron, Zillan</td>
<td>PL20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Netherland, Maria</td>
<td>POB19, POB71</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ng, Cathy</td>
<td>POT35, POT36</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ngan, Elly</td>
<td>OR13*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nguyen, Le</td>
<td>OR76</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Niemann, Catherina Annika</td>
<td>POC26</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Niemeyer, C M</td>
<td>POT78</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Niewisch, Marena</td>
<td>POT19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Niggli, Felix</td>
<td>POT78, SEL13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nilsson, Staffan</td>
<td>POB19, POB71, SEL4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nishikawa, Eri</td>
<td>POC35</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nishikawa, Masanori</td>
<td>SEL16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nishimura, Riki</td>
<td>OR70, POB83</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nishio, Nobuhiro</td>
<td>POC44</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nittner, David</td>
<td>POT73</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noesel, Max</td>
<td>POT21, POT22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noguera, Rosa</td>
<td>OR8, OR35, OR36, OR38, OR41, OR65, POB32, POB38, POB50, POB73, POC50, POT77, POT8*, POT62, POT67, SEL40, SEL41</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noguera, Rosa</td>
<td>POT55</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norberg, Erik</td>
<td>POC49</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nordell, Bo</td>
<td>SEL14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normand, Charline</td>
<td>POC27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norris, Geoffrey</td>
<td>PL19, PL5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norris, Murray</td>
<td>OR25, OR31, OR43*, OR47, PL20, POB70, POLB10, SEL17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nose, Kiaziuke</td>
<td>SEL16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nowacki, Sandra</td>
<td>OR66</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nowakowska, Natalia</td>
<td>POB80*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuchtern, Jed</td>
<td>CS*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuytens, Justine</td>
<td>POB55, POB91</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nyalendo, Carine</td>
<td>POC28</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oba, Shigeuyuki</td>
<td>OR67</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oberthuer, Andre</td>
<td>OR36, OR40, OR66, OR79, PL23*, POT1, POT77</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obexer, Petra</td>
<td>POB81*, SEL39</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ochiai, Hidemasa</td>
<td>OR50*, POT69</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Odenthal, Margarete</td>
<td>OR40</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oehme, Ina</td>
<td>OR45*, POT82*, POT54</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ogawa, Seishi</td>
<td>OR67, OR70, POB83</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ogura, Atsushi</td>
<td>POC101</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oh, Doo-Yoo</td>
<td>POC28</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ohira, Miki</td>
<td>OR50, OR65, OR67*, OR86, PL9, PL10, POB60, POT68, POT69, POT71, SEL6, SEL31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ohi, Kentaro</td>
<td>OR70</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ohkubo, Jun</td>
<td>SEL33</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ohita, Hiroshi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ohtaki, Megu</td>
<td>POT37</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Okimoto, Yuri</td>
<td>OR50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Okubo, Jun</td>
<td>POB83*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Okuma, Hirotugu</td>
<td>POC35</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olgun, Nur</td>
<td>POLB50, POLB5, POLB5, SEL20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oltra, Silvestre</td>
<td>POLB50, POC50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O'Meara, Anne</td>
<td>PL27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onciu, Mihaela</td>
<td>POC9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ooo, Myat Lin</td>
<td>POT71</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opel, Daniela</td>
<td>POC16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opolon, Paulé</td>
<td>POT18, POT66</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ora, Ingrid</td>
<td>OR60, POT25, SEL7, SEL11, SS1, WS15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ordóñez, José Luis</td>
<td>POB33</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orellana, Carmen</td>
<td>POB50, POC50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orengo, Anna Maria</td>
<td>POC16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orrego, Abiel</td>
<td>POLB3, SEL34</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orrenius, Sten</td>
<td>POB49</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oskarsson, Anna</td>
<td>POT10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osone, Shinya</td>
<td>POT84</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ostaijen ten Dam, MM</td>
<td>POT49</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O'Sullivan, Maureen</td>
<td>PL27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O'Toole, Kieran</td>
<td>POT6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oue, Takaharu</td>
<td>SEL16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Owens, Cormac</td>
<td>SEL15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ozaki, Toshinori</td>
<td>OR74, POT41, SEL31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ozkaynak, Fevzi</td>
<td>C8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ozogoul, Candan</td>
<td>POLB1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ozzahin, H</td>
<td>POT32</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pagnan, Gabriella</td>
<td>OR71, POT12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pålham, Sven</td>
<td>OR8, OR11, OR51, POB54, POB11, OR55, WS1*, WS13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pajtler, Kristian</td>
<td>OR66, OR72, PL22, SEL38</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paleari, Laura</td>
<td>POB31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palmberg, Ebba</td>
<td>POT60*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palmer, Ruth</td>
<td>OR68, POB72, POB95, POT34</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pandit-Taskar, Neeta</td>
<td>POC14, POC24, SEL12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Panigrahy, Ashok</td>
<td>OR59</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papachristou, Panagiotis</td>
<td>POB36</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papadakis, Vassilios</td>
<td>OR60, PL29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pardo, B</td>
<td>POT32</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paris, Larry</td>
<td>POB24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parisi, Marguerite</td>
<td>PL30, POC25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Park, Julie</td>
<td>OR48, OR61*, POC13, POC23, POT65</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parodi, Federica</td>
<td>SEL36</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parodi, S</td>
<td>SEL14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Passoni, Lorena</td>
<td>POB31, POB68</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paster, Ira</td>
<td>POT85</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pastorino, Fabio</td>
<td>OR29*, OR71, POT12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patterson, Danielle</td>
<td>OR24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pattr, Filip</td>
<td>POB34, POB55, POB73, SEL30*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pawel, Bruce</td>
<td>PL18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pawlowska, Anna</td>
<td>POC32</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pearson, Andrew</td>
<td>OR64, OR16, OR16, OR36, OR17, SEL10, SEL36, SEL40</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pegg, Anthony</td>
<td>POB47</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peirce, Susan K.</td>
<td>POT30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peirs, Maria</td>
<td>POT61*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pellegatti, Patrizia</td>
<td>POB11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peng, Steven Hsin-Feng</td>
<td>POC19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perek, Danuta</td>
<td>POT43, POT44</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perel, Yves</td>
<td>POT11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perez-Ataye, Antonio</td>
<td>POC10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perini, Giovanni</td>
<td>OR31, OR43, OR47, OR54, PL20, SELL5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perri, Patrizia</td>
<td>OR71, POC7*, POT12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pession, Andrea</td>
<td>OR14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petroni, Mariella</td>
<td>OR49, POC64*, POC109</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pettersen, Ingvid</td>
<td>POB85</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pezz, Annalisa</td>
<td>WS7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pezzolo, Annalisa</td>
<td>SEL35*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pfleffer, Ulrich</td>
<td>POC17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pfleffer, Matthias</td>
<td>POC17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pferdemenges, Doerthe</td>
<td>POT73</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pfister, Stefan</td>
<td>OR26</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Philippe, Cathy</td>
<td>POC78</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Philippe-Chomette, Pascale</td>
<td>POC27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pickert, Diana</td>
<td>OR45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pierron, Gaelle</td>
<td>OR34, OR37, POC27, POT11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pieters, R</td>
<td>OR1, POT21, POT22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pietras, Alexander</td>
<td>OR8*, OR11, POB11, POT55, WS1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pietsch, Torsten</td>
<td>OR1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pinkerton, Ross</td>
<td>PL36, SEL10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pinto, Navin</td>
<td>OR82*, POC29*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piotrowska, Izabela</td>
<td>OR54, POT86*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Author Name</td>
<td>OR/POT/POB/SEL Number</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>-----------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piqueras, Marta</td>
<td>OR36, POT7*, POT8, POT55, POT62*, POT67*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pique-Regi, Roger</td>
<td>OR16*, OR56, POB10, POB11, POB14, POB9, POT12, SEL35, WS7*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pistorio, Angela</td>
<td>SEL35</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pivarsc, Andor</td>
<td>OR51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pizer, Barry</td>
<td>OR12, WS9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plantaz, Dominique</td>
<td>OR34</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plegaria, Jeffrey</td>
<td>PL18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poehler, Christina</td>
<td>OR26*, POT12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poetschger, Ulrike</td>
<td>OR84</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ponthou, Frida</td>
<td>POT6, POT29, POT63</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ponzi, Mirco</td>
<td>OR29, OR71*, POT9, POT12*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poortinga, Gretchen</td>
<td>OR3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Popov, Alexander</td>
<td>OR29, OR71*, POB7, POT12*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Popova, Tatiana</td>
<td>OR65</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porcu, Michael</td>
<td>OR31, OR43, SEL5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porre, Antonia</td>
<td>POB73</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Powel, Rob</td>
<td>PL15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prakesch, Michael</td>
<td>OR87</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prehn, Jochen</td>
<td>OR16, OR21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prentter, Suzanne</td>
<td>POB84</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prigione, Ignazio</td>
<td>POLOB8*, POLB9*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prince, Chengyu</td>
<td>SEL15*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prosodoski, Andrea</td>
<td>POC29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prosskovskaya, Innna</td>
<td>OR7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proust-Houdemout, Stéphanie</td>
<td>OR19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pu, Yonlin</td>
<td>OR25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Puigvert, Jordi C.</td>
<td>OR25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Puisieux, Alain</td>
<td>OR19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pule, Martin</td>
<td>POB87*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Puppo, Maura</td>
<td>POLB7, POT81, SEL30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pöhler, Christina</td>
<td>OR23, OR38</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pötschger, Ulrike</td>
<td>POC32</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Qian, Dajun</td>
<td>OR17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quarta, Carmelo</td>
<td>OR16, OR56, POB10, POB11, POB9, POT9, SEL35</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Qureshi, Sajid</td>
<td>POB61</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>POB19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raabe, Tobias</td>
<td>OR17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabino, Gabriel A</td>
<td>OR16, OR56, POB10, POB11, POB9, SEL35</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raffagnello, Lizzia</td>
<td>POB61</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rafi, Islam</td>
<td>POT5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rahbar, Afsar</td>
<td>POB68</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rahman, Ajjur</td>
<td>OR85, POB96</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rahmann, Sven</td>
<td>OR25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raff, Anna</td>
<td>POC15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rajan, MGR</td>
<td>POC1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rajic, Lubica</td>
<td>POLB10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rakmanaliev, Elian</td>
<td>POLC15, POLC30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ramadvar, Mukta</td>
<td>POT23, POT61</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ramani, Pramila</td>
<td>OR10, POB99</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rappaport, Eric</td>
<td>OR75*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raquel, Fernandez</td>
<td>POB86, SEL22*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rasmusson, Agnes</td>
<td>POB87</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raynal, Virginie</td>
<td>POLB11, POLB12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Re, Angela</td>
<td>POB88*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Redden, Robert</td>
<td>POT64</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Redding, Nicole</td>
<td>POT6, POT63</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Redfern, Christopher</td>
<td>OR69, POB78</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regariaz, Marie</td>
<td>POB76</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regan, Kelly</td>
<td>OR10, POB89, POB90</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reh, Jerold E.</td>
<td>OR44</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rehn, Matilda</td>
<td>OR8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reid, Joel M.</td>
<td>OR57</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reiff, Tobias</td>
<td>OR42</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reisfeld, Ralph</td>
<td>SEL26</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renard, Marleen</td>
<td>OR65</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renaud, Arnaud, Céline</td>
<td>OR69</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rens, Willem</td>
<td>OR80</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renshaw, Jane</td>
<td>OR1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Revel, Ingrid</td>
<td>POC46</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reynolds, Patrick</td>
<td>OR57, PL18, POT28*, POT30, WS27*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhaman, Nazneen</td>
<td>OR76</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ribacke, Ulf</td>
<td>OR51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ribatti, Domenico</td>
<td>OR29, OR71</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ribeiro, Agnes</td>
<td>OR34, OR87, POB21, POC27, POT11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Richard, Lauren</td>
<td>POT65*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Richon, Catherine</td>
<td>POC45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Richardson, Linda</td>
<td>POT60</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ridderstråle, Karin</td>
<td>OR55</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rieder, Dietmar</td>
<td>SEL42</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rigo, Valentina</td>
<td>POT16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rihan, Ali</td>
<td>POT55, POB73, POB91*, POT3, POT79*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rinaldi, Christian</td>
<td>POC84</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rinaldo, Cinzia</td>
<td>POC84</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ringmer, Markus</td>
<td>POC73</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ringstedt, Thomas</td>
<td>POC36</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rios, José</td>
<td>POT27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roberts, Steve</td>
<td>POT64</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Robins, Simon</td>
<td>POCS1*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roda, Aldo</td>
<td>PL16, POT17, SEL36</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rodríguez, Eva</td>
<td>POT14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rohrer, Hermann</td>
<td>POC100*, POT39</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rokita, Hanna</td>
<td>POC48</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roman, Pimenov</td>
<td>SEL43</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rommelaere, Jean</td>
<td>OR19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rooney, Cionla</td>
<td>POB27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rosasco, Lorenzo</td>
<td>POC96</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rosenstiehl, Philipp</td>
<td>POC32</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ross, Kenneth</td>
<td>OR19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rossig, Claudia</td>
<td>OR7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roth, Wilfried</td>
<td>OR7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotman, Maarten</td>
<td>OR7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rouffiac, Valérie</td>
<td>POB18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rubansky, Mikhail</td>
<td>POLB6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rubie, Hervé</td>
<td>OR34, OR64, SEL41</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ruiz-Sauri, Amparo</td>
<td>POC6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rupp, Martina</td>
<td>POT67</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rusakiewicz, Sylvie</td>
<td>SEL39</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Russell, Amanda</td>
<td>POB97</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Russell, Heidi</td>
<td>OR31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ruud, Ellen</td>
<td>OR19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ruuth, Kristina</td>
<td>OR68</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ryabov, Andrey</td>
<td>OR68</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ryan, Jacqueline</td>
<td>OR68, POT72, POT34</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rössler, Jochen</td>
<td>POLB6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>OR65</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Safdie, Fernando</td>
<td>OR56</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saito, Erik</td>
<td>POC92*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saito, Kengo</td>
<td>POC92</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saito, Takeshi</td>
<td>POC92</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sakai, Ryuchi</td>
<td>OR56</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sala, Arturo</td>
<td>OR27, OR27*, OR54, POC22, POC96</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salatino, Mariana</td>
<td>OR71</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salo, Jil</td>
<td>POT1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salvador, Christina</td>
<td>SEL39</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salvi, Sandra</td>
<td>POB30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sandan, Masashi</td>
<td>OR70, POB83</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sandstedt, Bengt</td>
<td>SEL34</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SantaMaria, Jaione Simon</td>
<td>SEL22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Santana, Victor</td>
<td>POC9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sappino, AP</td>
<td>POT32</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sapra, Puja</td>
<td>OR29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarnacki, Sabine</td>
<td>POC27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Sartelet, Hervé
Sartoletti, Alan
Sato, Yoshiharu
Sawada, Tadashi
Saveliev, Leonid
Savelyeva, Larissa
Savendahl, Lars
Savich, Tatyana
Savoldo, Barbara
Savva, Natalia
Scarlett, Chris
Scarfuffi, Paola
Scheidt, Katharina
Scheding, Stefan
Scheel-Walter, Hans-Gerhard
Schiapparelli, Paula
Schiaffio, Amalia
Schild, Linda
Schilderink, Nathalie
Schilling, Freimit H.
Schlehofer, Jörg R.
Schleiermacher, Guadrun
Schlierf, Stefanie
Schmid, I
Schmidt, Mary Lou
Schmidt, Mirko
Schmidt, Ulrike
Schnepp, Robert
Schonherr, Christina
Schreiber, Stefan
Schroff, Hubert
Schouten, Jan
Schramm, Alexander
Schrappe, Martin
Schreier, Günther
Schulte, Johannes
Schultz, C
Schumacher-Kuckelkorn, Roswitha
Schwab, Manfred
Schweigerer, Lothar
Schönherr, Christina
Scotlandi, Katie
Screpanti, Isabella
See, Viola
Seeger, Robert
Seethalaxmi, V
Segers, Stephanie
Segerström, Lova

POB28
OR31
POB92
POC12, POC45
PL28, POC36
POB13, SEL3*
SEL19
POLB8, POLB9
OR47
OR36, OR79, POB30,
POB93, POLB11, POLB12,
POT77
OR36
SEL11
PL35
POB94*
POLB4*
SEL47
POB46
POT78, SEL13
SEL43
C6*, OR34*, OR36, OR38,
OR78, OR87, POB73,
POC27, POT11*, SEL41,
WS19
PL22*, POC84, POB96,
WS14
OR85, PL22
OR35, OR41, OR65, OR66,
OR72, OR85*, PL22, POB32,
POB38, POB73, POB74,
POB96, POT3, POT19,
SEL18*, SEL38, WS14, WS18
POC37
POB96
OR38
OR5, OR35, OR41, OR65,
OR66*, OR72, OR85, PL6,
PL22, POB32, POB38,
POB73, POB74, POB96,
POT19, POT54, POT78,
SEL7, SEL18, SEL38*, WS14,
WS18*
POC33, POT78
OR26, OR31, OR36, PL7,
POB12, POB13,
POB46, POB77, POT81,
SEL2, SEL3, SEL30
POC17
OR81
OR68, POT34
OR1
OR49, POB84, POB109
OR12, WS9
OR18, OR36, PL8, POB4,
POB5, POB98, POC13,
POT1, POT2, SEL9, SEL24,
WS20
POC15
OR1
PL11, POT5, POT29, POT72*,
SEL44
POC6
OR25*, OR47, POB70
SEL37
POT57
POB97*
SEL39
POT82
OR1
POT14
POT38
POC46
POC73, POT76
POC35*
POC29
POT18
POT69
POC1
OR4
OR3
OR18, SEL24
POC34
POT78
POC94
OR24, OR25
SEL46
OR53*, POT74, SEL21*
PL28, POC36*
POC22
OR8
POT58
POT33
POT32
POC10
C4*
POC44
POT19
POC4
POC2
POC33, POC37*,
PL26, SEL13
POC11
POB99*, POB103, POT38,
POT52, POT65
POC18
POB19, POB71, SEL8,
SEL28
OR8
POC1
POC38*, POC39*
OR17
POC18
POC2
PL8, POB5, POT2
POC24, SEL12
OR26
POB93
POC8
POC43, POT70, POT75*
<table>
<thead>
<tr>
<th>Author</th>
<th>Index Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speleman, Frank</td>
<td>C1*, OR35, OR36, OR38, OR41, OR65, PL6, PL22, PL27, POB32, POB34, POB38, POB55, POB73, POB74, POB91, POB108, POT3, POT79, SEL30, SEL38, SEL41, SEL42, WS4, WS5</td>
</tr>
<tr>
<td>Spence, Yunyu</td>
<td>POT36</td>
</tr>
<tr>
<td>Spitz, Ruediger</td>
<td>SEL13</td>
</tr>
<tr>
<td>Spoto, Richard</td>
<td>POB5, POC13, POT1, POT2</td>
</tr>
<tr>
<td>Spuller, Ekkehart</td>
<td>POB15</td>
</tr>
<tr>
<td>Stallings, Raymond</td>
<td>OR6, OR35, OR38, OR46, OR75, PL21, PL27, POB2, POB17, POB18, POB29, POB34, POB113, SEL4, SEL7, WS12*</td>
</tr>
<tr>
<td>Stambuk, Hilda</td>
<td>POC14</td>
</tr>
<tr>
<td>Stark, Batia</td>
<td>POT46</td>
</tr>
<tr>
<td>Starke, Sven</td>
<td>OR39</td>
</tr>
<tr>
<td>Stavropoulos, Dimitri</td>
<td>POC38</td>
</tr>
<tr>
<td>Stegmaier, Kimberley</td>
<td>POT4</td>
</tr>
<tr>
<td>Stein, Jerry</td>
<td>POT45</td>
</tr>
<tr>
<td>Stermann, Alexander</td>
<td>POT76*</td>
</tr>
<tr>
<td>Stetler-Stevenson, Maryalice</td>
<td>POT85</td>
</tr>
<tr>
<td>Stewart, Clinton</td>
<td>POCC7, POCC9</td>
</tr>
<tr>
<td>Stewart, Rodney</td>
<td>PL12</td>
</tr>
<tr>
<td>Stigliani, Sara</td>
<td>OR79, POB30, POB93, POT77</td>
</tr>
<tr>
<td>Stoker, Andrew</td>
<td>POB26</td>
</tr>
<tr>
<td>Stossi, Fabio</td>
<td>PL20</td>
</tr>
<tr>
<td>Stroeken, Peter</td>
<td>OR1, POB114</td>
</tr>
<tr>
<td>Stubbs, James</td>
<td>OR59</td>
</tr>
<tr>
<td>Studer, Lorenz</td>
<td>WS5</td>
</tr>
<tr>
<td>Stuehler, Kai</td>
<td>POT50</td>
</tr>
<tr>
<td>Sturm, Dominik</td>
<td>OR26</td>
</tr>
<tr>
<td>Stutterheim, J</td>
<td>POT78*</td>
</tr>
<tr>
<td>Suenaga, Yusuke</td>
<td>POC41</td>
</tr>
<tr>
<td>Sugimoto, Tohru</td>
<td>POC12, POC45</td>
</tr>
<tr>
<td>Suh, Yeon Lim</td>
<td>POC41</td>
</tr>
<tr>
<td>Sumineo, Aiko</td>
<td>POC43, POT70</td>
</tr>
<tr>
<td>Sumitomo, Naokata</td>
<td>POC35</td>
</tr>
<tr>
<td>Sun, Can-Lan</td>
<td>OR82</td>
</tr>
<tr>
<td>Sun, Jianping</td>
<td>OR18, SEL24</td>
</tr>
<tr>
<td>Sung, Ki Woong</td>
<td>POC40*, POC41*, POC42*</td>
</tr>
<tr>
<td>Suniol, Mariona</td>
<td>POT27</td>
</tr>
<tr>
<td>Suragh, Cecelia</td>
<td>PL8</td>
</tr>
<tr>
<td>Sveinbjörnsson, Baldur</td>
<td>PL11, POB85*, POB3, POT5, POT72, SEL22, SEL34, OR88, PL29</td>
</tr>
<tr>
<td>Swerts, Katrien</td>
<td>OR30*</td>
</tr>
<tr>
<td>Sylvain, Baruchel</td>
<td>T</td>
</tr>
<tr>
<td>Tabori, Uri</td>
<td>T</td>
</tr>
<tr>
<td>Tadeo, Irene</td>
<td>T</td>
</tr>
<tr>
<td>Taguchi, Tomoaki</td>
<td>T</td>
</tr>
<tr>
<td>Tajiri, Tatsuro</td>
<td>OR30</td>
</tr>
<tr>
<td>Takagi, Daisuke</td>
<td>OR30</td>
</tr>
<tr>
<td>Takahashi, Yoshiyuki</td>
<td>OR30</td>
</tr>
<tr>
<td>Takano, Ryo</td>
<td>OR30</td>
</tr>
<tr>
<td>Takatori, Atsushi</td>
<td>OR30</td>
</tr>
<tr>
<td>Takenobu, Hisanori</td>
<td>OR30</td>
</tr>
<tr>
<td>Takenouchi, Ayako</td>
<td>OR30</td>
</tr>
<tr>
<td>Takita, Junko</td>
<td>OR30</td>
</tr>
<tr>
<td>Tam, Paul</td>
<td>OR30</td>
</tr>
<tr>
<td>Tamura, Yutaka</td>
<td>OR30</td>
</tr>
<tr>
<td>Tan, Choon-Yee</td>
<td>OR30</td>
</tr>
<tr>
<td>Tan, Owen</td>
<td>OR30</td>
</tr>
<tr>
<td>Taniaka, Sakura</td>
<td>OR30</td>
</tr>
<tr>
<td>Tanaka, Takeo</td>
<td>OR30</td>
</tr>
<tr>
<td>Tang, Xiao</td>
<td>OR30</td>
</tr>
<tr>
<td>Tanyeli, Atilla</td>
<td>OR30</td>
</tr>
<tr>
<td>Tapscott, Stephen</td>
<td>OR30</td>
</tr>
</tbody>
</table>

** = Presenting author
<table>
<thead>
<tr>
<th>Name</th>
<th>OR/PL/POT</th>
<th>Sel./WS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uchisaka, Naoki</td>
<td>OR70</td>
<td></td>
</tr>
<tr>
<td>Ueda, Yasuji</td>
<td>OR14, PL15</td>
<td></td>
</tr>
<tr>
<td>Uehling, David</td>
<td>PL11</td>
<td></td>
</tr>
<tr>
<td>Uhlén, Per</td>
<td>OR45</td>
<td></td>
</tr>
<tr>
<td>Ulrich, Scott M.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unpakar, Urmila</td>
<td>POT23</td>
<td></td>
</tr>
<tr>
<td>Urakami, Tatsuhiko</td>
<td>POC35</td>
<td></td>
</tr>
<tr>
<td>Urban, Christian</td>
<td>OR84</td>
<td></td>
</tr>
<tr>
<td>Urbanski, Laura</td>
<td>POB88</td>
<td></td>
</tr>
<tr>
<td>Wahleström, Therese</td>
<td>OR51</td>
<td>PL36, SEL10</td>
</tr>
<tr>
<td>Vaidya, Sucheta</td>
<td>OR25</td>
<td>SEL33</td>
</tr>
<tr>
<td>Wainwright, Brandon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wakayama, Teruhiko</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valdora, Francesca</td>
<td>OR34, OR36, OR38, SEL40</td>
<td>OR34, OR38, PL29, PL33, POB45, POB97, SEL15</td>
</tr>
<tr>
<td>Valent, A</td>
<td>OR34, OR36, OR38, SEL40</td>
<td>OR34, OR38, PL29, PL33, POB45, POB97, SEL15</td>
</tr>
<tr>
<td>Valenti, Marcus</td>
<td>OR44</td>
<td></td>
</tr>
<tr>
<td>Walker, Erin</td>
<td>OR30</td>
<td></td>
</tr>
<tr>
<td>Valli, Emanuele</td>
<td>OR54, SEL5*</td>
<td></td>
</tr>
<tr>
<td>Valochnik, Alena</td>
<td>PUBL8</td>
<td></td>
</tr>
<tr>
<td>Valteau-Couanet, Dominique</td>
<td></td>
<td></td>
</tr>
<tr>
<td>van Bekkum, Margo</td>
<td>OR52</td>
<td></td>
</tr>
<tr>
<td>van Crieke, Wim</td>
<td>OR7</td>
<td></td>
</tr>
<tr>
<td>van de Water, Bob</td>
<td>OR7</td>
<td></td>
</tr>
<tr>
<td>van den Abbeele, Thierry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>van der Ploeg, Ida</td>
<td>POC27</td>
<td></td>
</tr>
<tr>
<td>van der Schoot, C E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>van Groningen, Tim</td>
<td></td>
<td></td>
</tr>
<tr>
<td>van Kuik-Romeijn, Petra</td>
<td></td>
<td></td>
</tr>
<tr>
<td>van Kuilenburg, André</td>
<td></td>
<td></td>
</tr>
<tr>
<td>van Maerken, Tom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>van Nes, Johan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>van Noesel, M M</td>
<td>OR35, OR36, OR65, POB38, SEL40</td>
<td>OR35, OR36, OR65, POB38, SEL40</td>
</tr>
<tr>
<td>van Peer, Gert</td>
<td>OR7</td>
<td></td>
</tr>
<tr>
<td>van Roy, N</td>
<td>OR7</td>
<td></td>
</tr>
<tr>
<td>van Roy, Nadine</td>
<td>OR7</td>
<td></td>
</tr>
<tr>
<td>van Sluis, Peter</td>
<td>OR7</td>
<td></td>
</tr>
<tr>
<td>van Tol, MJD</td>
<td>OR7</td>
<td></td>
</tr>
<tr>
<td>Wan, Zesheng</td>
<td>OR7</td>
<td></td>
</tr>
<tr>
<td>Vanderstraeten, Nathalie</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vanderaalder, Valerie</td>
<td>OR7</td>
<td></td>
</tr>
<tr>
<td>Vandesompele, Jo</td>
<td>OR7</td>
<td></td>
</tr>
<tr>
<td>Wang, Chunxi</td>
<td>OR76</td>
<td>PL5</td>
</tr>
<tr>
<td>Wang, Kai</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Wang, Lifeng</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Wang, Wenchao</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Wang, Yu</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Vanni, Steven</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Varela, Carly</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Varesio, Luigi</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Varhol, Richard</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Warnaert, Patrick</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Vasily, Boyarshinov</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Vassal, Gilles</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Watt, Fujiko</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Watters, Karen</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Vaughn, Lynsey</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Wayne, Alan</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Weber, Axel</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Webster, Keith</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Wegener, Dennis</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Wei, Yung</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Wei Yau Liu, Cathy</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Wei, Jie</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Wei, Jun</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Wei, Renn-Shian</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Wei, Xiao</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Weinmar, Joel</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Weiss, Brian</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Weiss, William</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Wels, Winfried S.</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Wen Fong, Ooi</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Wen, Xinyu</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Weng-En, Lui</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Verboon, Lonneke</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Verissimo, Carla S.</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Vermeulen, Joelle</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Verney, Åsa</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Verri, Alessandro</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Verschuur, Arnaud</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Versteeg, Rogier</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Westermark, Ulrica</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>White, Mike</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>White, Peter S.</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Vialard, Jorge</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Vicha, A</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Vicha, Ales</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Wickstrom, Malin</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Vidal, Marc</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Wieczorek, Aleksandra</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Wigerup, Caroline</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Vigny, Marc</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Vilborg, Anna</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Wilhelm, Margaretta</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Villalba, Judith</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Villamor, E</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Villamor, Eva</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Wiman, Klas</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Winberg, Jan-Olof</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Winter, Cynthis</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Viprey, Virginie</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Virden, Ryan</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Vishvanath, Anasuya</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Vishvanathan, Seethalakshmi</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Vita, Marina</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Witt, Olaf</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Witte, John</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Voermans, C</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Vojvodic, Milijana</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Volchenbom, Samuel L.</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Volckmann, Richard</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Walden, Suzanne L.</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Wolmer-Solberg, Nina</td>
<td>OR32</td>
<td></td>
</tr>
<tr>
<td>Volobuev, Andrey</td>
<td>OR32</td>
<td></td>
</tr>
</tbody>
</table>

* = Presenting author
Author Index

* = Presenting author

Wolter, Jennifer POB116*
von Deimling, Andreas OR45
von Schweinitz, Dietrich OR81
von Stedingk, Kristoffer POB67, POB111*
Woo, Chan-Wook OR32, POC28
Woo, Jana OR13
Wood, Andrew PL18
Wood, Andrew OR3*
Vora, Tushar POC15
Wozniak, Agnieszka POT40
Wozniak, Wojciech POT44
Wrana, Jeff PL15
Vree, F POT78
Vreugdenhil, Erno OR7
Vu, Annette POB66, POT30, SEL17
Wu, Bing POT51
Wu, Hong-Wei OR18, SEL24
Wu, Pei-Yi POC11
Vyatkin, Igor POC36

X
Xu, Hong POT83
Xu, Ning OR47
Xu, Yibing OR18*, SEL24*
Xue, Cheng OR31

Y
Yagasaki, Hiroshi POC35
Yagyu, Shigeki POT84*
Yamada, Chizu SEL31
Yamaguchi, Yohko OR50, POT69
Yamashiro, Chika POT42
Yan, Qinzi OR55
Yan, Shuang POT85*, WS24
Yañez, Yania POB50, POC50
Yang, Hai-Ling POB95
Yang, Rong POT82
Yang, Yong-Li PL31, POC19
Yanik, Gregory OR58*, OR59, PL30*, POC7,
POT13, POC23, POC25
Yaniv, Isaac OR36, POB6*, POT45*
Yao, Xiaopan POC10
Yao, Zichen POB40
Yataghene, Karima OR15, POC16
Yeger, Herman POB8, POT51, POT86
Yeh, Susan OR28, OR37, PL24
Yigit, Nurten POB55, POB91, POT79
Yilmaz, Sema POB58*, POB59*
Yin, Chen POB117*
Yokochi, Tomoki OR74, OR86, SEL31*
Yokoyama, Tomoaki POT71
Yoneda, Akhiro SEL16*
Yonemitsu, Yoshikazu POT70
Yoo, Keon Hae POC40, POC41, POC42
Yoshida, Hideo POB92
Young, Song POB52
Yu, Alice C8*, WS23*
Yu, Fan SEL31
Yu, Fei POC51
Yu, Meng PL9
Yusuke, Suenaga POB61, SEL6*
Yuzawa, Yukio POT42
Yvon, Eric OR19
Yılmaz, Osman SEL20

Z
Zabrocki, Piotr OR65
Zahedi, Sarah SEL3
Zakaria, Siti Mariam OR55
Zanzonico, Pat POC24, SEL12
Zapata, Marc POB13, SEL2
Zappeij - Kannegieter, L POT78
Zettini, Samantha SEL28
Zencuslussen, Ana C OR17, OR20
Zhang, Haitao OR76
Zhang, Haito PL5
Zhang, Junghui PL18, POB105*, POB118*,
POC8, POC51*, POT2
Zhang, Libo POB8, POT51, POT86*
Zhang, Yan POB93
Zhao, Hongyu PL18, POB99
Zhao, Huaqing PL8
Zhao, Yongjun POT49*
Zhong, Quan SEL45
Zhu, Kejin OR44
Zhu, Yuyan PL9
Zhuang, Tiangang POC119*, POT47, POT48
Ziegler, Andrea OR23, OR38, OR84
Ziegler, David OR51, WS13
Zirath, Hannu POT26, SEL44*
Zivovogel, Laurence POB97
Zollo, Massimo POT74
Zorzoli, Alessia POT12
Zou, Huawei POC8
Zykova, Svetlana POC85

Ä
Ährlund-Richter, Lars SEL34

Ö
Özoðul, Candan SEL20
<table>
<thead>
<tr>
<th>Keyword</th>
<th>OR accession numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK</td>
<td>OR65, OR66, OR68, OR70, OR71, PL18, POB108, POB114, POB21, POB31, POB68, POB7, POB72, POB76, POB77, POC38, POT11, POT34, POT4, SEL4</td>
</tr>
<tr>
<td>Angiogenesis</td>
<td>OR29, OR3, OR71, OR8, PL17, POB111, PLB5, POT36, POT51, POT61, POT66, POT86, SEL12, SEL34, SEL35, SEL39</td>
</tr>
<tr>
<td>Animal Models</td>
<td>OR31, OR44, OR68, OR69, OR71, PL12, PL16, PL17, POB101, POB119, POB37, POB51, POB52, POB66, POB86, POT16, POT18, POT29, POT30, POT36, POT39, POT51, POT53, POT86, POT76, SEL17, SEL23, SEL26, SEL33, SEL34, SEL35, SEL38</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>OR1, OR24, OR25, OR3, OR4, OR40, OR6, OR7, OR71, OR74, PL9, POB104, POB109, POB118, POB20, POB26, POB37, POB4, POB43, POB44, POB49, POB50, POB64, POB66, POB75, POB76, POB77, POB8, POB80, POB81, POB84, POB86, POB92, POB94, POC6, POLB10, POT10, POT26, POT29, POT30, POT31, POT40, POT46, POT50, POT59, POT66, POT76, SEL17, SEL20, SEL23, SEL26, SEL33, SEL34, SEL35, SEL38</td>
</tr>
<tr>
<td>Cancer Stem Cells</td>
<td>OR10, OR11, OR12, OR13, OR14, OR50, OR8, OR9, PL10, POB1, POB103, POB113, POB115, POB117, POT26, POT29, POT33, POT39, POT40, POT50, POT54, POT56, POT64, POT80, SEL10, SEL16, SEL29, SEL33, SEL34</td>
</tr>
<tr>
<td>Caspases</td>
<td>OR4, OR44, PL9, POB105, POB118, POT46, SEL20, SELEN27</td>
</tr>
<tr>
<td>CGH</td>
<td>OR34, OR38, OR60, OR67, OR75, OR77, OR79, OR83, PL23, PL27, PL6, POB13, POB2, POB21, POB32, POB38, POB5, POB53, POB62, POB71, POB93, POC38, POT2, POT77, SEL2, SEL3, SEL40</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>OR56, OR61, OR63, OR81, OR82, OR84, PL33, PL35, PL36, POB116, POB28, POB39, POB64, POB78, POB80, POB81, POB88, POB9, POT10, POT12, POT16, POT18, POT20, POT22, POT44, POT57, SEL13, SEL33, SEL34</td>
</tr>
<tr>
<td>Chromosome</td>
<td>OR30, OR32, OR39, OR80, OR83, POB112, POB119, POB52, POB58, POB59, POB93, POC38, POLB8, POT45, SEL28</td>
</tr>
<tr>
<td>Circulating Tumor Cells</td>
<td>OR23, OR39, OR88, PL29, POT50, POT51, POT52, POT54, POT80, SEL21, SEL8</td>
</tr>
<tr>
<td>DNA Damage</td>
<td>OR11, OR12, OR31, OR40, OR41, OR42, OR43, OR45, OR50, OR51, OR73, OR8, PL12, PL7, POB1, POB103, POB113, POB115, POB117, POT26, POT29, POT33, POT39, POT40, POT46, POT54, POT55, POT56, POT61, POT66, POT76, SEL17, SEL20, SEL23, SEL26, SEL33, SEL34, SEL35, SEL38</td>
</tr>
<tr>
<td>Differentiation</td>
<td>OR49, OR77, PL11, PL24, PL6, POB12, POB13, POB20, POB48, POB53, POB78, POT84, POT87, POT13, POT19, POT23, POT58, POT83, SEL28, SEL3, SEL31, SEL47</td>
</tr>
<tr>
<td>Epidemiology</td>
<td>OR33, OR64, OR76, OR82, OR83, PL5, POB63, SEL13, POLC5, POLB4, POT37</td>
</tr>
<tr>
<td>Epigenetics</td>
<td>OR10, OR11, OR26, OR32, OR44, OR45, OR46, OR50, OR54, PL21, POB17, POB18, POB19, POB29, POT33, POT40, POT55, POT88, POT90, POT10, POT22, POT47, POT54, POT57, SEL10, SEL30, SEL5</td>
</tr>
<tr>
<td>Epistemology</td>
<td>OR57, POT28, POT50, OR23, OR80, POB15, POB58, POB68, POLB6, POLB8, POT43, POT45, POT55, POT62, POT67, POT77, POT75, POT8, SEL35, SEL41</td>
</tr>
<tr>
<td>FGL2</td>
<td>OR15, OR19, OR21, OR23, OR63, OR89, PL28, PL34, POB14, POB16, POC3, POLC33, POC4, POT39, POT53, POT73, SEL12, SEL26, SEL35</td>
</tr>
<tr>
<td>FISH</td>
<td>OR61, OR81, POB1, POB3, POB8, POB85, PC10, PC14, PC45, PC33, PC43, POT44, POT57, SEL13, SEL33, SEL34</td>
</tr>
<tr>
<td>Genome-wide screens</td>
<td>OR15, OR16, OR17, OR18, OR19, OR20, OR21, OR22, OR63, OR89, PL34, POB37, POB41, POB51, POB6, POB97, PC17, PC22, PC33, PC37, PC43, PC4, PC48, PC80, POLB10, POLB3, POT12, POT16, POT39, POT49, POT53, POT70, POT73, POT76, POT85, SEL11, SEL12, SEL24, SEL26, SEL48</td>
</tr>
<tr>
<td>Immunotherapy</td>
<td>OR18, OR22, POB100, POB51, POB80, POB85, POB100, POB88, POB85, POLB10, POLB3, POT1, POB6, POT92, SEL24</td>
</tr>
<tr>
<td>Inflammation</td>
<td>OR64, OR83, PL32, POT67, OR77, PL35, PL36, PC26, PC32, PC35, PC51, PC8, SEL15, SEL19, SEL20</td>
</tr>
<tr>
<td>INRG</td>
<td>OR64, OR83, PL32, POT67, OR77, PL35, PL36, PC26, PC32, PC35, PC51, PC8, SEL15, SEL19, SEL20</td>
</tr>
<tr>
<td>Keyword Index</td>
<td>Novel Therapeutic Agents</td>
</tr>
<tr>
<td>---------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Metastasis</td>
<td>OR13, OR39, OR44, OR63,</td>
</tr>
<tr>
<td></td>
<td>OR64, OR79, PL10, PL31,</td>
</tr>
<tr>
<td></td>
<td>PL33, POB106, POB107,</td>
</tr>
<tr>
<td></td>
<td>POB22, POB23, POB24,</td>
</tr>
<tr>
<td></td>
<td>POB25, POB30, POB4, POB50,</td>
</tr>
<tr>
<td></td>
<td>POB98, POC12, POC14,</td>
</tr>
<tr>
<td></td>
<td>POC17, POC36, POC44,</td>
</tr>
<tr>
<td></td>
<td>POC47, POC50, POC6, POLB2,</td>
</tr>
<tr>
<td></td>
<td>POLB5, POT18, POT23, POT51,</td>
</tr>
<tr>
<td></td>
<td>POT78, POT80</td>
</tr>
<tr>
<td></td>
<td>OR58, OR59, OR81, PL30,</td>
</tr>
<tr>
<td></td>
<td>PL31, POC13, POC24, POC25,</td>
</tr>
<tr>
<td></td>
<td>POC29, POC34, POC39,</td>
</tr>
<tr>
<td>Micro RNA</td>
<td>POC46, POC7, POT58, SEL11,</td>
</tr>
<tr>
<td></td>
<td>SEL13</td>
</tr>
<tr>
<td>MIBG</td>
<td>OR35, OR41, OR51, OR6,</td>
</tr>
<tr>
<td></td>
<td>OR75, PL22, PL27, POB108,</td>
</tr>
<tr>
<td></td>
<td>POB115, POB32, POB34,</td>
</tr>
<tr>
<td></td>
<td>POB38, POB45, POB74,</td>
</tr>
<tr>
<td></td>
<td>POB91, POB96, POLB11,</td>
</tr>
<tr>
<td></td>
<td>POT3, POT54, POT77, POT80,</td>
</tr>
<tr>
<td></td>
<td>SEL1, SEL7</td>
</tr>
<tr>
<td>MLPA</td>
<td>OR38, OR84, POB68, POT32,</td>
</tr>
<tr>
<td></td>
<td>POT45, POT57, POT67, POT77,</td>
</tr>
<tr>
<td></td>
<td>SEL40, SEL41</td>
</tr>
<tr>
<td>MYCN</td>
<td>OR10, OR2, OR25, OR26,</td>
</tr>
<tr>
<td></td>
<td>OR27, OR3, OR39, OR47,</td>
</tr>
<tr>
<td></td>
<td>OR48, OR49, OR50, OR51,</td>
</tr>
<tr>
<td></td>
<td>OR52, OR53, OR54, OR6,</td>
</tr>
<tr>
<td></td>
<td>OR60, OR61, OR64, OR75,</td>
</tr>
<tr>
<td></td>
<td>OR83, OR85, PL11, PL16,</td>
</tr>
<tr>
<td></td>
<td>PL17, PL20, PL21, PL22,</td>
</tr>
<tr>
<td></td>
<td>PL27, POB101, POB102, POB108,</td>
</tr>
<tr>
<td></td>
<td>POB109, POB110, POB113,</td>
</tr>
<tr>
<td></td>
<td>POB115, POB12, POB13,</td>
</tr>
<tr>
<td></td>
<td>POB15, POB17, POB20,</td>
</tr>
<tr>
<td></td>
<td>POB22, POB3, POB41, POB43,</td>
</tr>
<tr>
<td></td>
<td>POB44, POB45, POB46,</td>
</tr>
<tr>
<td></td>
<td>POB47, POB48, POB49, POB5,</td>
</tr>
<tr>
<td></td>
<td>POB52, POB53, POB57,</td>
</tr>
<tr>
<td></td>
<td>POB58, POB61, POB66,</td>
</tr>
<tr>
<td></td>
<td>POB71, POB74, POB75,</td>
</tr>
<tr>
<td></td>
<td>POB79, POB84, POB86,</td>
</tr>
<tr>
<td>Neurotrophins</td>
<td>POB88, POB89, POB90,</td>
</tr>
<tr>
<td></td>
<td>POB96, POC1, POC11, POC34,</td>
</tr>
<tr>
<td></td>
<td>POC36, POC45, POB1,</td>
</tr>
<tr>
<td></td>
<td>POLB2, POLB7, POLB8,</td>
</tr>
<tr>
<td></td>
<td>POT14, POT17, POT19, POT20,</td>
</tr>
<tr>
<td>MLPA</td>
<td>POT25, POT26, POT27, POT32,</td>
</tr>
<tr>
<td></td>
<td>POT41, POT55, POT60, POT61,</td>
</tr>
<tr>
<td></td>
<td>POT62, POT68, POT71, POT72,</td>
</tr>
<tr>
<td></td>
<td>POT74, POT75, POT76, POT78,</td>
</tr>
<tr>
<td></td>
<td>POT81, SEL13, SEL17, SEL23,</td>
</tr>
<tr>
<td></td>
<td>SEL29, SEL3, SEL30, SEL31,</td>
</tr>
<tr>
<td></td>
<td>SEL32, SEL35, SEL36, SEL37,</td>
</tr>
<tr>
<td>Neurotrophins</td>
<td>SEL38, SEL41, SEL42, SEL44,</td>
</tr>
<tr>
<td></td>
<td>SEL46, SEL5, SEL6, SEL7,</td>
</tr>
<tr>
<td>Osteoclasts</td>
<td>SEL8</td>
</tr>
<tr>
<td>Neurotrophins</td>
<td>OR11, OR13, OR69, PL9,</td>
</tr>
<tr>
<td></td>
<td>POB103, POB64, POC23,</td>
</tr>
<tr>
<td></td>
<td>POT37, POT52, POT69, SEL18,</td>
</tr>
<tr>
<td>Neurotrophins</td>
<td>p53</td>
</tr>
<tr>
<td>Neurotrophins</td>
<td>PHO2B</td>
</tr>
<tr>
<td>Prognostic Factors</td>
<td>Structural Abberation</td>
</tr>
<tr>
<td>----------------------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>OR23, OR31, OR34, OR35,</td>
<td>OR34, OR38, OR67, OR77,</td>
</tr>
<tr>
<td>OR36, OR38, OR43, OR51,</td>
<td>OR78, OR79, PL23, PL7,</td>
</tr>
<tr>
<td>OR60, OR64, OR66, OR67,</td>
<td>POB15, POB21, POB38,</td>
</tr>
<tr>
<td>OR72, OR75, OR81, OR82,</td>
<td>POB53, POB71, POB93,</td>
</tr>
<tr>
<td>OR84, OR85, OR86, OR87,</td>
<td>POC38, POLB11, POLB12,</td>
</tr>
<tr>
<td>OR88, PL22, PL23, PL27, PL29,</td>
<td>POLB8, POT47, POT48, POT57,</td>
</tr>
<tr>
<td>PL30, PL32, PL7, POB115,</td>
<td>POT62, POT67, POT77, SEL3, SEL40, SEL41, SEL42</td>
</tr>
<tr>
<td>POB117, POB14, POB16,</td>
<td></td>
</tr>
<tr>
<td>POB19, POB27, POB3, POB41,</td>
<td></td>
</tr>
<tr>
<td>POB46, POB47, POB59,</td>
<td></td>
</tr>
<tr>
<td>POB67, POB71, POB88,</td>
<td></td>
</tr>
<tr>
<td>POB96, POB97, POC13, POC3,</td>
<td></td>
</tr>
<tr>
<td>POC36, POC39, POC45,</td>
<td></td>
</tr>
<tr>
<td>POLB11, POLB12, POLB4,</td>
<td></td>
</tr>
<tr>
<td>POLB8, POT1, POT2, POT21,</td>
<td></td>
</tr>
<tr>
<td>POT25, POT27, POT32, POT33,</td>
<td></td>
</tr>
<tr>
<td>POT42, POT43, POT44, POT47,</td>
<td></td>
</tr>
<tr>
<td>POT48, POT52, POT54, POT55,</td>
<td></td>
</tr>
<tr>
<td>POT62, POT68, POT77, POT78,</td>
<td></td>
</tr>
<tr>
<td>POT8, POT80, POT84, POT86,</td>
<td></td>
</tr>
<tr>
<td>SEL10, SEL15, SEL39, SEL4,</td>
<td></td>
</tr>
<tr>
<td>SEL40</td>
<td></td>
</tr>
<tr>
<td>Proliferation</td>
<td>Structural Abberation</td>
</tr>
<tr>
<td>OR12, OR13, OR27, OR42,</td>
<td>OR34, OR38, OR67, OR77,</td>
</tr>
<tr>
<td>OR46, OR47, OR49, OR5, OR6,</td>
<td>OR78, OR79, PL23, PL7,</td>
</tr>
<tr>
<td>OR68, OR69, OR73, OR79,</td>
<td>POB15, POB21, POB38,</td>
</tr>
<tr>
<td>PL11, PL17, PL20, PL5, PL7,</td>
<td>POB53, POB71, POB93,</td>
</tr>
<tr>
<td>POB101, POB115, POB26,</td>
<td>POC38, POLB11, POLB12,</td>
</tr>
<tr>
<td>POB28, POB30, POB33,</td>
<td>POLB8, POT47, POT48, POT57,</td>
</tr>
<tr>
<td>POB3, POB39, POB54,</td>
<td>POT62, POT67, POT77, SEL3, SEL40, SEL41, SEL42</td>
</tr>
<tr>
<td>POB65, POB66, POB67,</td>
<td></td>
</tr>
<tr>
<td>POB69, POB70, POB72,</td>
<td></td>
</tr>
<tr>
<td>POB73, POB76, POB77,</td>
<td></td>
</tr>
<tr>
<td>POB79, POB80, POB85,</td>
<td></td>
</tr>
<tr>
<td>POB94, POB95, POB99,</td>
<td></td>
</tr>
<tr>
<td>POC10, POLB12, POT10,</td>
<td></td>
</tr>
<tr>
<td>POT13, POT18, POT19, POT19,</td>
<td></td>
</tr>
<tr>
<td>POT29, POT38, POT41, POT44,</td>
<td></td>
</tr>
<tr>
<td>POT54, POT69, SEL1, SEL22,</td>
<td></td>
</tr>
<tr>
<td>SEL29, SEL33</td>
<td></td>
</tr>
<tr>
<td>Prostaglandins</td>
<td></td>
</tr>
<tr>
<td>OR15, OR57, OR63, POB1,</td>
<td></td>
</tr>
<tr>
<td>POB113, POB26, POB61,</td>
<td></td>
</tr>
<tr>
<td>POB70, POC1, POC37, POC47,</td>
<td></td>
</tr>
<tr>
<td>POLB1, POT50, SEL6</td>
<td></td>
</tr>
<tr>
<td>Psychosocial factors</td>
<td></td>
</tr>
<tr>
<td>OR58, OR59, OR64, PL36,</td>
<td></td>
</tr>
<tr>
<td>POC20, POC24, POC35, POC7,</td>
<td></td>
</tr>
<tr>
<td>POT58</td>
<td></td>
</tr>
<tr>
<td>Radiotherapy</td>
<td></td>
</tr>
<tr>
<td>OR15, OR57, OR63, POB1,</td>
<td></td>
</tr>
<tr>
<td>POB113, POB26, POB61,</td>
<td></td>
</tr>
<tr>
<td>POB70, POC1, POC37, POC47,</td>
<td></td>
</tr>
<tr>
<td>POLB1, POT50, SEL6</td>
<td></td>
</tr>
<tr>
<td>Retinoic Acid</td>
<td></td>
</tr>
<tr>
<td>OR15, OR28, OR43, OR48,</td>
<td></td>
</tr>
<tr>
<td>OR68, OR66, PL19, PL27,</td>
<td></td>
</tr>
<tr>
<td>POB108, POB14, POB14,</td>
<td></td>
</tr>
<tr>
<td>POC22, POC22, POC69, POC14,</td>
<td></td>
</tr>
<tr>
<td>POLB3, POC38, POC43,</td>
<td></td>
</tr>
<tr>
<td>POT11, POT21, POT27, POT37,</td>
<td></td>
</tr>
<tr>
<td>POT42, POT65, POT84, SEL14,</td>
<td></td>
</tr>
<tr>
<td>SEL16</td>
<td></td>
</tr>
<tr>
<td>Screening</td>
<td></td>
</tr>
<tr>
<td>OR33, OR38, OR72, OR76,</td>
<td></td>
</tr>
<tr>
<td>PL24, PL5, PL6, POB110,</td>
<td></td>
</tr>
<tr>
<td>POB15, POB93, POT2, POT77,</td>
<td></td>
</tr>
<tr>
<td>SEL1</td>
<td></td>
</tr>
<tr>
<td>SNP</td>
<td></td>
</tr>
<tr>
<td>OR58, OR62, OR84, OR88,</td>
<td></td>
</tr>
<tr>
<td>PL33, PL35, POB6, POC1,</td>
<td></td>
</tr>
<tr>
<td>POC17, POC20, POC32,</td>
<td></td>
</tr>
<tr>
<td>POC34, POC36, POC40,</td>
<td></td>
</tr>
<tr>
<td>POT42, POC48, POC69,</td>
<td></td>
</tr>
<tr>
<td>POT78, SEL11, SEL15, SEL9</td>
<td></td>
</tr>
<tr>
<td>Stem Cell Transplantation</td>
<td></td>
</tr>
<tr>
<td>OR58, OR62, OR84, OR88,</td>
<td></td>
</tr>
<tr>
<td>PL33, PL35, POB6, POC1,</td>
<td></td>
</tr>
<tr>
<td>POC17, POC20, POC32,</td>
<td></td>
</tr>
<tr>
<td>POC34, POC36, POC40,</td>
<td></td>
</tr>
<tr>
<td>POC42, POC48, POC69,</td>
<td></td>
</tr>
<tr>
<td>POT78, SEL11, SEL15, SEL9</td>
<td></td>
</tr>
<tr>
<td>Proliferation</td>
<td></td>
</tr>
<tr>
<td>OR12, OR13, OR27, OR42,</td>
<td></td>
</tr>
<tr>
<td>OR46, OR47, OR49, OR5, OR6,</td>
<td></td>
</tr>
<tr>
<td>OR68, OR69, OR73, OR79,</td>
<td></td>
</tr>
<tr>
<td>PL11, PL17, PL20, PL5, PL7,</td>
<td></td>
</tr>
<tr>
<td>POB101, POB115, POB26,</td>
<td></td>
</tr>
<tr>
<td>POB28, POB30, POB33,</td>
<td></td>
</tr>
<tr>
<td>POB35, POB39, POB54,</td>
<td></td>
</tr>
<tr>
<td>POB65, POB66, POB67,</td>
<td></td>
</tr>
<tr>
<td>POB69, POB70, POB72,</td>
<td></td>
</tr>
<tr>
<td>POB73, POB76, POB77,</td>
<td></td>
</tr>
<tr>
<td>POB79, POB80, POB85,</td>
<td></td>
</tr>
<tr>
<td>POB94, POB95, POB99,</td>
<td></td>
</tr>
<tr>
<td>POC10, POLB12, POT10,</td>
<td></td>
</tr>
<tr>
<td>POT13, POT18, POT19, POT19,</td>
<td></td>
</tr>
<tr>
<td>POT29, POT38, POT41, POT44,</td>
<td></td>
</tr>
<tr>
<td>POT54, POT69, SEL1, SEL22,</td>
<td></td>
</tr>
<tr>
<td>SEL29, SEL33</td>
<td></td>
</tr>
<tr>
<td>Proliferation</td>
<td></td>
</tr>
<tr>
<td>OR12, OR13, OR27, OR42,</td>
<td></td>
</tr>
<tr>
<td>OR46, OR47, OR49, OR5, OR6,</td>
<td></td>
</tr>
<tr>
<td>OR68, OR69, OR73, OR79,</td>
<td></td>
</tr>
<tr>
<td>PL11, PL17, PL20, PL5, PL7,</td>
<td></td>
</tr>
<tr>
<td>POB101, POB115, POB26,</td>
<td></td>
</tr>
<tr>
<td>POB28, POB30, POB33,</td>
<td></td>
</tr>
<tr>
<td>POB35, POB39, POB54,</td>
<td></td>
</tr>
<tr>
<td>POB65, POB66, POB67,</td>
<td></td>
</tr>
<tr>
<td>POB69, POB70, POB72,</td>
<td></td>
</tr>
<tr>
<td>POB73, POB76, POB77,</td>
<td></td>
</tr>
<tr>
<td>POB79, POB80, POB85,</td>
<td></td>
</tr>
<tr>
<td>POB94, POB95, POB99,</td>
<td></td>
</tr>
<tr>
<td>POC10, POLB12, POT10,</td>
<td></td>
</tr>
<tr>
<td>POT13, POT18, POT19, POT19,</td>
<td></td>
</tr>
<tr>
<td>POT29, POT38, POT41, POT44,</td>
<td></td>
</tr>
<tr>
<td>POT54, POT69, SEL1, SEL22,</td>
<td></td>
</tr>
<tr>
<td>SEL29, SEL33</td>
<td></td>
</tr>
<tr>
<td>Prognostic Factors</td>
<td></td>
</tr>
<tr>
<td>OR23, OR31, OR34, OR35,</td>
<td></td>
</tr>
<tr>
<td>OR36, OR38, OR43, OR51,</td>
<td></td>
</tr>
<tr>
<td>OR60, OR64, OR66, OR67,</td>
<td></td>
</tr>
<tr>
<td>OR72, OR75, OR81, OR82,</td>
<td></td>
</tr>
<tr>
<td>OR84, OR85, OR86, OR87,</td>
<td></td>
</tr>
<tr>
<td>OR88, PL22, PL23, PL27, PL29,</td>
<td></td>
</tr>
<tr>
<td>PL30, PL32, PL7, POB115,</td>
<td></td>
</tr>
<tr>
<td>POB117, POB14, POB16,</td>
<td></td>
</tr>
<tr>
<td>POB19, POB27, POB3, POB41,</td>
<td></td>
</tr>
<tr>
<td>POB46, POB47, POB59,</td>
<td></td>
</tr>
<tr>
<td>POB67, POB71, POB88,</td>
<td></td>
</tr>
<tr>
<td>POB96, POB97, POC13, POC3,</td>
<td></td>
</tr>
<tr>
<td>POC36, POC39, POC45,</td>
<td></td>
</tr>
<tr>
<td>POLB11, POLB12, POLB4,</td>
<td></td>
</tr>
<tr>
<td>POLB8, POT47, POT48, POT57,</td>
<td></td>
</tr>
<tr>
<td>POT62, POT67, POT77, SEL3, SEL40, SEL41, SEL42</td>
<td></td>
</tr>
</tbody>
</table>