NOPHO Annual Meeting Programme
May 31st – June 4th 2013, Copenhagen

Welcome to Wonderful Copenhagen!
Welcome

Dear NOPHO Friends and Colleagues

The Childhood Cancer Unit of Rigshospitalet, Copenhagen is honored and excited to welcome you to the NOPHO 31st annual meeting.

We offer you one of the oldest and most vibrant capitals in Europe and at the same time an exciting chance to get updated on Childhood Cancer with an interesting scientific programme including excellent guest speakers from around the globe. It is our vision that the coming days will be a source of inspiration and open new fields of research and treatment for our patients.

Next to our scientific programme we also offer you a series of social events to obtain what we here in Denmark refer to as the term “Hygge” (have fun, relax, have a good time, get into a good mood). You can find more information about the various days' schedule on the following pages.

We offer a huge thanks to our sponsors, exhibitioners, guest speakers and everyone who has contributed to this years’ annual meeting. And we thank you for visiting and your participation.

The NOPHO 31st annual meeting has something to offer for everyone.

Welcome. We wish you a great time.

With best regards – On behalf of the organizing Committee,

The Childhood Cancer Unit of Rigshospitalet
Contents

Welcome 1
Contents 2
General Information 3
Working Group Meetings 10
Scientific Programme 12
Social Events 20
Sponsors & Exhibitors 22
Guest Speakers 24
Abstracts; Oral Presentation 41
Abstracts; Poster Presentations 63
NOPHO Annual Meeting 2014 85
Farewell Greetings from Copenhagen 86
General information

Meeting venue
Copenhagen Mariott Hotel
Kalvebod Brygge 5
DK-1560 Copenhagen, Denmark

With 401 spacious air-conditioned accommodations & luxury suites offer Marriot Copenhagen the finest water views in the city of Copenhagen, Denmark. The venue is within walking distance to Copenhagen Central Station or Tivoli Gardens and only 15 minutes' drive from the Copenhagen Airport. And with its 887 sqm of pure daylight conference venue it is one of Copenhagen’s best venues.

Insurance and liability
Neither the organizers, nor Marriot take any responsibility for injury or damage involving persons or property during the congress. Participants are advised to take their own health and travel insurance.

Language
The congress will be conducted in English.

Travel Information
Getting to Denmark is easy. Denmark is a peninsular in Northern Europe, which means you can go by car or train from any of the North European countries. Aarhus is close to the E45 motorway.

By plane
There are flights from several destinations throughout the world to three different airports in Denmark.

One of them is Copenhagen Airport (Kastrup) - with 65 airlines operating from Copenhagen Airport, and with more than 20 million passengers every year, it is Scandinavia’s biggest airport.

From the airport you can take the train or metro to Copenhagen. Please find useful information at http://www.cph.dk/CPH/UK/MAIN/Parking+and+Transport/
Local Information

**Banks**
Banks are open from 09:30 to 16:00 on weekdays with late hours until 18:00 on Thursdays (closed Saturdays and Sundays). There are numerous cash machines throughout the city and the plentiful money transfer offices are open during weekends, for example Forex at Nørreport Station and Copenhagen Central Station.

**Currency**
The currency in Denmark is Danish Kroner (DKK). One krone is divided into 100 øre.

**Electricity**
Copenhageners use 220 volt for electric appliances. Denmark, like most other European countries, has 220-volt AC, 50Hz current and uses two-pin continental plugs. If you visit from the UK and Ireland, you will need an adaptor for electric appliances, whereas North Americans need a transformer in order to use their 110/125V appliances.

**Language**
The mother tongue in Copenhagen is Danish, which is closely related to both Swedish and Norwegian. In General Danes speak English extremely well, and some even German and French.

**Time zone**
Denmark follows Central European Time (CET) which is one hour ahead of Greenwich Mean Time (GMT) and six hours ahead of Eastern Standard Time (EST).

**Tipping**
Tipping is greatly appreciated, but not expected, and you should only do so if you feel you are getting exceptionally good service. If you do so 10 percent of the bill is sufficient.

**Transportation**
It is very easy and safe to travel around the city by bus, train or Metro. You can buy tickets at almost every station or at the kiosk. You can also purchase your tickets directly in the bus. Tickets can be used regardless of the form of transportation (Metro, bus, train).
Copenhagen – Open for you

Welcome to Copenhagen, Scandinavia’s most fantastic city and the world’s oldest monarchy, where culture, art and cuisine unite. Copenhagen is the Capital and largest city of Denmark with an urban population of approximately 1.2 million. The city consists of a multitude of areas, each with its own charm, history and distinctive character.

Things to see in Copenhagen
We have listed here a small sample of sights and experiences available in Copenhagen. August is one of the busiest months in Copenhagen and the town and surrounding areas are blooming with activities – no matter where your interest may lie. For more information and ideas please visit www.visitcopenhagen.com.

The Royal residence

Amalienborg palace is considered one of the greatest works of Danish Rococo architecture and was constructed in the 1700’s. It is made up of four identical buildings - The Christian VII’s Palace is also known as Moltke’s Palace, The Christian VIII’s Palace is also known as Levetzau’s Palace, The Frederik VIII’s Palace is also known as Brockdorff’s Palace and The Christian IX’s Palace or Schack’s Palace – spread around the octagonal courtyard. This is the main residence of Her Majesty the Queen. When the flag is raised, it signals that the Queen is present.
Copenhagen's largest shopping area is centered around Strøget in the heart of the city. Strøget is the world's longest pedestrian street with a wealth of shops, from budget-friendly chains to some of the world's most expensive brands. The stretch is 1.1 kilometers long and runs from City Hall Square (Rådhuspladsen) to Kongens Nytorv. Strøget is a nickname from the 1800s and covers the streets Frederiksberggade, Nygade, Vimmelskaftet and Østeregade and Nytorv square, Gammeltorv Square and Amagertorv Square. The stretch was originally called Routen, and it was not before 1962 and in the years after it was converted to a pedestrian street.
A few minutes’ walk from the Town Hall Square, right in the middle of the city, you will see the gates of the famous old Tivoli Gardens. An enchanted world of exotic architecture, exquisite gardens and scenery, theatres, open air stages, restaurants, cafés, and 26 rides ranging from monstrous rides, that will twirl and spin their victims, to gentle children's rides.
A visit to Carlsberg will give you an idea how the world-famous Carlsberg Beer is made. Carlsberg is among the largest tourist attractions in Copenhagen and now you have the opportunity to get closer to the roots of Carlsberg, the history and the beer. The Old Carlsberg Brewery from 1847 has been converted into a modern centre for visitors covering 10,000 m².
Congress Secretariat
Registration forms, hotel reservations, and all correspondence concerning registration and accommodation (payment, cancellation, changes, social programme etc.) **must be sent to:**

KongresKompagniet A/S
Nordhavnsgade 4
DK-8000 Aarhus C
Phone + 45 8629 6960
Fax + 45 8629 6980
E-mail: nopho2013@kongreskompagniet.dk
www.kongreskompagniet.dk

**The Childhood Cancer Unit of Rigshospitalet, Copenhagen**
**Organizing committee**
Thomas Frandsen
Kjeld Schmiegelow
Catherine Rechnitzer
Birgitte Lausen
Karsten Nysom
Lisa Hjalgrim
Marianne Olsen
Jesper Brok
Marianne Hoffmann
Aisha Saroya
Rene Mathiassen
Annual Meeting 2013 Copenhagen

Working Group Meetings
**NOPHO Annual Meeting 2013 Programme**

Venue: Hotel Marriott, The Waterfront, Copenhagen.

May 31st – June 4th 2013

**Working Group Meetings:**

<table>
<thead>
<tr>
<th>Date</th>
<th>Working Group</th>
<th>Time</th>
<th>No. Attending</th>
<th>Room</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Friday May 31st</strong></td>
<td>Thrombosis and haemostasis</td>
<td>11:00-18.00</td>
<td>10-15</td>
<td>Samsøbælt</td>
</tr>
<tr>
<td></td>
<td>Young NOPHO</td>
<td>12:00-18:00</td>
<td>30</td>
<td>Skagerrak &amp; Kattegat</td>
</tr>
<tr>
<td></td>
<td>LLC</td>
<td>13:00-18:00</td>
<td>25</td>
<td>Østersøen &amp; Øresund</td>
</tr>
<tr>
<td></td>
<td>GCT</td>
<td>13:00-16:00</td>
<td>10</td>
<td>Lillebælt</td>
</tr>
<tr>
<td><strong>Saturday June 1st</strong></td>
<td>ECC</td>
<td>07:45-08:45</td>
<td>8</td>
<td>Lillebælt</td>
</tr>
<tr>
<td></td>
<td>LLC</td>
<td>09:00-12:00</td>
<td>20-25</td>
<td>Østersøen &amp; Øresund</td>
</tr>
<tr>
<td></td>
<td>Brain Tumor</td>
<td>09:00-12:00</td>
<td>12</td>
<td>Lillebælt</td>
</tr>
<tr>
<td></td>
<td>NOBOS</td>
<td>All day</td>
<td>16</td>
<td>Samsøbælt</td>
</tr>
<tr>
<td></td>
<td>Board Meeting</td>
<td>13:00-17:00</td>
<td>30</td>
<td>Nordsøen</td>
</tr>
<tr>
<td><strong>Sunday June 2nd</strong></td>
<td>ITP</td>
<td>07:00-09:00</td>
<td>10</td>
<td>Lillebælt</td>
</tr>
<tr>
<td></td>
<td>Extra Medullary</td>
<td>07:15-08:15</td>
<td>10-15</td>
<td>Wine Room</td>
</tr>
<tr>
<td></td>
<td>Late Effects</td>
<td>07:30-10:30</td>
<td>15</td>
<td>Nordsøen</td>
</tr>
<tr>
<td></td>
<td>NOBOS</td>
<td>09:00-14:00</td>
<td>16</td>
<td>Storebælt</td>
</tr>
<tr>
<td><strong>Monday June 3rd</strong></td>
<td>ALL2008</td>
<td>07:00-09:00</td>
<td>15-20</td>
<td>Nordsøen</td>
</tr>
</tbody>
</table>
Annual Meeting 2013 Copenhagen

Scientific Programme
NOPHO Annual Meeting 2013 Scientific Programme

Venue: Hotel Marriott, The Waterfront, Copenhagen.

June 1st – June 4th 2013

Saturday June 1st:

12:00               REGISTRATION OPENS

13:00-14:00         Lunch buffet at Restaurant “the Midtown Grill” (Marriott)

14:00-17:00         Young NOPHO Educational Session
                    Chair Lisa Hjalgrim

14:00-14:45         Challenges in running clinical Trials in NOPHO
                    Dr. Mats Heyman and Jenny Juhlin (“The NOPHO secretariat“)

14:45-15:15         Coffee Break

15:15-16:15         Challenges in running clinical trials in Europe
                    Professor Pamela Kearns, Birmingham, UK

16:15-17:00         Understanding outcome within the frame of host genomics – illustrated by childhood ALL
                    Professor Kjeld Schmiegelow and Ramneek Gupta, Copenhagen

18:00-20:00         Opening reception: The City Hall.
                    The Health Mayor of Copenhagen
                    Sct. Annae Girls Choir

20:00 -              After: Tivoli on your own
Sunday June 2nd:

08:30-08:45  Official Opening

08:45-09:45  The NOPHO Lecturer.
Chair: Secretary General, NOPHO, Marit Hellebostad

Professor Per Kogner, Stockholm

09:45-10:15  Coffee Break

10:15-11:45  FREE PAPERS. Leukemias
Chair: Birgitte Lausen

10:15-10:30  O1
Central Nervous System Disease in childhood acute lymphoblastic Leukemia. Mette Levinsen.

10:30-10:45  O2
Asparaginase Associated Pancreatitis in Childhood Acute Lymphoblastic Leukaemia. Raheel Raja.

10:45-11:00  O3
Deletions of IKZF1 and SPRED1 in pediatric B-cell precursor acute lymphoblastic are associated with poor prognosis. Linda Olsson

11:00-11:15  O4

11:15-11:30  O5
Does low dose PEG-asparaginase treatment lead to successful asparagine depletion in cerebrospinal fluid of children treated according to the NOPHO ALL-2008 protocol? Louise Tram Henriksen.

11:30-11:45  O6
Applicability of the WHO classification in pediatric AML. Julie Damgaard Sandahl.

12:00-13:00  Lunch buffet at “The Foyer” (Marriott)

13:00-14:15  FREE PAPERS: Late effects/Solid Tumors
Chair: Jesper Brok.

13:00-13:15  O7
Cardiovascular morbidity in long-term survivors of early onset cancer. Liisa Järvelä.

13:15-13:30  O8
Performance of male survivors of childhood leukemia and non-Hodgkin lymphoma during military service in general and in cognitive and physical fitness tests. Minna Honkila.

13:30-13:45  O9
Renal disorders in childhood cancer survivors - Results from the Adult Life after Childhood Cancer in Scandinavia (ALiCCS) study (www.alics.org). Trine Gade Bonnesen.
13:45-14:00  O10
Childhood high-grade bone sarcomas in Estonia. Sirje Mikkel.

14:00-14:15  O11

14:15-17:00 Theme 1: Sarcomas
Chair: Catherine Rechnitzer

14:15-15:00 Rhabdomyosarcoma past, present and future
Dr. Meriel Jenney, Cardiff, UK

15:00-15:30 Coffee Break

15:30-16:15 Significance of fusion genes in rhabdomyosarcomas
Dr. Janet Shipley, London, UK

16:15-17:00 Clinical Trial Strategies for Evaluating New Treatments for Osteosarcoma
Dr. Katherine Janeway, Boston, USA

17:30-19:00 Canal Boats

19:00 - Dinner at Spiseloppen.
Participants walk home afterwards. Short walk of 10-15 minutes.
**Monday June 3rd:**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>07:00-08:00</td>
<td>Fun and Run. The Waterfront.</td>
</tr>
<tr>
<td>08:30-08:40</td>
<td>Information from the organizers</td>
</tr>
<tr>
<td>08:40-13:00</td>
<td><strong>Theme 2: Personalized Medicine</strong></td>
</tr>
<tr>
<td></td>
<td>Chair: Kjeld Schmiegelow</td>
</tr>
<tr>
<td>08.45-09.30</td>
<td><strong>Mary Beve Symposium: Clinical Implementation of Preemptive Pharmacogenetic Testing</strong></td>
</tr>
<tr>
<td></td>
<td>Dr. Mary Relling, St. Jude, USA</td>
</tr>
<tr>
<td>09.30-10.00</td>
<td><strong>Pharmacogenetically tailored therapy with high-dose methotrexate</strong></td>
</tr>
<tr>
<td></td>
<td>Dr. Torben Stamm Mikkelsen, Aarhus, Denmark</td>
</tr>
<tr>
<td>10.00-10.30</td>
<td>Coffee Break</td>
</tr>
<tr>
<td>10.30-11.15</td>
<td><strong>Asparaginases against ALL and T-cell lymphomas</strong></td>
</tr>
<tr>
<td></td>
<td>Dr. Vassillios Avramis, Los Angeles, USA</td>
</tr>
<tr>
<td>11.15-12.00</td>
<td><strong>Personalized approaches in the treatment of childhood brain tumors</strong></td>
</tr>
<tr>
<td></td>
<td>Dr. Stefan Pfister, Heidelberg, Germany</td>
</tr>
<tr>
<td>12.00-13.15</td>
<td><strong>Lunch buffet at Restaurant “the Midtown Grill” (Marriott)</strong></td>
</tr>
<tr>
<td>13.15-15.00</td>
<td><strong>Theme 3: Implementation of Novel Therapy</strong></td>
</tr>
<tr>
<td></td>
<td>Chair: Karsten Nysom</td>
</tr>
<tr>
<td>13.15-14.00</td>
<td><strong>Will Cellular immunotherapies for cancer ever become a standard of clinical care?</strong></td>
</tr>
<tr>
<td></td>
<td>Dr. Malcolm K. Brenner, Houston, USA</td>
</tr>
</tbody>
</table>
14.00-15.00  FREE PAPERS. Leukemias  
Chair: Kjeld Schmiegelow  
14:00-14:15  O12  
Incorporation of 6-thioguanine nucleotides into DNA during maintenance therapy of childhood acute lymphoblastic leukemia – the influence of thiopurine methyltransferase genotypes. Maria Schou Ebbesen.  
14:15-14:30  O13  
Myelotoxicity following High-dose methotrexate in Children with Down syndrome and Acute Lymphoblastic Leukemia. Cathrine Bohnstedt.  
14:30-14:45  O14  
Pharmacokinetics: Renal and hepatic clearance following High-Dose Methotrexate in children with acute lymphoblastic leukaemia. Regitse Højgaard Christensen  
14:45-15:00  O15  
Treatment-related death in relapsed childhood acute lymphoblastic Leukemia. Trausti Oskarsson.  

15.00-15.30  Coffee Break  

15:30-18:00  General Assembly  

19:00-19:30  Welcome Drinks in the Foyer at Marriott Hotel.  

19.30-01:00  Annual Dinner at Marriott Hotel.
Tuesday June 4th:

08.30-08.40  Information from the organizers

08.40-10.15  FREE PAPERS. Miscellaneous
Chair: Astrid Sehested
08:45-09:00  O16  Use of Glucarpidase (Voraxaze®) in ALL-2008 in connection with HD-MTX-induced severe toxicity. Jesper Heldrup.

09:00-09:15  O17  Nordic Recommendations on fertility preservation for children and Adolescents. Cecilia Petersen.

09:15-09:30  O18  Nordic Centre for Fertility Preservation of Boys after Cancer Treatment. Jan-Bernd Stukenborg.


09:45-10:00  O20  The NOPHO-AML late effect study - cardiac muscle function in patients treated with chemotherapy only. Marianne Jarfelt.

10:00-10:15  O21  The level of ETV6-RUNX1-positive cells in umbilical cord blood from healthy newborns. Marianne Olsen.

10.15-10.45  Coffee Break

10.45-12.00  Theme 3: Implementation of Novel Therapies
Chair: Thomas Frandsen

10.45-11.15  Clinical Trials Center – Copenhagen Phase I/II studies
Dr. Karsten Nysom, Copenhagen

11.15-12.00  New drugs in ALL relaps IntReALL 2010
Dr. Arend Von Stackelberg, Charité, Berlin, Germany

12.00-12.15  Short Break

12.15-13.00  Theme 3: Implementation of Novel Therapies
Chair: Karsten Nysom

Implementation of Novel Therapy
Professor Pamela Kearns, Birmingham, UK
<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.00-13.10</td>
<td>Presentation of the next NOPHO-NOBOS Annual meeting in Bergen 2014</td>
</tr>
<tr>
<td>13.15-13.30</td>
<td>Closing Time</td>
</tr>
<tr>
<td>13.30</td>
<td>Lunchbag</td>
</tr>
</tbody>
</table>

See you next year in Bergen!!
Annual Meeting 2013 Copenhagen

Social Events
NOPHO 31st Annual meeting, Copenhagen
31st May - 4th June 2013

Social events you need to sign up for.

June 1st
Saturday evening: Opening ceremony at the City Hall of Copenhagen
After the opening ceremony there is a free time at Tivoli Gardens

June 2nd
Sunday evening: We take a canal boat to the Freetown of Copenhagen - Christiania.
The freetown Christiania was founded in 1971 by a group of hippies who developed their own rules, completely independent of the Danish government.

June 3rd
Monday morning: Fun & Run at 7:00.
Monday Evening: The Annual dinner at Marriott Hotel at 19:00
Gold Sponsors

www.barncancerfonden.se

børne cancer fonden

www.bornecancerfonden.dk

www.sobi.com

www.sigmatau.com

www.pharmanovia.com

www.novartis.com
Other Sponsors & Exhibitors

- Bristol Meyers
- Gilead Sciences Sweden AB
- Kræftens Bekæmpelse
- Mary Beve Foundation
- Medac
- Norpharma
- Pierre Fabre

We offer a huge thanks to the sponsors and exhibitors without whom this event could not take place.

Thanks to NOPHO-webmaster Elisabeth Broby for support and help.
Annual Meeting 2013 Copenhagen

Guest Speakers
Pamela Kearns, Prof. Director, Cancer Research UK Clinical Trials Unit, Birmingham, UK

p.r.kearns@bham.ac.uk

After being awarded a PhD, Pamela Kearns was appointed as a Senior Lecturer and Honorary Consultant in Paediatric Oncology at the University of Bristol and in 2007 relocated to Birmingham where she further developed her research in new therapeutic approaches for refractory leukaemias focusing on pre-clinical models for evaluating novel treatments and the design and development of early phase clinical trials investigating novel therapies for childhood leukaemias. In 2010, she established the Children’s Cancer Trials Team, within the University of Birmingham’s Cancer Research UK Clinical Trials Unit (CRCTU). This is UK’s designated national children’s cancer trials unit. In July 2012, she was appointed Director of the CRCTU, one of the largest cancer trials units in the UK specializing in the design, conduct and analysis of phase I to IV cancer clinical trials for investigators for a wide range of cancers and leukaemias across all ages.

Saturday June 1st 15.15 – 16.15
Challenges in running clinical trials in Europe

Tuesday June 4th 12.15 - 13.00
Implementation of Novel therapy

Overall survival from childhood malignancies in economically developed countries has dramatically improved of the last three decades with survival rates reaching over 75%. Nevertheless, some types of childhood cancer remain a difficult challenge and even for those with a good outcome, the burden of treatment in terms of short and long-term toxicity can be considerable and needs to be reduced.

The current paradigm for new cancer therapies is to harness our increasing knowledge of the molecular basis of carcinogenesis and the cellular processes that maintain the malignant phenotype in the development of exquisitely targeted therapies. The pre-clinical and clinical development of novel therapies is generally driven by the need to improve survival from adult malignancies. Drug development programmes are most commonly focused on adult cancers and the potential efficacy of new drugs in childhood malignancies is not considered. Moreover, there is a perception that molecular targets identified in adult cancers are not always as relevant in paediatric malignancies, however there is increasing evidence that this is not the case. Academic initiatives, for example, in Europe; the Innovative Therapies for Childhood Cancer Group, ITCC [http://www.itccconsortium.org/] and in the US; POETIC [http://www.poeticphase1.org] and TACL [https://ipcr.chla.usc.edu/tacl/], have been developed to systematically address this unmet need. Both initiatives are focused on the evaluation novel agents with specific relevance to childhood malignancies. Until recently, clinical development of
new drugs in childhood cancer was restricted by the limited accessibility of new agents for evaluation in Paediatric Phase I/II studies. The rarity of childhood cancers is little incentive for pharmaceutical companies to develop new drugs specifically for this area of medicine, however the arena is changing. Changes in EU and US legislation now incentivize pharmaceutical companies to provide paediatric clinical data for all new drugs relevant to children, including anti-cancer drugs. This is already affording the paediatric oncology community greater access to potentially beneficial novel therapeutic agents.

Paediatric consortiums like ITCC and similar US initiatives have established networks of expertise with the specific aim of evaluating new drugs for the treatment of childhood cancers. This aim is starting to be realised through close collaboration with academic institutions, the pharmaceutical industry, legislative authorities and, importantly, patients and their families.

Examples of recent successes in the development of novel agents in for paediatric malignancies will be presented. The landscape for drug development in children is rapidly changing and it can be anticipated that the next decade will see a vast increase in the availability of many new therapies. Through proper evaluation in collaborative clinical trials will we learn how best to use these new therapeutic approaches and improve the survival rates for children with cancer.
Saturday June 1st 16:45-17:00
Understanding outcome within the frame of host genomics

The 5-years overall survival of many childhood cancers now exceeds 80% in the best contemporary protocols due to an overall intensification of therapy. Still, as many as 20-30% of all childhood cancer deaths may be caused by toxicities or second neoplasms, and many survivors are burdened by lifelong effects, which emphasizes the necessity of developing more individualized treatment approaches.

In general, the pathways (and genes) affecting pharmacokinetics of anticancer agents are well known. Anticancer agents target both the malignancies (cure rates) and the host (toxicities), but our understanding of the pathogenesis of most toxicities is very limited. The individual host genome varies as much as the cancer genome, which affects both pharmacokinetics and –dynamics. This genetic variation includes millions of single nucleotide polymorphisms (SNPs) i.e. single base differences in the DNA sequence occurring in at least 1% of the population or on average at every 100-300 base site. Other variations are insertions, deletions, variable number of tandem repeats (VNTR) of 2-60 bases, copy number variation (CNV), and DNA methylation patterns and histone modifications.

Some of the most complex treatment protocols - like NOPHO ALL2013 - may include more than ten different chemotherapeutic agents, and the impact of individual SNPs in the multiple affected pathways are thus difficult to evaluate.
Furthermore, due to the complexity of the treatment, single pharmacogenetic variants will in general have little influence on cure rates or risk of toxicities. Instead extensive panels of genetic variants need to be explored using both candidate gene/pathway approaches and more genome wide strategies.

Mapping both cancer and host genomes and improving the capture and classification of toxicities in large patients cohorts are one of the major tasks for improving anticancer therapy. We will in this talk address some of the current clinical, logistic, technical, and bioinformatic challenges we face when exploring host genotype-phenotype associations.
Meriel Jenney, Children’s Hospital for Wales, Cardiff, UK

Meriel Jenney is a Consultant Paediatric Oncologist at the Children’s Hospital for Wales, Cardiff, UK. Her research interests include rhabdomyosarcoma, the late effects of treatment for childhood cancer and the assessment of quality of life in children.

Sunday June 2nd 14.15 - 15.00
Rhabdomyosarcoma past, present and future

Rhabdomyosarcoma (RMS) is a challenging disease. It occurs in children of all ages (particularly in the first five years of life) and in challenging sties from head to toe.

Whilst it is a highly chemo-responsive tumour, surgery and often radiotherapy are required to maximize the chance of cure. In the past, the outcome for children with RMS was poor and had been dramatically improved by the use of intensive chemotherapy including alkylating agents. The so-called ‘local control’ of the disease has been underpinned by two different philosophies: In the US the IRS (now COG) Studies there has been an emphasis on cure during first line therapy with a high use of radiotherapy in most treatment strategies. The European (particularly SIOP) strategy uses risk stratification and response to chemotherapy to identify those patients that may be cured without ‘significant local therapy’.

Whilst overall survival is similar, the latter approach results in a lower event free survival with the creation of a ‘salvage gap’. These different philosophies will be discussed in more detail.

There is now a greater understanding of the biology of rhabdomyosarcoma, in particular the importance of fusion status in identifying those patients with poorer outcome. The role of PET imaging is under evaluation and an important aim is to improve outcome overall and to identify those patients who can be cured without radiotherapy or surgery with significant morbidity. Techniques for local control are improving. Brachytherapy (with surgery) is useful at certain sites, with proton therapy being increasingly used for patients with RMS.

Looking to the future there is a recognition that the treatment for these patients often needs to be considered by National or even International specialist teams (given their rarity at very different anatomical sites). Whilst there has been a lack of new targeted agents showing clear benefit in RMS to date, there is considerable research underway in this area and it is hoped that new targeted agents will be available to be brought into future clinical trials in RMS.
Janet Shipley, The Institute of Cancer Research, London, UK
Janet.Shipley@icr.ac.uk

Janet Shipley is the Team Leader of Sarcoma Molecular Pathology, Divisions of Molecular Pathology and Cancer Therapeutics, The Institute of Cancer Research, London, UK. She has worked in the DNA Repair and Cell Biology Group at the Pediatric Research Unit at Guy’s Hospital in London, the Genetics Division of Harvard at Children’s Hospital in Boston, USA and the Imperial Cancer Research Fund UK (now the Cancer Research UK London Research Institute). She moved to The Institute of Cancer Research (ICR) in 1992 to establish a laboratory characterizing chromosomal rearrangements in tumours, and was awarded tenure in 1996 and a readership in 2003. She has fostered links with clinicians and National and European organizations in order to address key clinical issues in specific types of soft tissue sarcomas and testicular cancers through increasing our understanding of their underlying molecular biology.

Sunday June 2nd 15.30 - 16.15
Significance of fusion genes in rhabdomyosarcomas

Rhabdomyosarcomas (RMS) are the most common soft tissue sarcoma in childhood and are a leading cause of death from cancer in this age group. Their defining feature at the cellular level is that they resemble developing skeletal muscle, although are unable to terminally differentiate.

The two major histological subtypes, Alveolar (ARMS) and Embryonal (ERMS) carry distinct morphological and genetic alterations. The majority of ARMS, but not all, are associated with chromosome translocations that fuse PAX genes with FOXO1. The resultant PAX3-FOXO1 or PAX7-FOXO1 chimeric transcription factors drive gene expression changes and thereby the tumorigenic process.

We have investigated the clinical and biological significance of histology and fusion gene status in RMS through analyzing genomic and gene expression profiles in well-annotated material from patients. We demonstrated that this has implications for risk stratification.

We have also identified and investigated specific downstream targets altered by the PAX3-FOXO1 fusion protein. This is increasing our understanding of the underlying molecular biology of RMS and may provide novel therapeutic opportunities for high-risk patients.
Katherine Janeway, Dana-Farber Cancer Institute, Boston, USA
Katherine_Janeway@dfci.harvard.edu
Assistant Professor of Pediatrics, Harvard Medical School

Dr. Janeway received her medical degree from Harvard Medical School in 2000. She subsequently completed her residency in Pediatrics at Children’s Hospital, Boston. She was a Chief Resident at Children’s Hospital, Boston, and then completed her fellowship in Pediatric Hematology-Oncology at Dana-Farber Cancer Institute / Children’s Hospital, Boston. In 2007, Dr. Janeway joined the staff of Dana-Farber and Children's Hospital, Boston, where she is a pediatric hematologist-oncologist and investigator with a research focus of pediatric sarcomas.

Sunday June 2nd 16.15 - 17.00
Osteosarcoma

Clinical Trial Strategies for Evaluating New Treatments for Osteosarcoma

5-year overall survival ranges from 65 to 75% for children, adolescents and young adults presenting with localized osteosarcoma and generally less than 30% for those presenting with metastatic disease.

While recent insights have improved our understanding of the biology of osteosarcoma, they have not yet led to therapeutic advances that clearly improve outcome nor has one mechanism clearly stood out as the central event in osteosarcoma genesis.

Consequently, in the past 25 years therapeutic advances have been limited in this disease. The major priority of clinical research in osteosarcoma therefore is to identify novel agents with potential clinical activity.

The Novel agents being considered for study and considerations in trial design will be discussed.
Mary V. Relling, St. Jude, USA
mary.relling@stjude.org

Pharm.D. Our group has published extensively on clinical pharmacology and pharmacogenomics.
Mary Relling’s career is focused on identifying the basis for interpatient variability in response to medications, desired antileukemic and undesired adverse effects, and using that information to improve care of patients—especially, children with leukemia.
Implementing pharmacogenomics in the clinic is an important part of the overall program.

Monday June 3rd 08.45 - 09.30
Clinical Implementation of Preemptive Pharmacogenetic Testing

Although there has been considerable progress in the technical ability to perform genomic tests using DNA from patients, the adoption of such test results into clinical practice has been slow (Relling et al, Lancet Oncol 11:507-9, 2010). At St. Jude, a few pharmacogenetic test results have already become the standard of care, and are documented in the medical record. It is anticipated that over a period of many years, there will be a gradual increase in compelling evidence to support increased adoption of pharmacogenetic tests into clinical practice. For practical reasons, such results must be available for pre-emptive use by clinicians. St. Jude has opened a research protocol, PG4KDS (www.stjude.org/pg4kds ), the purpose of which is to establish processes by which pharmacogenomic test results are moved from the research laboratory to the clinical laboratory, used for patient care, and appropriately documented in the medical record for preemptive use (Crews et al, Clin Pharmacol Ther. 92:467-75, 2012). Throughout this process, we will identify barriers and solutions, assess acceptance of the process by clinicians and research participants/families, and share our knowledge with clinicians and researchers in settings outside of St. Jude. Our goal is to enroll all eligible patients who receive drug therapy from St. Jude; we have enrolled over 1000 patients. We perform genotyping using the DMET Array, supplemented with a CYP2D6 copy number assay (Fernandez et al, Clin Pharmacol Ther 92:360-5, 2012). We have created an automated system for generating pharmacogenetic interpretations in the medical record (Hicks et al, Clin Pharmacol Ther. 92:563-6, 2012). Our top priority genes and drugs for clinical implementation are those identified by the Clinical Pharmacogenetics Implementation Consortium http://www.pharmgkb.org/page/cpic (CPIC, Relling & Klein, Clin Pharmacol Ther 89:464-7, 2011). Thus far at St. Jude, we have implemented two genes (TPMT and CYP2D6) and 9 drugs (Codeine, Tramadol, Amitriptyline, fluoxetine, paroxetine, ondansetron, MP, thioguanine, azathioprine). These efforts also contribute to the Pharmacogenomics Research Network’s Translational Pharmacogenetics Program (TPP), in which multiple organizations develop common approaches to implementation, of pharmacogenetic testing, identify and solve logistic barriers to adoption, and disseminate 'best practices.'
Monday June 3rd 09.30-10.00
Pharmacogenetically tailored therapy with high-dose methotrexate

Infusions with high-dose methotrexate (MTX) are used in the treatment of acute lymphoblastic leukemia, non-Hodgkin lymphoma and osteosarcoma. The large inter-patient variation in MTX clearance complicates the treatment because patients with fast MTX clearance have an increased risk of relapse whereas patients with slow MTX clearance have more toxicity.

Pharmacogenomic and pharmacogenetic research is used to determine if genetic variation between patients can be used to predict the MTX clearance of each individual patient. The aim of personalized therapy is to minimize toxicity, and decrease the risk of relapse by tailoring the MTX treatment based on genetic variation. At present, several single nucleotide polymorphisms (SNPs) located in genes encoding drug transporting proteins have been associated with MTX clearance, but it remains a challenge to translate these findings into clinical practice.

Genetic variation in the gene SLCO1B1 is associated with MTX clearance, and can explain some of the pharmacokinetic differences between patients, but the question is, how this knowledge can be used to personalize the MTX treatment. Can genotype information combined with clinical variables be used to predict the MTX dosage? How can current knowledge on MTX pharmacogenetics be implemented in future clinical trials?
Asparaginases against ALL and T-cell lymphomas

The uniform approach to risk-based treatments has yielded advances in the treatments of childhood malignancies. These efficient treatment methods have resulted in a high cure rate (~80%) for ALL. Asparaginases (ASNase) have become the cornerstone modality in many combination regimens against lymphoid diseases. Some ASNases contain glutaminase activity, thus depleting both asparagine (Asn) and glutamine (Gln) in serum, two conditionally essential amino acids for leukemia cells.

We have studied the pharmacokinetics and pharmacodynamics (PK-PD) of ASNases in pediatric ALL and similarly, in adult ALL patients. Based on its unique PK and PD characteristics reduced pegasparagase doses (1000 to 2000 IU/m²), together with toxicity and CR profiles have been administered for safer results in pediatric and adult ALL patients. The pegasparagase IV dosing (2000 IU/m²) strategy has produced significant long-term survival rates in adult patients. Depletion of Asn and Gln inhibit global protein biosynthesis, thus disturbing the homeostasis of key proteins like, albumin, AT-III, etc. However, their depletion can enhance stress induced heat shock protein (HSP) expression in vitro, and we presume in vivo, thus improve ALL cell survival via a variety of up regulated stressful stimuli (ASNS, cMyc, Bcl-2, etc.).

In addition to ASNS up regulation, the most important mode of resistance to ASNase is the high titer antibody (Ab) against ASNase (IgG4 or IgE), which was detected in patients with and without overt clinical allergy (silent hypersensitivity, SH). When the Ab remains undetected, early disease relapse occurs with poor outcomes. In some patients treatment failure results from inadequate drug dosing rather than from an intrinsic drug resistance of the leukemia cells. Moreover, Ab against E. coli ASNase results in a high percentage of cross-reactions with pegasparagase, but not with Erwinia chrysanthemi ASNase (ErA). ErA has rescued many Ab+ patients with statistical significance. Thus, ErA administration is considered an individualized ASNase in Ab+ ALL patients.

Furthermore, the careful monitoring of ASNase activity, Anti-ASNase Ab(+), Asn and Gln deamination as percent of control, BM Status on Day 14 & Day 28, AT-III & albumin depletions expressed as % of control, and if possible, ASNS & GS activities in blasts, adipose and stromal
tissues is needed. Meticulous monitoring of these parameters will result in an individualized approach applicable in treating ALL patients. Moreover, the uniform treatment of ALL is supplemented by individualized treatments based on DNA gene expression by identifying each of the important leukemia subtypes and treating them accordingly. However, the development of individualized therapy against childhood malignancies with monoclonal Ab (mAbs) and signal transduction pathways inhibitors remains in early, but promising development. Should the toxicity profiles of these treatments be minimized, these new approaches hold the promise for improved outcomes.

Furthermore, stromal, mesenchymal, adipose, and other microenvironment tissues have been shown to aid the MRD leukemia cells. Recently, adaptively activated T-lymphocyte (tumor-infiltrating lymphocytes, TIL) treatments preferentially infiltrated melanoma and osteosarcoma tumors with positive results.

These new approaches are truly individualized uses of patient’s TILs, which alone or in combination with mAb can be utilized effectively in bone marrow malignancies. Lately, it has been shown that non-coding RNA sequences (ncRNA) potentially play an active role in controlling the epigenetics of DNA & Histone methylation, thus modulating gene transcription.

These new areas of individualized treatments remain elusive and are being investigated with fervor.
Stefan Pfister was appointed head of the Division Pediatric Neurooncology at the German Cancer Research Centre (DKFZ) in 2012. Being a paediatrician by training, Pfister received his MD from Tübingen University, and his clinical education at Mannheim and Heidelberg University Hospitals. As a physician-scientist, he completed postdoctoral fellowships with Christopher Rudd at the Dana-Faber Cancer Institute/Harvard Medical School, and with Peter Lichter at the German Cancer Research Centre, Division of Molecular Genetics. Pfister’s research focuses on the genetic and epigenetic characterization of childhood brain tumours by applying next-generation profiling methods and subsequently translating novel findings into a clinical context.

Monday June 3rd 11.15 - 12.00
Personalized approaches in the treatment of childhood brain tumors

Résumé:
Pfister’s research focuses on the genetic and epigenetic characterization of childhood brain tumors by applying next-generation profiling methods and subsequently translating novel findings into a clinical context. This might be achieved by establishing prognostic biomarkers and classification systems, by identifying new drug targets and genetic cancer predispositions, or by providing models for preclinical drug testing. For his translational neurooncology projects, Pfister received several prestigious awards, including the Kind-Philipp Award for Pediatric Oncology 2009, the Alfred-Müller Award for Neurooncology in 2011, and the German Cancer Award in 2013.

Together with Peter Lichter he is currently conducting the whole genome sequencing part of the PedBrainTumor project, the first German contribution to the International Cancer Genome Consortium (ICGC), in which whole genome sequencing is being performed on 600 tumor and 600 normal samples.

Amongst others, Pfister is also coordinating the BMBF-funded project "Molecular diagnostics in medulloblastoma", which aims to prospectively validate a number of highly promising molecular biomarkers in this aggressive childhood brain tumor for future clinical application.
Malcolm Brenner, Houston, USA
mbrenner@bcm.edu

Dr. Malcolm Brenner is a British clinical scientist working mostly in the field of gene therapy and immunotherapy applied to malignancy. He was educated at Forest School London and Emmanuel College, Cambridge England. He received his medical degree and subsequent Ph.D. from Cambridge University, England. In the 1980s, he left the UK to work in St. Jude Children's Research Hospital in Memphis. There, he conducted one of the first human gene therapy studies when he transduced bone marrow stem cells with a retroviral vector with the intention of marking them to study their survival and fate. This seminal study demonstrated that engrafted bone marrow stem cells contribute to long-term hematopoiesis and also that contaminating tumor cells in auto grafts can cause relapse. More recently, his group has become interested in the genetic modification of T-cells for cancer therapy, cancer vaccines and monoclonal antibodies. He was President of the International Society for Cellular Therapy and President of the American Society of Gene Therapy in 2002–2003. He was appointed Editor in Chief of the journal Molecular Therapy in 2009.

Monday June 3rd 13.15 - 14.00
Will Cellular immunotherapies for cancer ever become a standard of clinical care?

Despite more than two decades of clinical application, cellular immunotherapies for cancer have, almost without exception, failed to make the transition into licensed drugs that are standard of care for patients with malignant disease. This is in stark contrast to the success story of immunotherapy using monoclonal antibodies (MAb). At least part of the delay can be attributed to the dissimilarity between the business models needed to bring standard small molecule drugs/MAb to success and those required for cellular immunotherapies.

Unlike small molecules, cellular immunotherapies are usually individualized medicines, intended to be curative rather than ameliorative. They have complex IP, continuing high manufacturing costs, and they require iterative cycles of pre-clinical and clinical development to fine-tune their safety and effector function.

For the necessary critical mass of resources to be committed to break through these barriers, incremental advances in clinical benefit will be insufficient. Instead, it will be necessary for investigators to show that cellular immunotherapies are qualitatively better than conventional agents – able to safely cure otherwise intractable disease. In this presentation I will discuss one historic success story for cellular immunotherapy - the treatment of EBV-associated lymphoid and epithelial malignancies by tumor specific T cells - and show how the lessons learned from analyzing the mechanisms underlying this success can be implemented to treat a broader array of malignancies.

Thus initial studies of more than 125 patients with EBV immunoblastic lymphoma after allogeneic hemopoietic stem cell transplant showed adoptive transfer of EBV-specific T cells reduced a 12-15% incidence of fatal lymphoma to zero. Moreover, 11 of 13 patients with established, resistant lymphoma were successfully treated with these T cells without adverse effects and without recurrence.
At least 4 factors contributed to this success;

1) The infused T cells targeted strong and unique antigens, presented with ample accessory signals and co-stimulation;
2) The T cells contain polyclonal and multispecific memory population;
3) The tumor lacked potent immune escape mechanisms
4) The post-transplant environment favored (homeostatic) lymphoid expansion.

By developing a systematic approach to artificially introducing these characteristics to other T cell immunotherapies, we have been able to enhance their function.

As we and other investigators document an increasing level of success, we can reasonably hope that these therapies are indeed well on their way to becoming a standard of care.
Karsten Nysom, MD, Ph.d., Rigshospitalet, Copenhagen, Denmark
nysom@dadlnet.dk

Since 2007 consultant at the section of paediatric haematology and oncology, Rigshospitalet University Hospital, Copenhagen, heading the multidisciplinary paediatric CNS tumour programme and in charge of the care of children with Langerhans cell histiocytosis. Since September 2012 allocated full time to establishing a paediatric oncology phase 1 and phase 2 trial unit at Rigshospitalet.

Tuesday June 4th 10.45 - 11.15
Improved access to early clinical trials in paediatric haematology and oncology in the Nordic and Baltic region: the phase 1 and 2 trial unit at Rigshospitalet, Copenhagen

Denmark’s only dedicated phase 1 trial unit in adult oncology is located at Rigshospitalet in the same building as the section for paediatric haematology and oncology. Each year, 150-200 adult cancer patients are seen there for screening before potential inclusion in phase 1 trials, and approximately 100 of them are subsequently included in phase 1 trials. In close collaboration with the adult oncology phase 1 trial unit, we recently established a phase 1 and 2 trial unit at the section for paediatric haematology and oncology at Rigshospitalet, and we obtained membership of Innovative Therapies for Children with Cancer (ITCC) – the European organization for paediatric oncology phase 1 and 2 trial units. Currently, we work on attracting and opening phase 1 and 2 trials for childhood cancer patients, as well as creating early clinical trials of our own. In order to obtain a sufficient number of patients for inclusion in the trials we open, and to give as many Nordic and Baltic children with cancer as possible access to the newest experimental therapies with new targeted agents, we will be able to receive patients for inclusion in phase 1 and 2 trials from all Denmark, as well as from the paediatric oncology centres in the other Nordic and Baltic countries. The prerequisites for such referrals of patients from outside Denmark for potential inclusion in phase 1 or 2 trials will be that (1) there is a relevant phase 1 or 2 clinical trial currently open for such a patient at Rigshospitalet, (2) the patient and family seriously consider to let the patient participate in a phase 1 or 2 trial, (3) the general condition of the patient allows travelling for experimental cancer therapy, (4) the referring department guarantees payment of the basic fee for paediatric haematology and oncology treatment at Rigshospitalet, and (5) the individual case is discussed beforehand with one of the physicians involved in the phase 1 and 2 trial unit at Rigshospitalet.
We will inform our Nordic and Baltic colleagues about the currently available trials by sending a short message to all NOPHO members every time we open a new trial, and by maintaining an updated list of currently available trials at www.skaccd.org.
Arend Von Stackelberg, Charité, Berlin, Germany

Arend.Stackelberg@charite.de

Dr. von Stackelberg is attendant of the department for Paediatric Oncology/Hematology of the Charité University Hospital of Berlin. He is chair of the ALL-REZ BFM Study Group, coordinating investigator of the International Trial for Childhood Relapsed ALL (IntReALL 2010), and principle investigator of the phase I/II trial for Blinatumomab (anti CD3/19 BITE) in paediatric relapsed/refractory ALL. His interests are drug development and clinical trials in children with acute leukaemias.

Tuesday June 4th 11.15 - 12.00

New drugs in ALL relaps IntReALL 2010

Though survival of children with acute lymphoblastic leukaemia (ALL) has improved, relapse remains a leading cause of mortality in childhood cancer. Given the rarity of the disease, only a large international cooperative group can recruit sufficient patients for prospective studies with specific questions in biologic subgroups. Under the umbrella of the I-BFM SG all relevant mainly European study groups are creating the worldwide largest Study for Children with Relapsed ALL (IntReALL 2010). The aim is to develop optimized standard treatment as platform for systematic randomized phase II and III studies on the most promising new and targeted agents. An adequate trial structure, an optimized web based database, and standardized diagnostic methods need to be established.

Patients are stratified into a standard (SR) and a high risk (HR) group according to established factors. For SR patients, the best available treatment protocols ALL-REZ BFM 2002 and ALL R3 are randomly compared, and the additional effect on survival of the humanized monoclonal CD22 directed antibody epratuzumab is investigated. HR patients who have unsatisfying remission rates will receive an intensified induction with the proteasome inhibitor bortezomib compared to standard induction therapy to improve CR2 rates. Phase III randomization in late consolidation testing other new agents are planned. IntReALL 2010 allows for comprehensive tumour banking and systematic biologic research in subgroups with correlation to clinical outcome. SMEs will be involved into project management, data base development, and pharmaceutical and biotechnological research to ensure innovation in the respective areas. IntReALL 2010 is embedded in a network of European academic structures relevant for childhood cancer. It will be a cornerstone of drug development in childhood leukaemia and the only trial with the potential for well powered phase III studies in this indication. IntReALL 2010 will harmonize ALL-relapse therapy, establish highest diagnostic and therapeutic standard and improve survival of children with ALL.
Abstract Overview 2013

Oral Presentations
O1. Central Nervous System Disease in childhood acute lymphoblastic leukemia

Mette Levinsen, University Hospital Rigshospitalet, Department of Pediatrics and Adolescent Medicine, Denmark

Although the contemporary use of central nervous system (CNS) directed treatment has reduced the risk of CNS relapse for childhood acute lymphoblastic leukemia (ALL), it still accounts for 30-40% of initial relapses in most clinical trials. Patients with CNS leukemia at diagnosis, which is commonly defined as more than five leukocytes/microL cerebrospinal fluid (CSF) in the presence of lymphoblasts on cytospin preparations, seem to suffer from more relapses with CNS involvement compared with CNS-negative patients. Since increased focus on long-term CNS sequelae has led to reduction in use of cranial irradiation, it is even more important to identify patients with CNS leukemia at initial diagnosis to enhance their CNS-directed therapy. While conventional cytology (CC) is highly specific (>95%), it suffers from low sensitivity (<50%). Flow cytometry (FCM) having a higher sensitivity could provide better detection of CSF blasts. In this study, we explored the frequency and level of leukemic blasts in paraformaldehyde fixated CSF samples from newly diagnosed and relapsed ALL patients. Among de novo patients, 11 (42%) of 26 patients were CSF-positive by FCM, while CC was positive in only four patients (15%) (p=0.02). At relapse one (33%) of three patients was positive by both FCM and CC (p=1.00). FCM+/CC+ samples had a higher blast levels compared to FCM+/CC- samples (median 226 (range 5-49.451) vs. 8 (range 4-35) per mL CSF, p=0.04). In conclusion, FCM allows more frequent detection of leukemic blasts in CSF of children with ALL and the sensitivity is particularly improved in paucicellular samples. The prospective CNS leukemia study will explore the prognostic impact of FCM+/CC- samples.

Keywords: Acute lymphoblastic leukemia, central nervous system, flow cytometry, cytology, relapse

Authors: Mette Levinsen, Hanne Marquart, Birgitte Klug Albertsen, Thomas Frandsen, Line Groth-Pedersen, Arja Harila-Saari, Jesper Heldrup, Olafur Gisli Jonsson, Päivi Lähteenmäki, Birgitte Lausen, Riitta Niinimäki, Kees-Jan Pronk, Steen Rosthøj, Mervi Taskinen, Aina Ulvmoen, Goda Vaitkeviciene, Peder Skov Wehner, Kjeld Schmiegelow
O2. Asparaginase Associated Pancreatitis in Childhood Acute Lymphoblastic Leukaemia

Raheel Altaf Raja, The University Hospital Rigshospitalet, The Department of Paediatrics and Adolescent Medicine, Denmark

Purpose: To describe patients with acute lymphoblastic leukaemia (ALL) diagnosed with asparaginase-associated pancreatitis (AAP). Methods and material: Patients treated according to the NOPHO ALL 2008 protocol. 821 patients with ALL were included. AAP was defined as: - Signs and symptoms compatible with acute pancreatitis - Amylase or lipase levels 3 times above the upper normal limit - Radiographic signs of acute pancreatitis 2/3 of the above must be present. Patients with ALL that were treated with asparaginase at the time of pancreatitis, were eligible in the study. 47/821 patients were identified. The centres reporting the cases were asked to fill out questionnaires. 46 questionnaires were returned. 1 was excluded because of incorrect registration. 1 was excluded because no signs of acute pancreatitis on autopsy were detected. Results: 44 (5.4 %) patients were included. All patients met the criteria for AAP. 20 (5.2 %) were treated according to the standard risk protocol, 21 (7.8 %) were treated according to the intermediate risk protocol and three (1.8 %) were treated according to the high risk protocol. Median age: 6 years (1-17 years). Median number of asparaginase: 5 doses (1-13 doses). Median number of days AAP following asparaginase: 11 days (2-68 days). Complications: 30/44 (68.2 %) presented with signs of systemic inflammatory response syndrome (SIRS) or circulatory insufficiency. 13/44 (29.5 %) had pseudo cyst at presentation, 10 of these patients had intervention performed. 11/44 (25 %) patients had necrotizing pancreatitis. 12/44 (27.3 %) patients were re-introduced to asparaginase following AAP. Data on three patients were insufficient. 3/9 (33.3 %) experienced pancreatitis again after re-introduction. The nine patients received a median of 4 doses (1-9) of asparaginase. Conclusion: There is a high occurrence of pseudo cysts and necrotizing pancreatitis in AAP. A high percentage of patients presented with signs of SIRS, or circulatory insufficiency, making diagnosis difficult. It is feasible to re-introduce asparaginase after AAP, and there is a risk of recurrent AAP.

Keywords: ALL, pancreatitis, asparaginase

Authors: Raheel Altaf Raja (presenting author), Kjeld Schmiegelow, Birgitte Klug Albertsen, Maria Winther Gunnes, Kaie Prunsild, Merja Möttönen, Satu Ranta, Marit Hellebostad, Bernward Zeller, Jonas Abrahamsson, Goda Vaitkeviciene, Jonas Abrahamsson, Mats Heyman, Peder Skov Wehner, Mats Heyman, Mervi Taskinen, and Thomas Leth Frandsen
**O3. Deletions of IKZF1 and SPRED1 in pediatric B-cell precursor acute lymphoblastic leukemia are associated with poor prognosis**

*Linda Olsson, Clinical Genetics, Laboratory Medicine, Lund University, Sweden*

Despite the favorable prognosis of childhood acute lymphoblastic leukemia (ALL), a substantial subset of patients relapses. Since this occurs not only in the high risk but also in the standard/intermediate groups, the presently used risk stratification is suboptimal. The underlying mechanisms for treatment failure include presence of genetic changes causing insensitivity to the therapy administered. To identify relapse-associated aberrations we performed single nucleotide polymorphism array analyses of 307 uniformly treated, consecutive pediatric ALL cases accrued 1992-2011. Recurrent aberrations of 14 genes in patients who subsequently relapsed or had induction failure were detected. Of these, deletions/uniparental isodisomies of ADD3, ATP10A, EBF1, IKZF1, PAN3, RAG1, SPRED1, and TBL1XR1 were significantly more common in B-cell precursor ALL patients who relapsed compared with those remaining in complete remission. In univariate analyses, age (≥10 years), WBC counts (>100 x 10^9/l), t(9;22)(q34;q11), MLL rearrangements, near-haploidy, and deletions of ATP10A, IKZF1, SPRED1, and the pseudoautosomal 1 regions on Xp/Yp were significantly associated with decreased 10-year event-free survival, with IKZF1 abnormalities being an independent risk factor in multivariate analysis irrespective of risk group. High age and deletions of IKZF1 and SPRED1 were also associated with poor overall survival. Thus, analyses of these genes provide clinically important information.

**Keywords:** Pediatric ALL, SNP array analyses, relapse IKZF1, SPRED1

**Authors:** Linda Olsson, Anders Castor, Mikael Behrendtz, Andrea Biloglav, Erik Forestier, Kajsa Paulsson, and Bertil Johansson
O4. IMPROVEMENT OF CHILDHOOD CANCER SURVIVAL IN LITHUANIA: SINGLE CENTRE EXPERIENCE

Jelena Rascon, Center of Pediatric Oncology and Hematology, Children’s Hospital, Affiliate of Vilnius University H, Center for Pediatric Oncology and Hematology, Lithuania

BACKGROUND. The study aimed to analyze the long-time survival for children treated for malignant disorders at the Center of Pediatric Oncology and Hematology, Children’s Hospital (Vilnius) from 1982 to 2011. METHODS. Retrospective analysis of the data from the database of children treated for cancer in our institution was performed. The reviewed time period was divided into three decades: 1982-1991, 1992-2001 and 2002-2011. Estimates of ten-year overall survival (OS10y) were calculated using Kaplan-Meier method for each decade. RESULTS. In total 1294 patients were treated from 1982 to 2011. The diagnosis profile of the entire cohort consisted of all types of leukemia (n=775, 60%), Hodgkin and Non-Hodgkin lymphoma (n=227, 18%) and solid tumors (n=292, 23%). Children with lymphoma and solid tumors were started to be treated at our institution since 1992. During the first decade 236 patients were treated with median follow-up for long-term survivors (n=64, 33%) being 23.9 years (range 21.1-30.6); during the second decade the number of the treated patients increased up to 494, median follow-up for long-term survivors (n=286, 59%) was 14.6 years (range 10.2-20.9); during the third decade 564 patients were treated, the median follow-up for the long-time survivals (n=408, 74%) was 6.0 years (range 1.1-12.9). Comparative analysis of the long-term survival revealed significant increase in OS10y both for the entire cohort (32.3±3.3% vs. 58.7±2.2% vs. 70.4±2.3 treated in 1982-1991, 1992-2001 and 2002-2011, respectively (p<0.001)) and for the patients with leukemias (32,0±3,3% vs. 56.2±2.9% vs. 64.3±3.6, respectively (p<0.001)). The same tendency was detected in OS10y for the patients with lymphoma (67.2±4.4% vs. 88.5±3.1 (p=0.001)) and solid tumors (55.7±5.6% vs. 71.2±3.3 respectively (p=0.009)) for the periods 1992-2001 and 2002-2011 respectively. CONCLUSIONS. Improvement in diagnostic facilities, implementation of adequate supportive care and experience of the staff has led to the dramatic improvement of the treatment results in our institution.

Keywords: Overall survival, treatment results in Lithuania
Authors: Jelena Rascon, Sigita Stankeviciene, Goda Vaitkeviciene, and Lina Rageliene.
Does low dose PEG-asparaginase treatment lead to successful asparagine depletion in cerebrospinal fluid of children treated according to the NOPHO ALL-2008 protocol?

Louise Tram Henriksen, Aarhus University Hospital, Department of Pediatrics. Denmark

Asparaginase is an important component in the multi-drug treatment of childhood acute lymphoblastic leukemia (ALL). In the NOPHO ALL-2008 protocol low dose intramuscular PEG-asparaginase therapy with 1000 IU/m2 is used. Depending on randomization, standard and intermediate risk patients receive 8 or 15 doses of PEG-asparaginase during 30 weeks. The low dose differs from doses in many ALL-protocols and the question is whether it is high enough to ensure depletion in the cerebrospinal fluid (CSF). The objective of this study was to measure asparagine depletion in the cerebrospinal fluid in children receiving low dose PEG-asparaginase, and also to correlate the level of asparagine depletion in CSF to serum asparaginase activity. Danish ALL patients aged 1-17,9 years old, stratified in the NOPHO ALL-2008 protocol to standard, or intermediate risk treatment were included in the study. CSF samples were collected at specific treatment days, when the patients had a lumbar puncture for other reasons. Thus we collected serial CSF samples from each patient. The first sample was collected before the first dose of PEG-asparaginase. Depending on lumbar puncture schedule, the following CSF samples were collected either 7 days or 14 days after administration of PEG-asparaginase all through the 30 weeks of treatment. Serum samples were collected at the time of PEG-asparaginase administrations. We used liquid chromatography - mass spectrometry (LC-MS), for the concentration analysis of cerebrospinal fluid asparagine, aspartic acid, glutamine and glutamic acid. Asparaginase activity in patient serum and cerebrospinal fluid was analyzed using a spectrophotometric assay. We collected 113 cerebrospinal fluid samples and corresponding serum samples from 23 patients. Serum samples and cerebrospinal fluid samples are in the process of being analyzed. Results from these analyses will be presented.

Keywords: Acute lymphoblastic leukemia, PEG-asparaginase, cerebrospinal fluid, asparagine depletion

Authors: Louise Tram Henriksen, Steen Rosthøj, Thomas Leth Frandsen, Jacob Nersting, Henrik Schrøder, Birgitte Klug Albertsen
O6. Applicability of the WHO classification in pediatric AML

Julie Damgaard Sandahl, Aarhus University Hospital, Skejby, Department of Pediatrics, Denmark

The World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia was revised in 2008 and is based on recurrent genetic abnormalities and morphology. The new classification incorporates newly recognized entities and emphasizes, more than previously, the pivotal role of cytogenetic abnormalities. The classification of AML is primarily based on studies in adults. The value of WHO 2008 in pediatric AML has not been studied. Children, age 0-18 years, diagnosed with de novo AML in the Nordic countries, and treated according to the NOPHO-AML-1993 and 2004 protocols in the period 1993-2011, excluding ML-DS and t(15;17)PML/RARA, were identified in the NOPHO AML database. Clinical and genetic data were retrieved from the database. Karyotypes were centrally reviewed and patients classified according to WHO. Complete karyotype was available in 519 (98%) of 532 children of which 194 were classified as AML with recurrent genetic abnormalities; t(8;21)(q22;q22);RUNX1-RUNX1T1 (n=62), inv(16)(p13.1q22) or t(16;16)(p13.1;q22);CBFB-MYH11 (n=39), t(9;11)(p21;q23);MLLT3-MLL (n=51), t(6;9)(p22;q34);DEK-NUP214 (n=3), inv(3)(q21q26.2) or t(3;3)(q21;q26.2);RPN1-EVI1 (n=1), t(1;22)(p13;q13)/RB M15-MKL1 (n=3), NPM1 mutated (n=8), and CEBPA mutated (n=17). Furthermore, 14 children had FLT3-ITD. Fifty-six children were cytogenetically classified as AML with myelodysplasia-related changes (AML-MDS) and 269 as AML not otherwise specified (AML-NOS). Based on preliminary data, 5-year EFS for recurrent abnormalities was 58%, for AML-MDS 48% and AML-NOS 47%, (p=0.03). Five-year OS was also significantly better for the recurrent abnormalities (76%) compared with AML-MDS (66%) and AML-NOS (62%) (p<0.01). Median age was 7 years in the group of recurrent abnormalities vs. 4 years in both AML-MDS and NOS (p<0.01). There were no association between WBC, sex and the groups of recurrent abnormalities, AML-MDS or AML-NOS. The largest group in the WHO classification when applied in children is the unspecified group of AML-NOS limiting the applicability of classification in children with AML.

Keywords: AML, cytogenetics, WHO classification

Authors: Julie Damgaard Sandahl, Eigil Kjeldsen, Jonas Abrahamsson, Jesper Heldrup, Kirsi Jahnukainen, Ólafur G. Jónsson, Birgitte Lausen, Josefine Palle, Bernward Zeller, Erik Forestier, Henrik Hasle
O7. CARDIOVASCULAR MORBIDITY IN LONG-TERM SURVIVORS OF EARLY ONSET CANCER

Liisa Järvelä, Turku University Hospital, Department of Pediatrics, Finland

Background: Improvements in cancer therapy have resulted in an expanding population of early onset cancer survivors. In contrast to survivors of cancer in childhood and adolescence, there is a lack of data concerning late morbidities among survivors of cancer at young adulthood (YA). Methods: In a population-based setting, we explored the risk of cardiac and vascular disease in early onset cancer survivors compared with healthy siblings. Patients diagnosed with cancer below the age of 35 years since 1975 were identified from the Finnish Cancer Registry. 5-year survivors were included in this study (N = 14,649). Information on cardiovascular morbidity was collected from the national hospital discharge registry. Results: Cancer survivors aged 0-19 and 20-34 at diagnosis, respectively, were at significantly elevated risk of developing any of the studied outcomes compared to siblings: HR 17.6 (95%CI 12.1-25.7) and 4.1 (95%CI 3.2-5.2) for cardiomyopathy/cardiac insufficiency; 4.2 (95%CI 2.9-6.1) and 1.7 (95%CI 1.5-2.1) for atherosclerosis/brain vascular thrombosis; 3.5 (95%CI 1.8-6.6) and 1.8 (95%CI 1.5-2.0) for myocardial infarction/ischemia; and 2.1 (95%CI 1.4-3.1) and 1.4 (95%CI 1.2-1.7) for cardiac arrhythmia. The risk for adverse events was highest among survivors of lymphomas, acute lymphoblastic leukemia, brain tumors and testicular cancer in both age groups as well as among survivors of AML, renal tumors and bone tumors in the younger group. Conclusions: Our study provides novel information on cardiac and vascular morbidity concerning the population of survivors of cancer at young adulthood, and the results may help in planning risk-based long-term follow up for the survivors of early onset cancer.

Keywords: cancer, cardiac, heart, late effect, vascular

Authors: Andreina Kero, MD*, Liisa S. Järvelä, MD*, Mikko Arola, MD,PhD**, Nea Malila, MD, PhD^, Laura M. Madanat-Harjuoja, MD,PhD^, Jaakko Matomäki, MSc, biostatistician*, Päivi M. Lähteenmäki, MD, PhD* *Department of Pediatrics, Turku University Hospital, Turku, Finland ** Department of Pediatrics, Tampere University Hospital, Tampere, Finland ^Finnish Cancer Registry, Helsinki, Finland
O8. Performance of male survivors of childhood leukemia and non-Hodgkin lymphoma during military service in general and in cognitive and physical fitness tests

Minna Honkila, Oulu University Hospital, Pediatrics and Adolescence, Finland

PURPOSE: We evaluated the performance of male survivors of childhood leukemia and non-Hodgkin lymphoma (NHL) during military service in general and in cognitive and physical fitness tests. PATIENTS AND METHODS: Five hundred thirty-seven male patients with leukemia or NHL diagnosis before the age of 16 who were born in 1960–1992 and alive on their 18th birthday were identified from the Finnish Cancer Registry. Five matched controls were obtained for each patient from the Population Register Center of Finland. The military service data were gathered from the archives of the Finnish Defense Forces. RESULTS: Leukemia and NHL survivors were rejected from the military service more often than their healthy controls (P < 0.001, OR 5.5). The percentage of survivors placed in the best fitness class was 51 whereas the corresponding rate was 81% in the control group (P < 0.001, OR 5.5). There was no significant difference in the interruption or the attained level of military training between the survivors and the controls. Survivors attained lower results in the Cooper test than the controls (P < 0.001, OR 1.4), but non-irradiated patients did not show any decline. Survivors performed worse in the standing long jump test (P < 0.001, OR 2.1), but not in other muscular fitness tests compared to the control group. Cranially irradiated patients showed a decline in the standing long jump test (P < 0.05), sit-ups (P < 0.05), and push-ups (P < 0.05). Survivors achieved equally good scores in cognitive tests as their healthy controls. CONCLUSION: More than one third of survivors were rejected from the military service. Enlisted survivors, however, fared well in the service. Cancer and/or its treatment decreased the maximal aerobic capacity and leg muscle strength. Moreover, cranial radiation therapy was an indicator of poorer outcomes in the physical performance of the survivors. Keywords: Child, adolescent, cancer, late-effects, military service
Authors: Minna Honkila (the presenting author) Ritva Ahomäki Jaakko Matomäki Päivi Lähteenmäki Arja Harila-Saari
O9. Renal disorders in childhood cancer survivors - Results from the Adult Life after Childhood Cancer in Scandinavia (ALiCCS) study (www.aliccs.org)

Trine Gade Bonnesen, Aarhus University Hospital, Skejby, Department of Pediatrics, Denmark

Introduction: With remarkable improvement in treatment of childhood cancer over the past decades, a growing population of long-term survivors with a variety of adverse sequelae has become more apparent. Using the unique population-based registries in the Nordic countries with long-term and nearly complete follow-up, we investigated the risk of being hospitalized with a renal disorder in a large Nordic cohort childhood cancer survivors. Methods: Hospitalization for renal disorders were evaluated in a cohort of 32 454 children diagnosed with cancer before the age of 20. A randomly selected population comparison cohort consisting of 210 920 individuals from Denmark, Finland, Iceland, Norway and Sweden and matched by sex, country and year of birth was ascertained from Population Registries. Recruitment commenced with the implementation of each of the cancer registry in the respective country in 1940s and 50s, and continued until December 31st 2008. Cohort members were followed-up for renal disease in national registries which include all hospitalizations. Standardized hospitalization rate ratios (SHRRs) and absolute excess risks (AERs) per 100,000 person-years were calculated, with corresponding 95% confidence intervals (CI). Results: Survivors were at significantly increased risk of being hospitalized for any renal disorder compared with the general population (SHRR 2.2, 95 % CI 2.0-2.2). Significantly increased risk were seen for all main diagnostic groups of renal disorders, including glomerular diseases (SHRR 2.0, 1.6-2.4), renal tubule-interstitial diseases (SHRR 2.4, 2.2-2.7), renal failure (SHRR 6.0, 5.1-7.0), other disorders of kidney and ureter (SHRR 3.6, 3.0-4.4). Hospitalization for any renal disorders continued to be high for survivors even >20 years after diagnosis. Conclusion: Survivors of childhood cancer are at increased risk of renal late effects. Awareness of this excess risk may focus on improving treatment protocols, with fewer late effects and continuing high survival rates and focused follow-up.

Keywords: childhood cancer, late effects, renal disorders, hospitalization, clinical epidemiology

Authors: Trine Gade Bonnesen, MD, Jeanette Falck Winther, MD, DMSc, Peter Asdahl, MD, Sofie de Fine Licht, MSc, Thorgerdur Gudmundsdottir, MD, Jørgen H. Olsen, MD, DMSc, Henrik Hasle, Professor, MD, PhD, on behalf of ALiCCS.
Background: Osteogenic sarcoma and Ewing’s sarcoma are the most common malignant bone tumors in childhood. The intensification of chemotherapy has been shown to improve the overall survival of patients with bone sarcoma. Methods: We retrospectively analyzed the collected data of patients with newly diagnosed high-grade bone sarcomas treated between 1999 and 2012 in Estonia. Results: 23 patients with bone sarcomas were diagnosed and treated in Tartu University and Tallinn Children Hospital. Of the 23 patients (male, N = 12; female, N = 11) 15 presented with osteosarcoma (femur N = 7; humerus N = 2; tibia N = 5; scull N1) and 8 with Ewing sarcoma (vertebra N = 1; rib N = 1; humerus N = 1; pelvis N = 2; scapula N = 1; femur N = 1; mandible N = 1). Median age at initial diagnosis was 12 years. Most tumors (69%) arose in the extremity. Time from first symptoms to initial diagnosis was median 2.4 months. Primary metastases were detected in 2/23 children. All patients with osteosarcoma was treated by ISG/SSG I and Ewing sarcoma by SSG IX treatment protocol. 21 patients responded well to neoadjuvant chemotherapy. With a median follow-up of 7.2 years, 21/23 patients were alive. Fatal cases were observed only for two patients - one patient with osteosarcoma died from progression of disease and one patient with Ewing sarcoma of recurrent disease. Conclusions: The outcome of patients with bone sarcomas is good. 21 patients (91%) have complete remission by the end of treatment. There was no difference in survival between patients with osteosarcoma and Ewing’s sarcoma.

Keywords: bone sarcoma, pediatric, survival

Authors: Sirje Mikkel¹, Kadri Saks² Tartu University Hospital¹, Tallinn Children Hospital²
Optic pathway gliomas (OPGs) are a common type of low grade glioma in children which may occur sporadically or in association with neurofibromatosis type 1 (NF1), a cancer predisposing syndrome. There is large variety in the clinical pictures of these patients. In this retrospective, population-based study, we collected data from OPG-patients in east Denmark to describe the natural history of OPGs. The study population consisted of OPG-patients with or without NF1 diagnosed between 01.01.1996–31.12.2011, who were younger than 18 years old at time of diagnosis. Forty-eight eligible patients were identified through the Clinic of Rare Handicaps at Rigshospitalet and the Danish Childhood Cancer Registry. In our study, 69% of OPGs occurred in NF1-patients. We found no significant difference in age at diagnosis between the patients with or without NF1. At time of diagnosis, 80% of the non-NF1 children had decreased visual acuity (VA) compared to 33% of the NF1 patients. The VA increased or remained stable during chemotherapy in the majority of the treated patients. Of the non-NF1 patients, 93% were treated with chemotherapy, radiation and/or surgery compared to 24% of the NF1 patients. The currently preferred treatment for OPG in Denmark is chemotherapy according to the SIOP-LGG 2004 protocol. Overall, tumor growth and visual acuity loss were the most frequent causes of treatment. Analysis of radiological data and modeling of longitudinal changes of vision and tumour size will also be presented. Our study confirms the complexity of OPGs and the wide variety of clinical pictures they may give rise to.

Keywords: Optic pathway glioma Neurofibromatosis type 1 Visual acuity Treatment Epidemiology

Authors: C. Ehlers-Hansen1, K. Rothe Nissen2, K. Schmiegelow1, A. Sehested1, A. M. Jelsig3, C. Klausen4, K. Nysom1. 1) Pediatrics and Adolescent Medicine, University Hospital Rigshospitalet, Copenhagen, Denmark 2) Department of Ophthalmology, University Hospital Rigshospitalet/Glostrup, Copenhagen, DK 3) Department of Clinical Genetics, University Hospital Rigshospitalet, Copenhagen, DK and Odense University Hospital, Odense, DK.4) Department of Radiology, University Hospital Rigshospitalet, Copenhagen, DK
**O12. Incorporation of 6-thioguanine nucleotides into DNA during maintenance therapy of childhood acute lymphoblastic leukemia – the influence of thiopurine methyltransferase genotypes**

*Maria Schou Ebbesen, Juliane Marie Centre, Bonkolab 5704, Denmark*

**Purpose and background:** To explore the incorporation of 6-thioguanine nucleotides (TGN) into DNA (DNA-TG) during 6-mercaptopurine (6MP) therapy of childhood acute lymphoblastic leukemia and its relation to thiopurine methyltransferase (TPMT) genotypes. TPMT methylates 6MP and thereby reduces amount of drug available for DNA-TG formation. However, the methylated metabolites (MeMP) inhibit the purine de novo synthesis (PDNS). Methods: We studied DNA-TG levels and erythrocyte levels of 6MP metabolites in 507 blood samples from 13 TPMT heterozygote (TPMTLA) and 39 matched TPMT wild-type (TPMTWT) patients treated according to the NOPHO ALL2008 protocol during the 6MP/MTX maintenance therapy. DNA-TG was quantified by mass spectrometry. Key results: There was no difference between meanDNA-TG for TPMTLA and TPMTWT patients (Median (50% range): 434 (372–646) vs. 434 (302–527) fmol/µg, p = 0.25). The TPMTLA patients had significantly higher meanErythrocyte-TGN levels compared with TPMTWT patients (Median (50% range): 465 (428–647) vs. 192 (153–215) nmol/mmol Hb, p<0.001). MeanErythrocyte-MeMP was significantly lower in TPMTLA patients than in TPMTWT patients (Median (50% range): 1787 (680–3513) vs. 14,209 (7233–21,253) nmol/mmol Hb, p<0.001). To explore the relative incorporation of TGN into DNA in relation to cytosol TGN levels, a DNA-TG-index was calculated as DNA-TG/Erythrocyte-TGN. The DNA-TG-index increased with higher levels of MeMP (rs = 0.40, p = 0.004) reflecting enhanced DNA-TG incorporation. The majority of the TPMTLA patients had lower levels of mEry-MeMP and thereby lower DNA-TG-index. Conclusion: Our findings may explain why TPMTLA patients as well as patients on 6-thioguanine (6TG) therapy tolerate much higher cytosol TGN levels although they may not differ in their pharmacodynamics target, i.e. DNA-TG. Measurement of DNA-TG is potentially a combination of the effects of both cytosol 6TGN levels and TPMT activity (through MeMP) and is therefore a dose-adjustment parameter to be investigated.

**Keywords:** Acute Lymphoblastic Leukemia, Thiopurines, DNA-TG, Thiopurine methyltransferase, Maintenance therapy

**Authors:** Maria S. Ebbesen*, Jacob Nersting, Jack H. Jacobsen, Thomas L. Frandsen, Kim Vettenranta, Jonas Abramsson, Finn Wesenberg and Kjeld Schmiegelow.

*presenting author
O13. Myelotoxicity following High-dose methotrexate in Children with Down Syndrome and Acute Lymphoblastic Leukemia

Cathrine Bohnstedt, Pediatrics and Adolescent Medicine, The Juliane Marie Centre, Denmark

Purpose: Children with Down syndrome (DS) and acute lymphoblastic leukemia (ALL) have an inferior prognosis compared to non-DS ALL patients treated on the same protocol. Thus, the five year event-free survival for the 66 DS patients treated within NOPHO 1992-2000 was inferior to the 2602 non-DS patients (0.50 ± 0.07 vs. 0.77 ± 0.01, p<0.001) (Bohnstedt, Leukemia 2012). High-dose methotrexate (HD-MTX) given concurrently with oral 6-mercaptopurine (6MP) is frequently followed by myelotoxicity, which may necessitate treatment interruption and thus interfere with the efficacy of the treatment. HD-MTX may, through inhibition of purine de novo synthesis, enhance incorporation of the cytotoxic 6MP metabolites into DNA (Nygaard, Leukemia 2003). The increased dosage of chromosome 21 genes in DS-ALL may increase the cellular MTX uptake by the reduced folate carrier. This could lead to an increased inhibition of purine de novo synthesis, and thereby increase the cytotoxic effect of 6MP. We reviewed the courses of white blood cell (WBC) and absolute neutrophil counts (ANC) for 3 weeks following HD-MTX during MTX/6MP maintenance therapy for children in the Nordic countries. We compared the courses for DS-ALL diagnosed 1992-2008 to non-DS-ALL diagnosed 1992-1996. We had a total of 7238 blood samples following HD-MTX courses. The time to WBC nadir following HD-MTX was similar for DS and non-DS patients, however the DS-patients demonstrated lower WBC nadir (mean difference 0.42, stdErr = 0.19, p=0.02). The course for ANC differed for DS and non-DS patients (p<.0001), the levels being similar but DS patients obtained nadir at a later time point than non-DS (17.7 days vs. 14.8 days). These results are preliminary and further analysis on drug doses and pharmacokinetic will follow. In conclusion, the increased myelotoxicity after HD-MTX courses of DS-ALL patients may contribute to treatment interruptions and their poor prognosis.

Keywords: Acute lymphoblastic leukemia; Down syndrome; myelotoxicity; High-dose methotrexate; maintenance therapy.

Authors: Cathrine Bohnstedt1, Mette Levinsen1, Susanne Rosthøj2, Bernward Zeller3, Mervi Taskinen4, Solveig Hafsteinsdóttir5, Helga Björgvinsdóttir6, Mats Heyman6 and Kjeld Schmiegelow1,7. On behalf of the Nordic Society of Pediatric Haematology and Oncology (NOPHO)
Objective: To determine whether renal or hepatic function is responsible for the large intra-individual variation in methotrexate (MTX) elimination in paediatric patients with Acute Lymphoblastic Leukaemia (ALL), and to elucidate predictive parameters of MTX clearance.

Methods: 43 ALL children aged 2-20 years were enrolled in the study. Plasma samples were obtained from 116 High-Dose Methotrexate (HDM) courses and 89 combined with urinary collection. During each HDM course, blood samples were drawn at 23 hours after start of MTX infusion, at h24-27, at h27-30, and subsequently at 6h intervals until plasma cMTX<200nM. Urine was collected continuously from start of HDM infusion until h48-54. MTX and metabolites (7-OH-MTX and 4-amino-4-deoxy-N-methylpteroic acid (DAMPA)) were measured in plasma and urine by HPLC. Allelic discrimination of the genetic polymorphism 80G>A in the reduced folate carrier I (rfc-1 80G>A) was conducted using real-time PCR. Renal clearance, Cl_R, was calculated by Cl_R=MER_u/AUC_P, where MER_u is total amount of MTX excreted unmetabolised in urine and AUC_P is the corresponding area under the curve (AUC) of the plasma MTX concentration-time plot. Total clearance, Cl_T, was calculated by Cl_T=dose/AUC_P, where AUC_P is the total AUC of the plasma MTX concentration-time plot. Additionally, clearances and other pharmacokinetic parameters were generated from population pharmacokinetic modeling (using NONMEM VI).

Results: The association of the genetic polymorphism rfc-1 80G>A and MTX clearances in paediatric patients with ALL shown previously (1) could not be confirmed in this study. Thus, estimates of renal and hepatic clearances and other pharmacokinetic parameters will be presented to elucidate predictive parameters of MTX elimination. (1) Gregers J, Christensen I, Dalhoff K, Lausen B et al. Blood. 2010; 115: 4671

Keywords: Acute Lymphoblastic Leukaemia (ALL) Pharmacokinetics MTX clearance HDM Reduced Folate Carrier I

O15. Treatment-related death in relapsed childhood acute lymphoblastic leukemia

Trausti Oskarsson, Karolinska University Hospital, Department of Pediatric Oncology, Sweden

Background: Patients with relapsed ALL are more susceptible to the adverse effects of chemotherapy both because of the accumulation of organ specific toxicities and the higher intensity of the relapse treatment. Furthermore, a higher proportion of patients undergo stem cell transplantation (SCT) in second complete remission (CR2), where prolonged severe immunosuppression and graft versus host disease (GVHD) further increase the risk for life threatening events. Careful selection of patients for the most appropriate treatment intensity is highly important since over-treatment increases the risk of TRDs but under-treatment the risk of treatment failures or subsequent relapses. Material and methods: In this retrospective observational study we included all patients >1 years and <15 years with pre-B or T-cell ALL that underwent treatment according to the NOPHO ALL-92 and NOPHO ALL-2000 protocols and relapsed before 1.1.2010 (484 of 2735 patients). Patients that relapsed after undergoing SCT in CR1 (n=35) were excluded from the study. Patient data was exported from the NOPHO ALL registry but in case of incomplete registration requests were sent to treating clinics. Results: Among the 449 patients included in the study, 231 (51.4%) died during the study period. We identified 47 patients with TRDs (10.5%), where 13 died before reaching CR2, 10 in CR2 treated with chemotherapy only and 24 patients that died after undergoing HSCT in CR2. In 11 cases disease status at death was unknown. The most common cause of death was infection, 28 of 47. Further results after data supplementation and sub analysis will be presented at the NOPHO annual meeting. Conclusions: Treatment related deaths are more than three times as common during relapse treatment than during primary treatment. Apart from developing novel therapeutic strategies and selecting patients for the most appropriate treatment intensity, finding ways to decrease the risk of TRDs are important for improved survival after relapsed childhood ALL.

Keywords: Acute lymphoblastic leukemia, relapse, risk factors, treatment related death, stem cell transplantation

Authors: Trausti Oskarsson and Mats Heyman on behalf of the NOPHO ALL relapse working group and NOPHO ALL SCT working group (the final list of authors will be sent later) Presenting author: Trausti Oskarsson
O16. USE OF GLUCARPIDASE (VORAXAZE®) IN ALL-2008 IN CONNECTION WITH HD-MTX-INDUCED SEVERE TOXICITY

Jesper Heldrup, Childhood Cancer & Research Unit, Sweden

Purpose HD-M induced nephrotoxicity is a medical emergency. Renal methotrexate excretion is delayed resulting in prolonged exposure to high methotrexate concentrations. The duration of exposure is the primary determinant of the drugs toxic effects. Early recognition and prompt efforts to lower methotrexate concentrations are critical to preventing severe systemic toxicity. HD-M induced renal dysfunction is signaled by an increasing serum creatinine concentration during or shortly after the methotrexate infusion. In NOPHO ALL-2008 it was decided to use Glucarpidase, which rapidly lowers the serum methotrexate concentration by providing an alternative route of elimination. Method It was recommended to give Glucarpidase if the 24 hour levels of MTX was >250, 36 hour levels >20 or 42 hours levels >10 μM together with a reduced kidney function (see NOPHO ALL-2008 appendix 33.9). Glucarpidase should optimal take place within 60 hours. Results Until now Glucarpidase has been used in ALL-2008 31 times (3 % patients). 29 times according to protocol. Median age 9 (range 3-17) at time point 45,5 (32-61) at a dose of 50 E/kg (28-76). Elimination time to cMtx ≤0,20 μM was 240 hours (114-336). Max creatinine T36 112 μM (56-221). Urine output was maintained despite a rapid decline in glomerular filtration. None of the patients suffered from other MTX-toxicity. A clinical problem is that most commercial methotrexate assays will underestimate the impact of Glucarpidase on serum methotrexate concentrations because of the interference by the inactive byproduct, DAMPA, leading to folinic acid over-rescue during the 1½-2 days DAMPA circulates in the bloodstream. Failure to recognize the interference could lead to even further unnecessary Glucarpidase and potentially harmful interventions. Conclusion Glucarpidase rapidly and efficiently lowers the serum methotrexate concentration by providing an alternative route of elimination and, when administered as soon as possible after the recognition of nephrotoxicity, can effectively prevent methotrexate toxicity.

Keywords: Glucarpidase, Voraxaze®, HDM, toxicity, emergency

Authors: Arja Harila-Saari, Kjeld Schmiegelow, Jesper Heldrup
O17. Nordic Recommendations on fertility preservation for children and adolescents

*Cecilia Petersen, Karolinska University Hospital, Pediatric oncology, Sweden*

**Background:** The risk of infertility after cancer treatment in childhood is a concern for treating physicians, the family and - if old enough- the patient. During the last decades, techniques for assisted reproduction and cryopreservation of germ cells and gonadal tissue have improved significantly, which raises questions on how these techniques should be applied to in children and adolescents with cancer. In 2008, a multidisciplinary Nordic Network addressing these questions was formed. There have been annual meetings with pediatric oncologists, pediatric endocrinologists, fertility specialists, andrologists and basic researchers. One of the major aims for the network was to produce common Nordic Recommendations on fertility preservation (FP) in childhood cancer patients. Results: Nordic Recommendations on FP for both boys and girls have been finalized and approved by the NOPHO Late effect group. The recommendations, which may serve as templates for more detailed national guidelines, are published on the NOPHO website. The recommendations emphasize that: All boys who are mature enough should be offered sperm cryopreservation prior to the start of chemotherapy, regardless of the risk of infertility. All girls and prepubertal boys could be offered gonadal tissue cryopreservation only if they are facing a treatment with very high risk for infertility. Whether it will be possible to use cryopreserved tissue for fertility treatment is not clear for several reasons; e.g. the tissue may be infiltrated by malignant cells and thus not suitable for transplant back to the patient. At present, no method has succeeded to produce sperm from a human immature testis and research in this field is therefore highly required. Post pubertal girls who have been treated with alkylating agents or abdominal irradiation, should be offered referral to a fertility specialist for evaluation and counseling as a part of the regular post treatment follow-up.

**Keywords:** Fertility preservation, nordic recommendations

**Authors:** Cecilia Petersen1, Kirsi Jahnukainen2, Einar Stensvold3, Catherine Rechnitzer4 Members of the Nordic Network for Gonadal Preservation after Cancer treatment in Children and Young adults 1Pediatric oncology unit, Karolinska University Hospital, Stockholm, Sweden, 2Pediatric oncology unit, Helsinki university Hospital, Helsinki, Finland, 3Pediatric oncology unit, Rikshospitalet, Oslo, Norway, 4Pediatric oncology unit, Rigshospitalet, Copenhagen, Denmark
**O18. Nordic Centre for Fertility Preservation of Boys after Cancer Treatment**

*Jan-Bernd Stukenborg, Karolinska Institute, Department of Women's and Children's Health, Sweden*

Clinical and scientific significance: The “Nordic Centre for Fertility Preservation” is a joint effort by experts (scientists and clinicians) in the Nordic countries to create a network of excellence with the aim to preserve future fertility and hormonal function in young boys with disorders threatening testicular function. Research projects connected to the project will generate a large body of basic information on human gametogenesis. The regulation which has been extremely difficult to study earlier. The knowledge gained will enable us to generate new research models of human gametogenesis. Pre-pubertal boys with childhood cancer will have testicular tissue taken before chemotherapy will be probably the first real beneficiaries. Structure: The present project host 12 participating centers from 5 Nordic countries, so far. Additionally to combined research activities, this network will include a research training program for young basic and clinical researchers, annual meetings, seminars and an interactive website with information to scientists and for patients and parents. Therefore, the Nordic centre has the potential to become a world leading centre of excellence hosting high quality research and high quality health care. Aims and outlook: We have established a network to initiate and form a “joint force” research strategy for fertility preservation of boys undergoing gonad toxic oncological treatments in the Nordic countries. Our network will focus on: a) Evaluation of all requirements needed to combine human testicular biopsies from the Nordic countries into a common research project and common high quality control procedure (ethical, legal and logistical issues); b) Evaluation of different techniques and strategies for differentiating early male germ cells into mature sperm and optimization of current protocols for cryopreservation; c) Education of young scientists and physicians in the field of reproductive medicine and biology in Northern Europe on a top level worldwide.

**Keywords:** Late effects, fertility preservation, gonadal dysfunction

**Authors:** Jan-Bernd Stukenborg1, Johan Arvidson2, Outi Hovatta3, Mirja Nurmio4, Henna Joki4, Kristin Rós Kjartansdóttir1, Ragnar Bjarnason5, Ahmed Reda1, Anders Juul6, Babak Asadi7, Henriette Magelssen8, Irma Oskam9, Einar Stensvold10, Jorma Toppari4, Olle Söder1, 11Catherine Rechnitzer, Cecilia Petersen2,12, Kirsi Jahnukainen13 Presenting Author: Jan-Bernd Stukenborg
Persistent fatigue in long-term survivors of childhood leukemia and lymphoma

Bernward Zeller, Oslo University Hospital, Pediatric, Norway

Purpose: Clinical experience and recent research suggest that there is a subgroup of long-term survivors of childhood cancers suffering from chronic fatigue. The aim of our study was to compare long-term survivors of childhood leukemia and lymphoma with and without persistent chronic fatigue on self-reported and objectively measured health variables. Methods: Survivors assessed for chronic fatigue in a previous cross-sectional study were re-evaluated at mean 2.7 years later. The investigation comprised questionnaires, clinical examinations, blood tests, algometric pain pressure evaluation, and assessment of physical activity by accelerometers. The Fatigue Questionnaire was used to assess chronic fatigue. Results: Of 102 included survivors (median age 34.6 years, median follow-up time 25.3 years), 15 had major comorbidities possibly explaining fatigue or were pregnant, and were excluded from the comparative analyses. Of the remaining, 27 were classified as “persistent fatigue (PF)” (chronic fatigue at two time points) and 35 survivors were “persistently non-fatigued” (controls). Twenty-five had changed their fatigue status between first and second assessment, and were excluded from comparative analyses. Compared to controls, PF cases reported significantly more sleeping problems (p < .01), depression (p < .001), anxiety (p < .001), pain (p < .001), and reduced physical function (p < .001). No difference in pressure pain sensitivity was found. PF cases were less physically active than the controls (mean number of steps per day 6861 vs. 8687, p=.009). In a multiple regression analysis, depression remained the only significant predictor of persistent fatigue. Conclusions: The group of long-term survivors of childhood cancer with persistent fatigue is characterized by more depression, anxiety, pain, insomnia and less physical activity. These findings indicate that the survivors may benefit from psychotherapy, possibly combined with physical training programs.

Keywords: Fatigue, survivors, childhood cancer, leukemia, lymphoma

O20. The NOPHO-AML late effect study - cardiac muscle function in patients treated with chemotherapy only

Marianne Jarfelt, Inst. of Clin. Sciences, Dep. of Pediatrics, Sweden

Purpose: To evaluate cardiac muscle function in patients treated according to the NOPHO-AML -84, -88, or -93 protocols, and alive by June 30, 2007 without relapse or transplantation. Method Ninety-eight out of 138 eligible patients were examined with standardized centrally reviewed echocardiogram and combined assessment of the systolic and diastolic function (Tei-index) was performed. Results were compared with 30 age and sex-matched controls. Results Mean age at diagnose was 5 (range 0-15) y and at study 17 (5-36) y, and the follow-up time was 12 (4-25) y. All patients had received doxorubicin (mean cumulative dose 148(+/-39) mg/m2) and the majority (N=88) had received combinations of doxorubicin and mitoxantrone (mean cumulative dose 37(+/-14) mg/m2). The mean total anthracycline dose was calculated (doxorubicin + (5 x mitoxantrone)) to 317(+/-67) mg/m2). Ten patients and one control had fractional shortening (FS) < 28 %, and 13 patients had left ventricular ejection fraction (LVEF) < 55 %. There was a significant difference in FS between patients 32.6(+/- 4.0) % and controls 35.2 (+/-3.4) % (p=0.002) and also in LVEF (59.5 (+/-6.8) % vs. 64.2 (+/-4.4) %), (p=0.001).The Tei-index was higher in patients 0.32 (+/- 0.081) than controls 0.26 (+/- 0.074) (p<0.001). Cumulative dose of doxorubicin was significantly related to lower FS (p=0.037) and LVEF (p=0.016). Longer follow-up time was associated with lower FS (p=0.034). Higher Tei-index was related to young age at diagnosis (p=0.04) and longer follow-up (p=0.031), but not to the calculated cumulative dose of anthracyclines or dose of mitoxantrone. Conclusions In this study of Nordic AML patients treated with chemotherapy only, there was a significant reduction in left ventricular function compared with healthy controls, although most patients had cardiac function within normal limits. Higher doses and longer follow-up were associated with decreased cardiac function. Keywords: acute myeloid leukaemia cardiac muscle function dose of doxorubicin long follow-up time Authors: Marianne Jarfelt Niels H Andersen Heidi Glosli Kirsi Jahnukainen Guðmundur K. Jónmundsson Johan Malmros Karsten Nysom Henrik Hasle Presenting author: Marianne Jarfelt
O21. The level of ETV6-RUNX1-positive cells in umbilical cord blood from healthy newborns

Marianne Olsen, Rigshospitalet, Pediatric and Adolescent Medicine, Denmark

Introduction: Translocation t(12;21)(p13;q22) [ETV6-RUNX1] is present in up to 25% of children with B-cell precursor acute lymphoblastic leukaemia (ALL) and has been used to explore the natural history of common childhood ALL. Increasing evidence suggests that childhood ALL develops as a multi-step process involving both pre- and postnatal genetic events (two-hit hypothesis). In addition, since 2001 it has been a commonly held belief that 1% of healthy newborns harbors ETV6-RUNX1-positive cells at high levels (10^{-3} – 10^{-4}) in umbilical cord blood (Mori et al, PNAS 2001). However, the interpretation that substantially more newborns (1:100) than the cumulative risk of t(12;21)-positive ALL (1:10,000) harbors pre-leukemic ETV6-RUNX1-positive cells at a high level has recently been challenged by the present research group. In scrutiny of two different Danish populations of umbilical cord blood of 1258 newborns enrolled in the Danish National Birth Cohort (Olsen et al, JPHO 2011) and in additional 1417 mature newborns including vigorous quality validations (Lausten-Thomsen et al, Blood 2011), we demonstrated that the < 1% of newborns that harbor t(12;21)-positive cells, only do so at levels below 10^{-5}. Purpose: The presentation will address the conflicting observations regarding levels of pre-leukemic ETV6-RUNX1-positive cells in healthy neonates. The above mentioned studies all used qRT-PCR for initial screening. qRT-PCR carries a significant risk of contamination errors and needs validation by other methods. Whereas the study by Mori et al used FISH for initial screening, the study by Lausten-Thomsen et al applied cell enrichment. The moderate sensitivity and low specificity of several FISH strategies as validation methods for low levels of ETV6-RUNX1-positive cells will be addressed. Conclusions: Clarification of the level of ETV6-RUNX1-positive cells in healthy neonates is important for the understanding and the mapping of the natural history of t(12;21)-positive ALL as well as for potential future disease preventive methods.

Keywords: Common ALL, childhood, qRT-PCR, FISH, ETV6-RUNX1

Abstract Overview 2013

Poster Presentations
BACKGROUND: CUSUM plots showing the cumulated sum of an event in a consecutive patient series are useful for monitoring quality of treatment, giving early warning of deviations from an accepted event rate and identifying “bad runs” for clinical scrutiny. Application to acute lymphoblastic leukaemia (ALL), however, is complicated: a patient series is a mix of cases with different risks, and final outcome is not known till five years after diagnosis. We here present an approach to overcome these difficulties.

METHODS: We applied different types of cusum plots to children treated in our department: 1) a simple plot showing the accumulated number of events, 2) a risk group-adjusted (O-E) plot showing the cumulated difference between observed and expected events, and 3) an (O-E) plot using individual risks based on time to event. In addition, we tested 4) a newly developed logistic plot showing accumulation of evidence for or against the hypothesis of treatment results corresponding to Nordic standard.

RESULTS: Of 27 NOPHO-2000 patients, all with completed 5-year follow-up, 4 had an event. Cusum plotting with risk adjustment showed 2.1 events fewer than expected. The curves suggested improvement after the first 7 patients; no “bad runs” were identified. The hypothesis of performance according to Nordic standard was 2.7 times more likely than that of substandard performance (corresponding to 73% certainty). Of 24 NOPHO-2008 patients, so far only one patient has had an event; for the remainder final outcome is unknown. Preliminary plots shows a deficit of 0.9 events compared to Nordic results, with adequate performance being so far 1.5 times more likely than poor performance (certainty 60%).

CONCLUSION: Cusum plots can be adapted to review the quality of ALL treatment and to monitor outcomes during an ongoing protocol period with unfinished follow-up, giving real-time warning of deviations from an accepted standard.

Keywords: Acute Lymphoblastic Leukemia, treatment results, CUSUM plots.
Authors: Rikke-Line Jacobsen, Jon Helgestad & Steen Rosthøj
P2. Absolute immature platelet count may predict imminent platelet recovery in thrombocytopenic children following chemotherapy

Mimi Kjaersgaard, Aarhus University Hospital Skejby, Pediatrics, Denmark

Background Intensive cytotoxic chemotherapy often causes severe thrombocytopenia due to bone marrow suppression. To prevent severe bleeding episodes, platelets are transfused. It is a long-standing controversy whether standard prophylactic platelet transfusions are necessary or could be replaced by a therapeutic transfusion strategy. The immature platelets are the youngest circulating platelets and they reflect the rate of thrombopoiesis. The immature platelet fraction (IPF) or the absolute immature platelet count (AIPC) may predict platelet count recovery, and could therefore play an important part when choosing transfusion strategy.

Material and methods We measured platelet count and IPF in 19 pediatric patients (0°C17 years) receiving chemotherapy for malignant diseases. Baseline values for IPF, AIPC and platelet counts were obtained prior to chemotherapy when platelet count was iÝ 50iÁ109/L. Severe thrombocytopenia was defined as a platelet count <20iÁ109/L and nadir was defined as the lowest platelet count following chemotherapy. Platelet recovery was defined as either increasing platelet counts on three consecutive measurements on different days or doubling of the nadir count with a platelet count iÝ10iÁ109/L. To test if IPF or AIPC were associated with CRP we analyzed half (416) of all blood samples taken from January 1st to February 1st 2012. The 416 samples with simultaneous measurements of IPF, CRP, and platelet count represented 37 pediatric patients.

Results A significant increase of 0.6iÁ109/L in AIPC was seen between 1 and 2 days prior to platelet count recovery. IPF showed no predictive day-to-day differences. IPF and AIPC showed no correlation to CRP. AIPC was in contrast to IPF not influenced by platelet transfusions. Conclusion AIPC increased significantly between 24 and 48 hours before platelet recovery whereas IPF showed no significant increase during the same time period. AIPC may be superior to IPF in predicting platelet recovery after chemotherapy in pediatric patients.

Keywords: Immature platelet fraction (IPF); Absolute immature platelet count (AIPC); Thrombocytopenia; Sysmex XE-2100; Platelet transfusion

Authors: Laerke Walther Junggreen Have MD1, Henrik Hasle MD, PhD1, Else Marie Vestergaard MD, PhD2, Mimi Kjaersgaard MD, PhD1 (presenting) 1 Department of Paediatrics, Aarhus University Hospital, Skejby, Denmark 2 Department of Clinical Genetics, Aarhus University Hospital, Skejby, Denmark
P3. Substitution of acquired asparaginase related antithrombin deficiency in children with ALL

Susanna Ranta, Astrid Lindgren Children’s Hospital Q6:05, Department of Children’s and Women’s Health, Sweden

Background After introduction of the ALL2008 protocol with prolonged continuous asparaginase (ASP) treatment three symptomatic deep venous thrombosis (DVTs) in 2/10 patients were observed at the Children’s Hospital, Helsinki, Finland. After a case of massive cerebral venous sinus thrombosis with secondary hemorrhage, an antithrombin (AT) substitution strategy during ASP treatment was adopted to prevent further DVTs yet ensuring continuous exposure to ASP. Aim Our aim is to describe the effect of prolonged asparaginase treatment on antithrombin and fibrinogen levels and the AT substitution practice in Helsinki. Procedure All children with standard/intermediate risk ALL diagnosed in Helsinki between June 2009 and November 2011 (the intervention group n=25) received AT concentrate when their AT activity decreased below 55%. The DVTs (diagnosed by ultrasound, venogram or MRI) during AT substitution regimen are compared with DVTs before the substitution in Helsinki. The AT and fibrinogen levels before (n=10) the antithrombin substitution in Helsinki and simultaneously at the Astrid Lindgren Children’s Hospital, Stockholm, Sweden (n=39) were evaluated to study the effect of prolonged ASP treatment.

Results In the intervention group 60% (15/25) received AT concentrate (median number of infusions 3, range 1-17). Eight of the 9 patients who received ≥2 asparaginase treatments received antithrombin. The median lowest AT activity for those who received ≥2 substitutions was 44% (range 36-51%), for controls in Sweden 55% (range 35-123%) and in Helsinki 64% (range 41-104%). Fibrinogen level of ≤1.0 g/l was found in 18/177 (10%) routine samples taken during the ASP treatment. In Helsinki, 2/10 (20%) children had 3 symptomatic DVTs before and 2/25 (8%) had one symptomatic DVT each after the initiation of AT substitution, all with concomitant steroids. Conclusions Most children are exposed to low AT activity during ASP treatment predisposing to thrombosis. Larger studies are needed to evaluate the benefit of prophylactic antithrombin treatment.

Keywords: asparaginase, thrombosis, ALL, antithrombin, fibrinogen

Authors: Susanna Ranta, Mats Heyman, Kirsi Jahnukainen, Mervi Taskinen, Ulla Saarinen-Pihkala, Tony Frisk, Stefan Söderhäll, Pia Petrini, Anne Mäkipernaa
Background. Childhood acute lymphoblastic leukemia (ALL) is the most common childhood cancer with high cure rate. Changes in function, content and number of mitochondria have been found in several cancer types. However, there are only a few publications on mitochondrial DNA (mtDNA) and leukemia. In 2011, Kwok et al. reported that reduction in the number of mitochondria and heteroplasmy of mtDNA mutations correlated with good therapy response in childhood ALL. Objective. We studied mtDNA polymorphisms and pathogenic mutations in ALL patients. We hypothesized that mtDNA genetic variations associate with ALL and mtDNA mutations could be used as remission markers. Methods. The study population consisted of diagnostic and remission follow-up blood and bone marrow samples from 16 childhoods ALL patients. In the pilot study, mtDNA analysis was performed for 3 out of 16 patients by conformation sensitive gel electrophoresis (CSGE) and subsequent sequencing. The novel heteroplasmic mtDNA mutation detected from blood and bone marrow samples was confirmed by radioactive PCR and restriction fragment length polymorphism (RFLP). The functional importance of the novel mutation is evaluated using E. coli complex I model. Results. MtDNA sequence at diagnosis and remission phase differed on CSGE for one patient. Subsequent sequencing revealed a novel heteroplasmic m.10578A>G mutation, present only in blood and bone marrow samples at diagnosis and not in remission phase. Interestingly, this nucleotide change in the subunit ND4L encoding gene of the mitochondrial respiratory chain complex I (NADH dehydrogenase) lead to substitution of methionine-37 to valine in the vicinity of the catalytically essential proton channel residue glutamate-72 of the enzyme. Conclusion. The novel m.10578A>G mutation found only in leukemia cells could be used as remission marker for ALL. This mutation probably has metabolic consequences and could either inactivate complex I enzyme or could lead into uncoupling of ATP synthesis from oxygen consumption.

**Keywords:** Acute lymphoblastic leukemia, mitochondrial DNA mutations, remission marker, mitochondrial respiratory chain, complex I

**Authors:** Järviaho Tekla, Kervinen Marko, Savolainen Eeva-Riitta, Möttönen Merja, Niinimäki Riitta, Hinttala Reetta, Majamaa Kari, Harila-Saari Arja and Uusimaa Johanna
P5. Myelodysplastic changes mimicking MDS following treatment for osteosarcoma

Ditte Juel Adolfsen Løhmann, Aarhus University Hospital, Skejby, Department of Pediatrics, Denmark

Therapy-related myelodysplastic syndrome/acute myeloid leukaemia (t-MDS/AML) is a feared long-term complication of paediatric cancer including osteosarcoma. Few develop t-MDS/AML, but it is not known how many have significant haematological changes after finishing treatment for osteosarcoma. In this study we reviewed biochemistry from a consecutive series of children for up to two years after finishing treatment. We included all children (n=14) who were diagnosed from October 2006 to January 2011 at our department and treated according to the EURAMOS-1 protocol. Four patients relapsed and died before the end of the study period. We found noteworthy changes in MCV, platelets and ANC. MCV increased from median 83 fl at diagnosis to a maximum of 98 six months after the last chemotherapy course and then stabilized around 93 fl for the rest of the study period. Platelets decreased from a median of 309 x10^9/L at diagnosis to 173 at six months, and stabilized at around 200 x10^9/L, and ANC decreased from a median of 4.64 x 10^9/L at diagnosis to a minimum of 2.18 at 18 months and increased slightly to 2.93 at 24 months. Haemoglobin normalized after one month and remained within the normal range. Within the study period two cases had a bone marrow examination performed. In one case MDS (refractory anemia with excess blasts) with monosomy 7 was found and a hematopoietic stem cell transplant was performed. In the other case MDS without excess of blasts was found and a spontaneous normalization of the biochemistry occurred. In conclusion in our study most patients treated for osteosarcoma developed haematological abnormalities similar to early MDS but few developed t-MDS/AML. Close monitoring of patients recovering from osteosarcoma is essential.

Keywords: Osteosarcoma, t-MDS/AML, Haematological abnormalities, Children

Authors: Ditte Juel Adolfsen Løhmann and Henrik Hasle
P6. Long term follow up of healthcare visits in adult survivors of childhood cancer

Marianne Jarfelt, Inst of Clinical sciences, Dep of Pediatrics, Sweden

Objectives: The high survival rates of patients with childhood cancer require increased knowledge of the late complications of the diseases and their treatment. The aim of this work was to determine the in- and outpatient hospital visits during the years 2009-2011, in adult survivors of childhood cancer. Methods: All individuals diagnosed with cancer before the age of 18 in the time period 1985-2001 in the Western region of Sweden, older than 18 years, at least 5 years past treatment and still living in the region in January 2009 (452 individuals) were identified. Of them, 170 had leukaemia/lymphoma (LL), 141 central nervous system tumours (CNST) and 141 solid tumours (ST). 1000 age- and gender-matched controls (C) were identified in the Population Register of Sweden. We evaluated and compared their healthcare visits during 2009-2011. One leukaemia patient was considered as outlier due to extensively many visits, and therefore excluded in all calculations. Results: Compared to controls, adult survivors of cancer had in total 2.1 times more hospital visits per individual during the years 2009-2011. In the adult survivor group 75% had hospital visits compared with 56% in the control group. Evaluation of each subgroup showed equal results; LL 1.8, CNST 2.2 and ST 2.3 times more often hospitals visits. The stay in the hospital care was 2.5 times longer for survivors of cancer in comparison with controls. The health care costs were for the adult survivors in general 2.8 times higher per year. Conclusions: This population-based study shows that survivors of childhood cancer have greater demand for healthcare contacts in adulthood. They have both higher frequency of hospital visits and increased length of stay in hospital. The higher health care costs indicate a high burden of chronic health conditions and motivates structured follow-up. Further analysis of the specific health conditions will be presented.

Keywords: Long-term follow up. Health care costs Hospital visits Morbidity Adulthood

Authors: Elizabeth Schepke, MD, Inst of Clinical sciences, Dep of Pediatrics Per Sjöli, BBA, Regional Cancer Centre West, Sweden Marianne Jarfelt MD, PhD, Inst of Clinical sciences, Dep of Pediatrics Presenting author: Elizabeth Schepke
**P7. p53 protein expression; a predictor of poor outcome in JMML, MDS and CML?**

*Emma Honkaniemi, Dpt. of Clinical Science, Intervention and Technology, CLINTEC, Karolinska Institute, Pediatrics, Sweden*

Aim: To determine if p53 protein expression could be used as a prognostic factor for relapse after hematopoietic stem cell transplantation (HSCT) for children diagnosed with juvenile myelomonocytic leukemia (JMML), myelodysplastic syndrome (MDS) and chronic myelogenous leukemia (CML). Furthermore, we performed DNA sequencing of TP53 and immunohistochemistry of p21 protein expression to investigate if alterations in p53 expression could be explained by mutations in TP53. Patients and Methods: Paraffin imbedded bone marrow samples from 33 pediatric patients treated at Karolinska University Hospital Huddinge were collected retrospectively from time of diagnosis, HSCT and follow ups at 3, 6, 12 and 24 months. Samples were prepared for analysis by tissue micro array (TMA). Immunohistochemistry was performed with protein antibodies for p53 and p21. Positive staining was calculated for each patient and protein. To identify possible mutations, exon 2-11 of the TP53 gene was sequenced in 14 out of the 33 patients (7 JMML and 7 MDS). Results: A significant difference in p53 protein expression between the two groups was revealed at time of diagnosis where the relapse group had a higher expression than the event free group. Logistic regression of p53 expression at diagnosis and risk of relapse revealed an odds ratio of 1.19 (95% CI: 1.02-1.40, p=0.028302). The statistical significance remained when diagnostic p53 expression was analyzed with relevant confounding factors. No mutations were found in the genetic analysis. p21 expression correlated positively with p53 expression. Conclusion: In JMML, MDS and CML, p53 protein expression appears to be elevated at diagnosis in children who experienced relapse post-HSCT. DNA sequencing and p21 expression analysis did not suggest a genetic mutation on the TP53 gene indicating an incident at protein level. Elevated p53 protein expression may be an indicator for children in risk of relapse and therefore could benefit from more intense therapy.

**Keywords:** p53, JMML, MDS, CML, relapse.

**Authors:** Honkaniemi Emma, Mattsson Kristin, Sander Birgitta, Barbany Gisela, Gustafsson Britt.
P8. Children and adolescents experience of donating bone marrow - stem cells to surviving siblings

Carina Rinaldo, Astrid Lindgrens Barnsjukhus, Barncancerforskningsenheten
Q6:05, Sweden

Family and sibling relationships are affected in both positive and negative ways when a child in the family is afflicted with a severe illness during a long period of time. There are few national studies conducted in Sweden on how siblings who were bone marrow - stem cell donors think and feel about their experience. The aim of this study was to describe children's and adolescents experiences of donating stem cells to a sick sibling in Sweden. Method: A descriptive interview study with an inductive approach was performed using qualitative content analysis. The six participants were of both sexes and between 11-21 years. They were recruited from three different childrens transplant centers, had donated stem cells before the age of 17 and all had surviving siblings. Result: The theme proud heroes without a choice summarize the results. The category proud but anxious to be a donor describes a desire and a joy to help, but also concerns how they would endure the procedures and a concern of not being good enough as a donor. They were very anxious for their sick sibling. The category heroes without real choices in need of support highlights the strong family ties make them not having a choice situation for the donation, but a need support from their environment, healthcare and from receiving information but also all of its weaknesses. Conclusion: These donors were happy to contribute to the sibling's recovery. They were proud and gained a positive view of life from this experience. However the questions remain who will consider the psychological risks of these children and adolescents and if it is right to expose young siblings to this risk.

Keywords: Stem cell transplantation, siblings, children, experience, donor

Authors: Carina Rinaldo,1,2 Katarina Wallin,1 Pernilla Pergert,2,3 Magaretha Stenmarker 4 and Britt-Marie Frost1 1Dept. of Women’s and Children’s Health, Uppsala University, Sweden 2Childhood Cancer unit, Karolinska University Hospital, Stockholm, Sweden 3Dept. of Womens and Childrens Health, Karolinska Institute, Stockholm, Sweden 4Dept. of Pediatrics/Fututrum-Academy for Health and Care, Jönköping, Sweden
P9. Primary subclavian vein thrombosis associated with factor V Leiden mutation

Sonata Saulyte Trakymiene, Children’s Hospital Affiliate of Vilnius University Hospital Santariskiu Klinikos, Pediatric Hematology/Oncology, Lithuania

Background/aim. Deep venous thrombosis of the upper extremity (UEDVT) is an unusual disorder and accounts to <4% of all cases of DVT. Primary UEDVT is even more rare. The aim of this report is to present a rare case of UEDVT in a young girl.

Methods. Case presentation. Results. A 13-year-old right-handed girl presented with 3 weeks of left brachial swelling, pain, erythema and limited range of motion of the shoulder joint. Additional signs included subcutaneous venous collaterals over the shoulder region and chest. The onset of the UEDVT was characterized with an acute swelling of the left wrist and forearm with bluish discoloration and irradiating pain to the left shoulder. US of forearm and MRI of left shoulder did not reveal evidence of DVT. The patient initially was treated with no steroidal anti-inflammatory drugs without any improvement until duplex ultrasound revealed sub acute deep vein thrombosis and complete occlusion of the left subclavian vein extending to the left auxiliary and brachial veins. Her past medical, surgical, and family history were unremarkable, except that the patient was a professional tennis player since 5 years. The presence of local anatomic abnormalities were excluded. The patient was heterozygous carrier of G1691A factor V. She has been receiving therapeutic doses of LMWH since 4 months following diagnosis. Patient responded well to treatment, with a complete recanalization of auxiliary and brachial veins, however, still with signs of chronic DVT in left subclavian vein.

Discussion/conclusions. “Effort thrombosis”, or also known as Paget–Schroetter syndrome, that may have developed secondary to intimal damage of the axillary–subclavian veins following repetitive heavy arm exertion, occurs principally in young healthy males who are engaged in sports activities usually without commonly recognized pro-thrombotic risk factors. Coexistence of inherited thrombophilia may have contributed to the development of DVT in this rare case.

Keywords: thrombosis, subclavian vein
Authors: Valentina Daugelaviciene, Lina Rageliene
P10. THE CLINICAL SPECTRUM OF ACUTE LYMPHOBLASTIC LEUKEMIA: REVIEW OF 100 CONSECUTIVE CASES WITH EVALUATION OF REFERRAL CRITERIA

Ninna Brix, Aalborg University Hospital, Denmark, Pediatric Oncology Section, Pediatric Department., Denmark

Purpose and methods: Acute Lymphoblastic Leukaemia (ALL) may not always be easy to recognize in general practice, and consequently guidelines with criteria for prompt referral have been specified by health authorities. We reviewed the clinical presentation (symptoms, signs and laboratory findings at the time of diagnosis) in a cohort of 100 children with ALL in order to describe the clinical spectrum, to determine the frequency of atypical features, and to evaluate the sensitivity of the referral criteria. In addition, we explored the clinical differences between precursor B-cell ALL (n=89) and T-cell ALL (n=11). Results: Symptoms had been present for more than one month in 36%. Skeletal pains were reported in 49% (average duration 7-10 weeks), constitutional symptoms in 82% (3-5 weeks), and symptoms referable to bone marrow insufficiency in 77% (appr. 2 weeks). Only 23% presented with the classical clinical triad of pallor, fever and purpura, and only 36% had involvement of all three hematopoietic cell-lines. Almost one quarter (22%) presented with no or only one hematologic abnormality. In this group bone and joint pain was more frequent while other symptoms and signs were less frequent, and the average symptom interval was longer (approximately two months versus one month). In the subgroup with T-ALL, skeletal pains and lesions did not occur and bone marrow symptoms were less frequent, but enlarged glands or organomegaly and LD elevation were present in all cases compared to two-thirds of children with pre-B ALL. Overall, Danish and UK criteria for referral were fulfilled in 96% and 86%, respectively. Conclusion: The clinical presentation of ALL is variable, and often symptoms have been present for some time before the diagnosis is made. Early detection prior to development of full-blown bone marrow insufficiency may be promoted by meticulous examination for organomegaly and by attention to subtle hematologic changes.

Keywords: ALL, clinical presentation, referral criteria, diagnostic delay

Authors: Ninna Brix, BSc & Steen Rosthøj, MD
P11. Testing in vitro sensitivity of minimal residual disease in childhood acute lymphoblastic leukemia

Line Groth-Pedersen, University Hospital Rigshospitalet, Pediatrics and Adolescent Medicine, Denmark

Acute lymphoblastic leukemia (ALL) is the most frequent malignant disease in children. Although improved risk grouping, anti-cancer treatment and supportive care have resulted in survival rates above 80%, 15-20% of patients experience a relapse, which is associated with an inferior survival. Relapse is caused by persistence of minimal residual disease (MRD) primarily in the bone marrow during chemotherapy. Methods for identification of drugs that are capable of eliminating the MRD cell population are necessary. In vitro sensitivity testing carried out at the time of diagnosis predicts correlation to MRD levels and relapse rates but has not been applicable for treatment stratification. We hypothesize that in vitro sensitivity testing of the more resistant MRD cells remaining in bone marrow after the induction therapy could help to stratify patients to individualized chemotherapy. The greatest challenge in carrying out in vitro sensitivity testing on MRD cell populations is the low number of leukemic cells available, i.e. 0.1-5%. We aimed at developing a cell death assay applicable on small ALL cell populations. We show that a flow cytometry based assay using annexin V (apoptosis marker) and 7-Aminoactinomycin D (necrosis marker) staining is a reliable method for evaluating early and late stages of cell death in small ALL cell populations. Both in ALL cell lines REH (B-cell precursor (BCP) ALL, t(12;21), RS4;11 (BCP ALL, t(4;11)) and Nalm-6 (BCP ALL, t(5;12)) and in primary ALL cell samples the results are highly reproducible and show the same relative sensitivity profile as when a large number of cells are used. This cell death assay only demands a total of 40,000 cells to determine the in vitro sensitivity for 3 drugs run in triplicates at 4 concentrations. It could thus be applicable for sorted MRD populations accounting for 0.1% of the mononuclear cells in the bone marrow.

Keywords: Pediatric cancer, acute lymphoblastic leukemia, minimal residual disease, in vitro sensitivity, resistance mechanisms.

Authors: Line Groth-Pedersen, Rebecca Valentin and Kjeld Schmiegelow Department of Pediatrics and Adolescent Medicine, University Hospital Rigshospitalet, Copenhagen Denmark.
Background: There are increasing numbers of adult survivors of childhood central nervous system (CNS) cancer. This is a result of an improved treatment and an increased survival rate. Late effects are defined as any chronic or late occurring physical or psychosocial outcome persisting or developing well after diagnosis of the tumour. The prevalence of late effects has increased. Aim: The aim of this study was to identify late complications in individuals diagnosed with CNS tumours in childhood and to describe possible relationship to location and treatment modality. Method: A retrospective descriptive population-based study of patients diagnosed with a CNS-tumour between the years 1985-2004 in western Sweden was performed. All had an age of 18 years or older. The study criteria were fulfilled by 290 patients and 283 medical records (142 females and 141 males) were available and analyzed. The records were coded and a protocol was used for data collection. The late effects were categorized as medical or neurocognitive complications. Result: The analyses revealed that 212 (108 females, 104 males) were survivors, i.e., the survival rate was 74.9 percent. The most common treatment was surgery. The mean value of complications in survivors was 2.5 per patient (range 0-9) and 84.9 percent had at least one late effect documented. Visual impairment was found in 37.3 percent. Thirty-four percent had at least one late endocrinological disturbance and 16 percent showed cognitive dysfunction. Radiation therapy was found to be a significant predictive factor for endocrinological disturbances. Conclusion: Survivors of CNS tumours are at high-risk of developing late effects. In this study, missing data and limitations concerning follow-up may underestimate the overall prevalence of complications. The complexity in this area, including aspects of quality-of-life, elucidates the need for long time follow-up at late effect centres.

Keywords: Childhood CNS tumours, Late effects, Cancer survivors

Authors: Cajsa Magnusson (presenting author), Marianne Jarfelt, Margaretha Stenmarker
Introduction: In the coming years, increased understanding of the genetic contribution to childhood cancer will likely influence both cancer treatment and surveillance. With this case report, we wish to illustrate some challenges related to this development. Case report: A 1.5 year old boy presented to our ward with pubarche. Evaluation revealed increased blood levels of DHEAS and testosterone and a large non-metastatic tumor in his adrenal cortex (MR abdomen) that was histologically diagnosed to be an adrenocortical carcinoma. Due to an increased family history of cancer and the rarity of adrenocortical carcinomas in children, the family was referred to genetic counseling. Genetic testing confirmed that the patient had a germ line TP53 mutation, and additional testing of the family detected the same mutation in the patient’s asymptomatic mother, mother’s siblings, and their children. Discussion: Germ line TP53 mutations are rare, and are one of few known mutations found to cause the Li-Fraumeni syndrome, a highly penetrant hereditary cancer syndrome (1). A long term family study found a lifetime penetrance for cancer of 80% by age 50, with a higher lifetime risk and younger age debut among women (2). In the only prospective observational TP53 surveillance study to our knowledge, a vigilant follow up with clinical examinations, blood tests and ultrasound every 4 months, together with total body/CNS MRI yearly, lead to early identification and treatment of asymptomatic cancer, resulting in a dramatic increase in survival (100% 3 year survival in surveillance group (n=18) versus 21% 3 year survival in non-surveillance group (n=15) (3). With the possibility of detecting TP53 mutations, together with reports of a feasible clinical surveillance protocol that decreases cancer mortality, two important questions need to be addressed: (1) Should TP53 testing routinely be performed in children with germ line TP53 associated childhood cancers (and if positive, their families)? (2) If a TP53 mutation is detected, how do we propose to follow these children, balancing an efficiently vigilant life-long follow up with the resources available at our hospitals, and the quality of life of otherwise healthy children? 

Keywords: hereditary cancer syndromes, tp53, surveillance

Authors: Monica Cheng Munthe-Kaas MD PhD (presenting author), Anne Grethe Myhre MD PhD, Ellen Ruud MD PhD, Klaus Beiske MD PhD, Anne Grete Bechensteen MD PhD
P14. Multiple aspergillus brain abscesses in a child with acute lymphoblastic leukemia- a case report

Aina Ulvmoen, Oslo University Hospital, Department of Pediatric Medicine, Norway

Invasive fungal infection is a severe and life threatening condition in immunocompromised patients with mortality rates ranging from 60-90 percent. We report a case of a five-year old boy who developed brain abscesses during induction treatment for acute lymphoblastic leukemia. At diagnosis there was CNS2 involvement with 3x10^9 cells/L. These were identified as lymphoblasts by cytospin and triple i.t was given according to NOPHO ALL 2008 non-high risk protocol. The CSF showed normal protein levels, and clearance of lymphoblasts after the first lumbar puncture. On day 14 he had sepsis with septic shock and cardiac arrest and was admitted to the ICU. Later he developed hallucinations and focal seizures. MRI of the brain revealed five nodular lesions and thoracic CT several nodular lesions in the lungs. The brain abscesses were drained and later totally surgically removed. A microbiological diagnosis of Aspergillus viridinutans was made by culture and polymerase chain reaction (PCR). Antifungal treatment included liposomal amphotericin B, voriconazole, caspofungin, flucytosine and micafungin. In our patient the immunosuppression was decreased by postponing the antileukemic treatment for almost 3 months. MRD day 29 and 79 were negative and the patient has shown a remarkable neurological recovery. It is preferable to have management and treatment guidelines for difficult to treat infections. The diagnostic approach should include lumbar puncture and abscess drainage to identify causative organisms. Newer surrogate markers of invasive fungal infections include antigen/antibodies or fungal metabolites. However these are not routinely in use at our hospital. The antifungal treatment, empirical and targeted, should be continued until all signs and symptoms of the infection have resolved and often longer in immunocomprised patients. In the case of documented fungal abscesses like in our case there is a huge risk of reactivation when neutropenia occurs, and thus secondary prophylaxis is necessary, probably as long as the chemotherapy lasts.

**Keywords:** Acute lymphoblastic leukemia, aspergillus, brain abscess, immunocomprised, antifungal treatment.

**Authors:** Aina Ulvmoen, Marit Hellebostad
P15. Hemostasis and asparaginase activities during non-HR asparaginase treatment in NOPHO ALL-2008

Birgitte Klug Albertsen, AUH in Skejby, Department of Pediatrics, Denmark

Introduction: In the NOPHO ALL-2008 protocol non-HR patients receive prolonged low-dose PEG-asparaginase treatment for 6 months. Asparaginase is a well-known risk factor for thromboembolic events in ALL-patients. Aim: To describe the fluctuations in the procoagulants and anticoagulants in relation to asparaginase activities in the blood. The relation between these parameters and clinical thrombosis as well findings on echocardiographies performed regularly during asparaginase treatment are also described. Material and methods: So far 16 patients in Aarhus have been included in the study. At several visits to the clinic the patients had blood samples drawn for measurement of ATIII, Protein S, Protein C, PP, APTT, Fibrinogen, Factor VIII, VWF, D-dimer and enzyme activities. Echocardiographs were performed by the same paediatric cardiologist once before treatment, 3 times during asparaginase treatment and once after cessation of asparaginase therapy. Shortly after completion of asparaginase therapy they were investigated for thrombophilia. Thromboembolic events were registered. Results: In spite of fluctuations in the coagulation parameters and enzyme activities during asparaginase treatment only one patient experienced a thrombotic event. Minor changes were found on echocardiographs. None of the investigated patients had thrombophilia. Conclusions: The preliminary results from this study show no significant relation between asparaginase activities, levels of the coagulations parameters and clinical thrombosis or findings on echocardiographs.

Keywords: asparaginase, thrombosis, ALL, procoagulants, anticoagulants

Authors: Birgitte Klug Albertsen, Jesper Vandborg Bjerre, Niels Clausen, Lone Hvitfeldt Poulsen
P16. Loss of chromosomes is the primary event in near-haploid and low hypodiploid acute lymphoblastic leukemia

Setareh Safavi, Laboratoriemedicin, Clinical Genetics, Sweden

Near-haploid (23-29 chromosomes) and low hypodiploid (HoL; 33-39 chromosomes) acute lymphoblastic leukemia (ALL) is associated with a poor prognosis. Both subtypes are characterized by chromosomal loss and duplicated stem lines, the latter which may lead to misclassification as high hyper diploid ALL. Nothing is known about the pathogenetic consequences and mechanisms underlying near-haploidy and HoL. In the present study, we used SNP array analysis to investigate 12 cases of near-haploid (n=8) and HoL (n=4) ALL. A characteristic pattern of monosomies/uniparental isodisomies and retained heterodisomies/tetrasomies was identified, with the latter involving chromosomes X/Y, 14, 18, and 21 in near-haploid cases and chromosomes 1, 5, 6, 8, 10, 11, 14, 18, 19, 21, and 22 in HoL. All cases displayed widespread loss of heterozygosity (LOH). Two cases were initially diagnosed as high hyper diploid childhood ALL and both relapsed; notably, these were unambiguously identifiable as near-haploid with the SNP array analysis, showing the potential of this technique for correct risk stratification. Cryptic genetic events were found in five cases, including four CDKN2A deletions. The pattern of deletions and LOH indicated that micro deletions occurred in a subsequent step in one case, strongly suggesting that the chromosome loss is the primary event in near-haploid and HoL ALL.

Keywords: SNP array analysis, near-haploidy, low hypodiploidy, loss of heterozygosity

Authors: Setareh Safavi, Erik Forestier, Irina Golovleva, Gisela Barbany, Karolin H. Nord, Anthony V. Moorman, Christine J. Harrison, Bertil Johansson and Kajsa Paulsson
P17. Bacteraemia in children with cancer – is the empiric antibiotic therapy sufficient?

Lotte Vinther Kiefer, Aarhus university hospital, Department of Paediatrics, Denmark

Intensive chemotherapy makes children with cancer exceedingly susceptible to infections. Infection-related death is the most common cause of death second to malignant disease. Nosocomial infections are increasingly associated with antibiotic resistance, which may lead to increased rates of mortality. Prompt initiation of appropriate antibiotic therapy is essential. More knowledge is needed about susceptibility patterns of bacteria isolated from paediatric patients in the Nordic countries to ensure sufficient empiric treatment. This study aimed to describe the epidemiology and susceptibility of bacteraemia among children with cancer. We conducted a retrospective cohort study of all patients registered at the Paediatric Department of Haematology and Oncology, Aarhus University Hospital from January 2000 to October 2012. A bacteraemia was defined as a blood culture isolate from a patient with clinical signs of infection. All bacteraemia during the study period were evaluated. A total of 527 patients were registered with cancer. Bacteraemia was seen in 570 cases and 677 microorganisms were isolated. Gram-negative bacteria dominated before 2004 but the relative amount of Gram-negative bacteria has decreased since 2002. Only 55.5% of the positive blood culture isolates were identified in children with neutropenia. Susceptibility to the antibiotic drug combination piperacillin-tazobactam and gentamycin was 89.7%. If the seven most pathogenic bacteria were evaluated susceptibility increased to 96.5%. The susceptibility of meropenem was 75.5% for all bacteria and 95.7 % for the seven most pathogenic bacteria. Escherichia coli / Enterococcus spp and Pseudomonas aeruginosa were the resistant bacteria towards piperacillin-tazobactam/gentamycin and meropenem respectively. During the study 15.7% died of malignant causes and 1.1 % died of infectious causes. In conclusion piperacillin-tazobactam/gentamycin provides sufficient antibacterial coverage in children with cancer. β-lactam antibiotic drugs covered Gram-positive and negative bacteria with more than 80 %. No concerning emergence of MRSA and ESBL-producing bacteria was observed. Continued surveillance should guide future empiric antibiotic treatment.

Keywords: Keywords: Childhood cancer, Infection, Bacteraemia, Susceptibility, Antibiotic treatment.

Authors: Lotte Vinther Kiefer1, Mette Møller Handrup1, Anne Mette Hvass2, Kurt Fuursted2, Henrik Schrøder1 1Department of Paediatrics, Aarhus University Hospital Skejby, Denmark, 2Department of Medical Microbiology, Aarhus University Hospital Skejby, Denmark
**P18. Differentiation of putative gonadal cells from male human embryonic stem cell lines in vitro.**

*Jan-Bernd Stukenborg, Karolinska Institute, Department Women s and Children s Health, Sweden*

Objective: The establishment of a functional in vitro system to study human spermatogenesis from primordial germ cells (PGCs) in the fetal up to mature sperm in the postnatal testis has been the topic of many studies. Due to the limited access to early human testicular material, most of the knowledge has been gained from animal studies, mainly rodent. Thus, studies on human pluripotent stem cells seem to be the best strategies available to study early human gonadal development. The knowledge obtained by these studies is needed for the establishment of a robust in vitro culture model for male germ cells differentiation to address the late side effects of infertility.

Material and Methods: Three undifferentiated male hESC lines (HS207, HS360 and HS401) were cultured on hFFs and as spheres in suspension without supporting feeder layer. Cells were cultured at 37°C and 5% CO2 for 21 days before stimulation with BMP7 and/or hCG and rFSH. The analysis of cell differentiation was done by using morphologically, immunohistochemically and molecular biological techniques (qPCR and gene expression analysis). Results: In addition to cell morphologies similar to those of early germ and immature somatic cells, the spheres showed also organization patterns of putative germ and somatic cells reminding of those found in pre-pubertal human testis. When analyzing the stimulated and un-stimulated cells with Q-PCR and immunohistochemical staining, we observed expression of specific markers for male germ and somatic cells at RNA and protein level after two weeks of differentiation. Conclusion: Here, we show that different hESC lines show different potentials for differentiation of towards putative male gonadal cells showing similar morphologies and gene and protein expression profiles similar to those for germ, Sertoli and Leydig cells. Therefore, the study provides an ideal platform for ongoing experiments to investigate specific cellular mechanisms in early human gonadal development.

**Keywords:** Testis, fertility preservation, gonadal dysfunction, stem cells, male germ cells

**Authors:** Kristín Rós Kjartansdóttir1,2,3, Ahmed Reda3, Kelly Day1, Olle Söder3, Outi Hovatta1, Jan-Bernd Stukenborg3 Presenting Author: Jan-Bernd Stukenborg
P19. Medium related effects on rat testicular cell survival and testosterone production in soft agarose culture systems.

Jan-Bernd Stukenborg, Karolinska Institute, Department of Women’s and Children’s Health, Sweden

Objectives: The late side effects affecting future fertility in children suffering gonad toxic treatments do still exist and so far, no treatment can be offered to rescue fertility in those patients, in particular due to the lack of relevant experimental models. Recently, a 3D cell culture system (SACS) was established for murine male germ cell differentiation. However, the overall efficiency of this system to create mature sperm still needs to be improved, in respect to establish a robust system for in vitro spermatogenesis. Therefore, this study aims to optimize the SACS for male germ cell differentiation in vitro. Methods: Single cell suspensions of pre-pubertal rat testes were cultured in six different culture media, with or without gonadotropin stimulation in SACS. Testosterone production was evaluated by radio immunoassay and apoptosis rates were evaluated by TUNEL assay after 1, 7 and 14 days of culture in the different media. qPCR was performed to evaluate the effects on two important makers for steroid genesis. Results: The results showed a positive effect of gonadotropins on colony formation and overall cell survival. DMEM + glutamine medium showed the highest testosterone production. DMEM + Glutamax had a better profile regarding cell survival. qPCR data revealed an up-regulation of Tspo (translocator protein) in case of the DMEM + glutamine medium. Star (steroid genic acute regulatory protein) expression was not clearly related to the superiority of DMEM + glutamine in testosterone production. Conclusions: The combination of gonadotropin stimulation and DMEM + Glutamax or DMEM + glutamine culture medium has a positive effect on overall testicular cell viability and Leydig cell functionality in vitro. The higher testosterone production might be due to the up-regulation of Tspo, which is important for steroid genesis. Therefore, we recommend DMEM + glutamine or Glutamax to be used for further studies of in vitro male germ cell differentiation.

Keywords: testis, late effects, in vitro spermatogenesis, fertility, germ cells

Authors: Ahmed Reda, Mi Hou, Luise Landreh, Kristín Rós Kjartansdóttir, Konstatin Svechnikov, Olle Söder, Jan-Bernd Stukenborg Presenting Author: Jan-Bernd Stukenborg
P20. Invasive fungal infections in children with acute lymphoblastic leukemia treated according to the NOPHO ALL-2008 protocol.

Goda Vaitkeviciene, 1Centre of Pediatric Oncology and Hematology, Children’s Hospital, Affiliate of Vilnius University H, , Lithuania

Children undergoing chemotherapy for acute lymphoblastic leukemia (ALL) are at an increased risk of developing an invasive fungal infection (IFI). Aim: to explore the incidence, pattern and outcome of IFI among pediatric patients treated for ALL. Patients and methods: retrospective analysis of clinical and laboratory data as well as treatment strategy for children with ALL aged 1.0-17.9 years and treated at Vilnius University Children Hospital, Department of Oncology and Hematology according to NOPHO ALL-2008 protocol from April 2009 to December 2012 was performed. IFI after stem cell transplantation were not analyzed. IFI were classified as proven, probable or possible. Results: 77 patients were treated for ALL during the study period. Proven IFI was diagnosed for 9% of patients (N=7). Patients with non-high risk induction with Prednisolone were more common (N=5) compared to high-risk induction with Dexamethasone (N=2). Candidaemia was detected in all cases except one (C. crusei, N=2; C. glabrata, N=1; C. laurentii, N=1; C. tropicalis, N=1). IFI developed during induction (N=2) or during consolidation phase (N=4). Probable or possible IFI was diagnosed to 11 patients (14%) based on Aspergillus or Candida antigens detected in peripheral blood (N=10) or bronchoalveolar lavage (N=1), combined with clinical data. Out of these eleven patients four patients had received high-risk and seven patients non-high risk inductions. IFI was the direct cause of death for one patient (combined C. crusei and Geotrichum tropicalis blood culture). Two patients underwent lobectomy as part of IFI treatment. One patient of the latter two died because of progress of the relapsed ALL. Conclusions: IFI in children with ALL is a common complication. However, diagnosis of IFI is often difficult and hyper diagnostics as well as hypo diagnostics is possible leading to over- or under treatment of the patients. Improved guidelines for prophylaxis and treatment of fungal infections are needed at our department.

Keywords: Key words: invasive fungal infection, acute lymphoblastic leukemia, children
Authors: Giedre Rydolyte, Grazina Kleinotiene, Goda Vaitkeviciene

Päivi Lähteenmäki, Turku University Hospital, Department of Pediatrics, Finland

In a nationwide, registry-based study, we explored the risk of mental health disorders i Y 5 yrs after cancer diagnosis in childhood (0-19 yrs, N= 4699) and young adulthood (20-34 yrs, N= 9950) compared with siblings. Results: Long-term cancer survivors had significantly increased risk for organic memory/personality disorders in both age groups. In childhood cancer survivors, risk for mental retardation and learning and developmental problems was significantly elevated. Female childhood cancer survivors had significantly increased risk for depression, psychotic disorders, anxiety/neurotic disorders, somatization/eating disorders, behavioral disorders, and personality disorders. Female survivors of young adulthood cancers had significantly increased risk for anxiety and neurotic disorders. When the risk for mental health disorders was analyzed by original cancer diagnoses, survivors of childhood ALL had higher risk than siblings for depression (HR 2.5) and learning/developmental problems (HR 5.0). Irradiation treatment did not explain the finding. In survivors of brain tumors, the risk for organic memory/ personality disorders (HR 21.5, after childhood ca; HR 10.9 after young adulthood ca), psychotic disorders (HR 1.8, and HR 3.1), and depression (HR 3.8, and HR 1.8) was significantly elevated. Significant effect of irradiation was seen only in the risk for organic memory/personality disorders. Survivors of soft tissue sarcomas had increased risk for depression both in childhood (HR 3.0) and adulthood (HR 1.9) ca survivors as well as for somatization/eating disorders (HR 6.3, and HR 3.4). The risk for alcohol and drug abuse was significantly increased among irradiated survivors of testicular cancer (HR 2.6). Conclusion: Significant amount of mental health problems was detected especially after childhood cancer. Morbidity was only partly associated with irradiation. Additional research on chemotherapy-only protocols and their impact on survivor’s mental health is a future task. Allocation of resources for systematic psychosocial care in the follow-up of long-term early onset cancer survivors is important.

Keywords: adolescent, cancer, child, mental health, survivor

Authors: Ritva Ahomäki, Erika Gunn, Nea Malila, Laura-Maria Madanat-Harjuoja, Jaakko Matomäki and Päivi M. Lähteenmäki
NOPHO 32nd Annual meeting, 2014 Bergen
9th - 13th May  NOPHO-NOBOS

Scientific themes:
MDS/aplastic anemias
Bone sarcomas
Infections

Venue
Radisson Blu Royal Hotel
Bryggen
N-5835 Bergen
Norway
http://www.radissonblu.com/royalhotel-bergen
We hope you enjoyed your stay and that you had a “hyggelig” time 😊

Farewell Greetings from Copenhagen
Thank you for attending NOPHO Annual Meeting 2013 and thank you for visiting Wonderful Copenhagen

The Childhood Cancer Unit of Rigshospitalet

Hope to see you again 😊