SIOPEN

Newsletter #11

October 2009

Edited by Sara Calmanti
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In this issue

Editorial

Dear colleagues and friends,

It is my pleasure to look on the growing prosperity of SIOPEN. There have been a number of big achievements over the last period.
In spring and at the ASCO meeting 2009 we learnt about a major break through of immunotherapy when the COG was able to stop accrual into the immunotherapy arm in the COG Study ANBL0032 for early significance. This also had major implications for our group as we felt the need to adapt the immunotherapy arm (R2-randomisation) of our current HR-NBL1/SIOPEN study. After intensive discussions and meetings the executive committee approved the change towards a new SIOPEN immunotherapy strategy:

The ch14.18 antibody will be given to all patients who are registered upfront on the high risk study and receive HDT/SCT. The randomised question will be the use of subcutaneous Interleukin 2 according to the previous favourable SIOPEN experience. The protocol has been amended accordingly and has already achieved ethical approval in Austria. Together with the IMPD of the antibody ch.14.18 this should allow all national coordinators to approve the study in their countries and thus making the antibody treatment accessible for high risk patients in their countries. We have indeed come a long way! We are currently the only organisation in Europe with access to the antibody having it developed over the last 8 years. This is the time to acknowledge again the major funding efforts in 2001 and the fantastic support of Polymun being such a cooperative and generous partner throughout this project when undertaking the recloning and starting a new GMP production venture after a production failure elsewhere.

We are also seeking opportunities to make this treatment accessible to patients in relapse. We can welcome a new partner in the SIOPEN group coming in with a new study. A new SIOPEN immunotherapy package has been made available with help of the colleagues from Tübingen: Rupert Handgretinger and Peter Lang. After their first contact with our group a year ago and participation to the SIOPEN meeting in Paris they have achieved to write the study protocol on haploidentical stem cell transplantation involving mIBG pre-treatment, an NK cell enriched haploidentical stem cell graft followed by immunotherapy consisting of the ch14.18 antibody and IL2. This new study protocol has been submitted to the University of Tübingen being the Sponsor for this study. It also is already submitted in Austria and will be open for SIOPEN centres with experience in haploidentical stem cell transplantation.

After long and thoughtful discussions the new low and intermediate risk study LINES is nearing completion. The core of the writing study committee in particular Gudrun Schleiermacher and Kate Wheeler have made major efforts in coordinating the finalisation of scientific content and structures needed. A good wind has brought Alisa Alspach from New Zealand to work at the S²IRP in Vienna thus enabling us to have a very strong and critic proofreading by a native speaker being also very experienced with study design according to her former role at the study centre with Rob Corbett in New Zealand. Alisa has been helping with the finalisation of the LINES protocol. The sponsor role will be taken on by the Hospital Universitario Infantil La Fe, Valencia, Spain.

Our OMS protocol equally has reached the final stage of editing and is nearly ready to be submitted to ethics with Institute Curie prepared to take on the sponsor role.

After SIOPEN has become a legal entity through forming an association, we are happy to announce that our membership assignment has worked out very well through the data bank system. So far 90 members have signed up and have been approved on the national and executive committee level. Printing of membership cards has kindly been taken on by the Parent HAYIM Association in Israel. We also wish to thank Claudia Zeiner for her investment in setting up the membership platform and to handle all the queries.

The support of our fundraising efforts have been taken on by our Treasurer, Isaac Yaniv, who also has invested an extra visit to Vienna to coordinate financial affairs with the administrative head of the CCRI, Claudia Hochweis, MBA, who has supported the setup of the financial platform with an bank account allowing English language. Fundraising in the respective countries is ongoing to raise the agreed upon resources. All those already having settled their payment are given a big thank you. Funds will be used for the minimum maintenances necessary for our IT platform and to set up the new IT environment of our new studies. Not to forget we are grateful to the continuous support of our IT partner who had changed name from ‘Seibersdorf’, but neither identity nor commitment, to AIT (Austrian Information Technologies).

SIOP Europe currently undertakes an effort to hand in a project under the FP7 top create a network of excellence. Neuroblastoma studies are foreseen to be part of this application, in particular LINES, AYA and our MIBG scoring/imagining tasks.

Our international cooperation's are well on their way. A number of publications have resulted from the INRG task group and SIOPEN members are well presented after the leading efforts of Andy Pearson. We thank and congratulate in particular Peter and Inge Ambros, Klaus Beiske, Sue Burchill, Tom Monclair, Maya Beck-Popovic, Joelle Vermoellen, Günter Schreier and Andy Pearson for engagement in these important publications (See last page of this issue for the references).

We are glad to be able to have a number of our COG colleagues and friends at the AGM meeting in Rome which promises to be a scientifically exciting one helping the group to flourish further and enhancing thus our international cooperation. In particular we are proud to have Alice Yu with us and congratulate her on years of stringent believe and investment in immunotherapy in neuroblastoma. Special thanks go to the university in Rome having provided travel funds as well as to Adam's Hat for their continuous support of the AYA cooperative study developments.

Special thanks go also to Susan Hay for not having lost trust in us and helping again to revitalise the CONE group. We welcome engaged charities and parent associations and thank them in advance for their willingness to join us in Rome. We feel together we will grow stronger and in view of European regulation as well as specific neuroblastoma issues we believe we need their advocacy for affected children and parents also on a European level. In particular, we do have a common need in Europe to provide access to immunotherapy to first line and relapse patients in safe study environments and all support to facilitate this, helps us to give our children a better outlook to overcome their disease.

Special thanks go to all our active group members even if not named expressively here, for their continuous support and belief in clinical and translational research for neuroblastoma! We know that without the numerous daily efforts at local investigating sites we could not create such an identity as SIOPEN spanning over 20 member countries currently.

I look forward to cordially welcoming all of you in Rome and we are grateful to the organisers in particular Alessandro Jenkner and his colleagues, especially Prof. Dr. Alberto Donfrancesco, for hosting us at the Ospedale Bambino Gesù IRCCS (children's hospital) and organising the meeting as well as to the Italian "Associazione Italiana per la Lotta al Neuroblastoma" for their support in organising the meeting as well as for the support of Pierre Fabre.

Focus on…

How to become a member of the SIOPEN Association?

In spring 2009 we formed the SIOPEN Association. Hence, this provides the possibility to create a common user list for our ongoing and future work.
We are happy to inform you that the website for the online registration to become a member of the SIOPEN Association is available at: http://membership.siopen-r-net.org/

If you are interested in becoming a SIOPEN member, we kindly ask you to create your user account by completing the SIOPEN CV in order to become a member of the SIOPEN Association.
When you register as member of the SIOPEN Association, you will be able to:
- access information relevant to neuroblastoma research
- announcements of SIOPEN meetings
- access specific member areas according to your role within SIOPEN
- receive the SIOPEN newsletter
- update your contact details

We cordially invite you to create your user account at your earliest convenience.

Kind regards
Ruth Ladenstein, president of the SIOPEN Association

SIOPEN – Association to foster Neuroblastoma Research

SIOPEN – Verein zur Förderung der Neuroblastomforschung

(SIOP Europe Neuroblastoma Group)

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Registration number (Central Register of Associations, Austria)
ZVR 396592912

Telephone + 43 1 40470 4750
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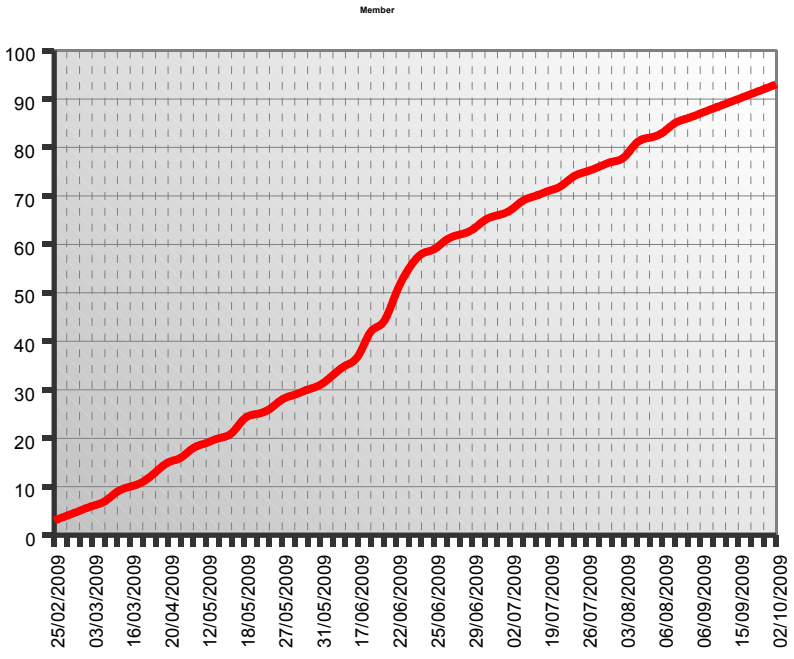
President:
Assoc. Prof. Ruth Ladenstein, MD, MBA, cPM ruth.ladenstein@ccri.at

Treasurer:
Dr. Isaac Yaniv
iyaniv@clalit.org.il

SIOPEN membership online registration - update as per 30th September 2009

Membership type	Member count
Associate member	14
Corporate member	1
Founding member	21
New Ordinary Member	55

Country	Member count
Australia	2
Austria	5
Belgium	3
Czech republic	2
Denmark	3
Finland	1
France	8
Germany	1
Greece	6
Hungary	1
Ireland	1
Israel	8
Italy	9
Norway	6
Poland	2
Portugal	3
Serbia	1
Slovakia	1
Spain	16
Switzerland	1
Turkey	1
UK	10
Total number	**91**



SIOPEN
Annual General Meeting
October 26-28, 2009 – Rome, Italy

By the Local Organising Committee

Dear Colleagues,

We are pleased to invite you to the SIOPEN Annual General Meeting that will take place in Rome, the Eternal City, October 26th-28th.

Meeting venue of the three-day conference will be Bambino Gesù Children's Hospital, celebrating this year its 140th anniversary. The hospital is within walking distance from most of the listed hotels, accommodation at the first three (in red) is at a special conference rate.

All meetings and conference activities, including coffee and lunch breaks, will take place at the third floor of Padiglione Salviati. Participants are cordially invited to the official dinner on Tuesday, October 27th.

Enclosed please find a registration form (please reply by fax or e-mail), hotel list and area maps. This information, as well as the final meeting agenda, can also be found on the SIOPEN-R-Net website, at the following address: https://www.siopen-r-net.org/

Contact addresses are as follows:

Secretariat:

Dr. Paola Volpi
Phone: +39-06-6859 2290 or +39-06-6859 2294
Fax: + 39-06 6859 2443
E-mail: congressi@opbg.net

We look forward to meeting you in Rome this autumn!

Best regards from the Neuroblastoma Team,

Alessandro Jenkner, MD, Aurora Castellano, MD, and Alberto Donfrancesco, MD

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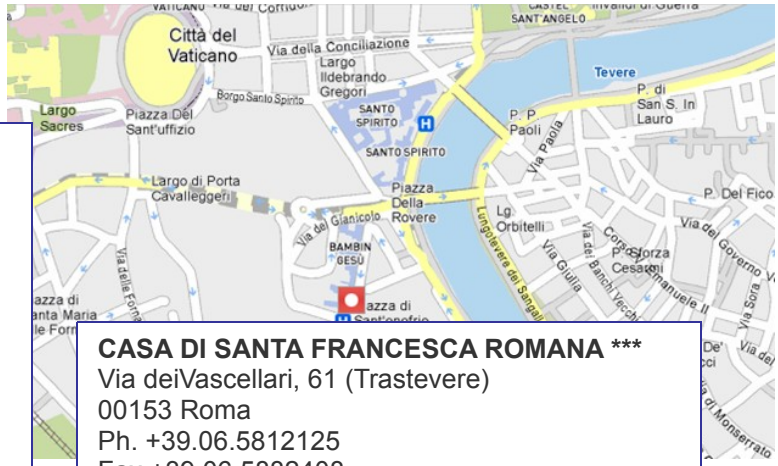
Where to overnight …



GRAND HOTEL DEL GIANICOLO ****
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E-mail: info@grandhotelgianicolo.it
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Fax +39.06.632359
http://www.starhotels.com

HOTEL LA ROVERE ***
Vicolo S. Onofrio, 4
00165 Roma
Ph. +39.06.68806739
Fax +39.06.68807062
www.hotellarovere.com

The first three hotels in the list offer special rates for participants in OPBG events, please mention when making your reservation

HOTEL COLUMBUS ******
Via della Conciliazione, 33
00193 Roma
Ph. +39.06.6865435
Fax: +39.06. 6864874
E-mail info@hotelcolumbus.net
www.hotelcolumbus.net

CROWNE PLAZA ROME ST. PETER'S ****
Via Aurelia Antica, 415
00165 Roma
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E-mail stpeters@hotel-invest.com
www.crowneplaza.com/rome-stpeters

CASA DI SANTA FRANCESCA ROMANA ***
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www.sfromana.it

HOTEL SAN PIETRO **
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PADRI TRINITARI *
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00165 Roma
Ph. +39.06.39367632
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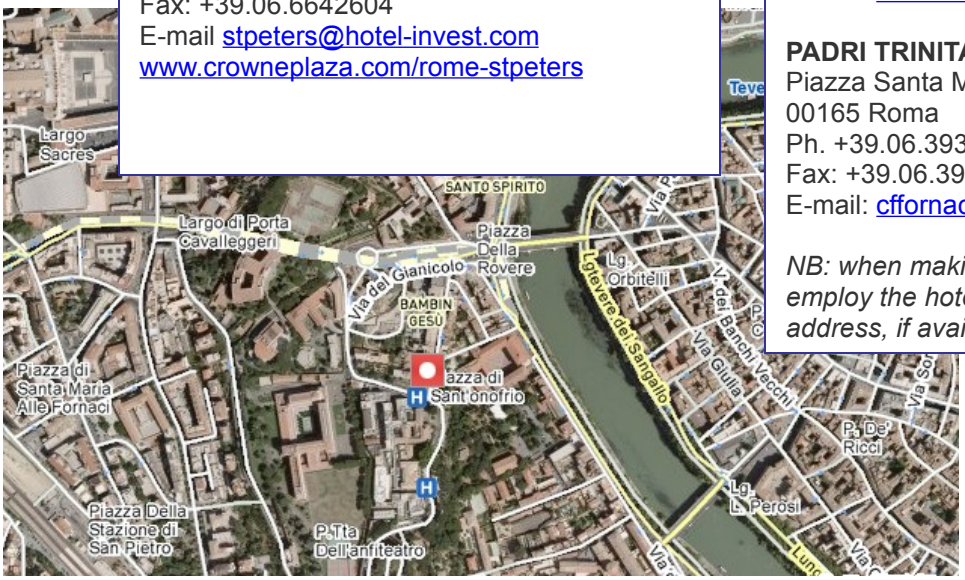
E-mail: cffornaci@tin.it

NB: when making your reservation, please employ the hotel website instead of the e-mail address, if available.



PROGRAMME of the

SIOPEN Annual General Meeting

26th – 28th October 2009, ROME

Bambino Gesù Children's Hospital, Padiglione Salviati, third floor
Ospedale Bambino Gesù IRCCS
Piazza S. Onofrio, 4,
00165 Roma, Italia

Sunday 25th October 2009 16:00 – 19:00

FP7 Pre Meeting

invited
SIOPEN Executive Committee Members, Clinical Trial Chairs,
Representatives of the SIOPEN Biology Committee, Image Group and Pharmacology and New Agents Committee

Venue:
Auletta Salviati or Aula Salviati, Padiglione Salviati, third floor
Ospedale Bambino Gesù IRCCS

Monday 26th October 2009
WORKING MEETINGS FOR SIOPEN COMMITTEES

8:00 – 10:00	Executive Committee Meeting	Executive Committee Members Advisory board members
8:00 – 10:00	Radiotherapy Committee Meeting	Mark Gaze

Coffee break 10:00 – 10:30

Clinical Trial Committee Meetings
PARALLEL sessions to specialty committees

9:00 – 10.00	LNESG 2	Maja Beck-Popovic
10:30 – 13:00	HR-NBL-1	Ruth Ladenstein
	– Study update	Ulrike Pötschger
	– AntiGD2 – Amendment	Holger Lode
	– IMPD and Ethical Committee Submissions	Update from NCs
	– Surgical Report	Keith Holmes
	– Updated results from the TVD Study	Alberto Garaventa

Lunch 13:00 – 14:00

14:00 – 17:00	AYA Study Committee Working Meeting	Isaac Yaniv SIOPEN AYA Committee members & COG committee members (John Maris, Yael Mosse, Alice Yu, Kate Matthay)
14:00 – 17:00	LINES Closed Writing Committee Meeting	Adela Canete Andrea di Cataldo Vassilios Papadakis Gudrun Schleiermacher Kate Wheeler Jean Michon
15:00 – 16:00	EUNB	Jan Kohler
17:00 – 18.30	Joint meeting of New Drug Development Group & ITCC Representatives & Immunotherapy Committee & Pharmacology Committee Topic : Achievements and future plans Update ITCC frontline research and testing for neuroblastoma	Andy Pearson Gilles Vassal Gareth Veal Holger Lode COG members invited

Specialty Committee Meetings

PARALLEL sessions to clinical trial meetings

14:00 – 16:00	Statistical Committee Meeting	Ulrike Pötschger and members
12 :00 – 13 :00	Joint meeting & Radiology SC & Radiotherapy SC & Surgery SC & Nuc Med C	Mark Gaze Marcus Hörmann Keith Holmes Nuc Med representative
16:00 – 17:00	INRG response criteria, joint meeting with Bone Marrow SC, Nuclear Medicine SC, Radiology SC, Biology SC	Dominique Valteau-Couanet Alberto Garaventa Klaus Beiske Mark Gaze Peter Ambros COG members invited

19:00 SIOPEN AGM Welcome Reception

Tuesday 27th October 2009
SIOPEN Annual General Meeting

TOPIC 1 Basic Science and New Therapies

8:00 – 10:00	**Chairs** **Per Kogner & Vito Pistoia**	
20’	AURORA Kineases and application in clinics	John Maris
15’	ALK research: role of ALK activation in neuroblastoma and ALK inhibitor TAE684	Gilles Vassal
15’	ALK mutation and expression in neuroblastoma	Gianpaolo Tonini
15’	Update on ALK and COG Phase I	Yael Mosse
15’	Molecular Profiling in Neuroblastoma	Angelika Eggert
15’	Bio Banking Experience & Strategies	Luigi Varesio, Geneviève Laureys

Coffee break 10:00- 10.30

10:30 – 12.00	**CHAIRS** **John Maris & Peter Ambros**	
20’	Ganglioside metabolism, the tumour microenvironment, and tumour progression. Implications for neuroblastoma	Stephan Ladisch
15’	MicroRNA involvement in the pathogenesis of neuroblastoma: potential for microRNA mediated therapeutics	Raymond Stallings
15’	Novel Phage Display-Derived Peptides for Tumour- and Vascular-Targeted Therapies against Neuroblastoma	Fabio Pastorino
15’	IGFR1 antibody, mTor And VEGF TKI inhibitors activity in Neuroblastoma Tumor Initiating Cells: a model for preclinical drug development	Sylvain Baruchel

TOPIC 2 Immunotherapies

12:00- 13.00	**Chairs** **Kate Matthay & Jean Michon**	
30’	Overview on antibody developments in USA Results of the COG ch14.18 randomised trial	Alice Yu
15’	Antibody based immunotherapy of neuroblastoma: Ganglidiomab, a novel anti-idiotype of the ch14.18 antid-GD_2 antibody family	Holger Lode
10’	Summary of Italy IL2 results	Arcangelo Prete

Lunch 13:00 – 14:00

14:00 – 16:00	Meeting with Charities and Parents	Susan Hay Members of the CONE group Executive Committee, SIOPEN National Deputies and Study and Committee chairs
14 :00 – 16 :00	Molecular Monitoring Group	Sue Burchill
16:00 – 19:00	SIOPEN BOARD MEETING	CLOSED MEETING for board members only: NCs, clinical trial chairs, Specialty Committee chairs and Executive Committee members and Advisory Board members

20:00 SIOPEN AGM Official Dinner

Wednesday 28th October 2009

TOPIC 3 mIBG & allogeneic approaches

8:00 -10:30	**Chairs** **Holger Lode & Alberto Garaventa**	
15’	International mIBG treatment: European results	Val Lewington
20’	Optimising neuroblastoma assessment and targeted radiotherapy with MIBG	Kate Matthay
15’	Immunogenicity of neuroblastoma - the insight from an experimental model	Shifra Ash
15’	A Multicenter Pilot Study of Reduced Intensity (RIC) Allogeneic Hematopoietic Stem Cell Transplantation with in-vivo T-cell pepletion to evaluate the role of NK cells and KIR mis-matches in relapsed or refractory high-risk Neuroblastoma	Sandeep Soni
15’	Haploidentical SCT for high risk NBL combined with immunotherapy A SIOPEN study	Peter Lang
15’	Allogeneic SCT for high risk NBL: Italy experience & proposal	Edoardo Lanino
30’	Discussion on allo approaches	

Coffee break 10:15 – 10:45

TOPIC 4 SIOPEN & COG Study Reports

10:45- 13:00	**Chairs** **Andy Pearson & Bruno De Bernardi**	
30’	Overview on ongoing COG Phase II-III trials and future INRG based strategies	John Maris
15’	New AYA Study	Isaac Yaniv
20’	LINES Study	Kate Wheeler, Andrea di Cataldo, Gudrun Schleiermacher, Adela Cañete

15’	Updated results from the European Unresectable Study	Jan Kohler
15’	LNESG2 Update	Maja Beck
10’	HR-NBL-1/SIOPEN Update	Ruth Ladenstein
10’	TOTEM Phase II	Hervé Rubie

Lunch 13:00 – 14:00

TOPIC 5 Communications & Discussion

14:00 - 16:30	**Chairs** **Dominique Valteau-Couanet & Keith Holmes**	
15’	Report from the board	Ruth Ladenstein
15’	Report from the treasurer	Isaac Yaniv
15’	Report from the Charity Committees	Susan Hay
90’	Presentation and discussion of case studies all members invited	Bruno De Bernardi Keith Holmes
10’	Concluding remarks	Ruth Ladenstein
16:30	**CLOSE**	

Bio Banking Experience & Strategies

Luigi Varesio[1] and Claudio Gambini[2]
[1]Laboratory of Molecular Biology, Giannina Gaslini Institute; [2] Department of Pathology, Giannina Gaslini Institute

Efficient, effective and coordinated Biobanking is a prerequisite for collaboration, and progress in the functional genomics of Neuroblastoma and efforts are being made under ANR mandate and on behalf of the SIOPEN. The limited number of neuroblastoma specimens and the wealth of information that can be derived by integration of functional genomics and clinical research suggest that centralization is the key word for a rapid progress. The centralization of neuroblastoma specimens seems highly impractical and too difficult to organize in the near future and beyond the scope of an international collaboration. In contrast, there is a greater support to the idea of a virtual biobank. Its function is to share the data derived from the analysis of the specimens performed in reputable centers according to Standard Operating Procedures. Prerequisite to this initiative is the agreement on basic items such the Standard Operating Procedures, the platforms for genomic studies, the bioinformatic and computer support. There are several initiatives along this line of thought in Europe and in the world. The challenge will be to find the most efficient and rapid way to implement a virtual exchange for benefiting from a treasure of information hidden sealed chests. A brief outline of the Italia experience in centralizing, processing and data analysis framework will be presented.

NCH 08-00234: A Multicenter Pilot Study of Reduced Intensity (RIC) Allogeneic Hematopoietic Stem Cell Transplantation with in-vivo T-cell depletion to evaluate the role of NK cells and KIR mis-matches in relapsed or refractory high-risk Neuroblastoma (NCT # 00874315;NMDP IRB # 2008-0230).

Sandeep Soni, MD
Nationwide Children's Hospital Columbus, OH, USA; Sandeep.Soni@nationwidechildrens.org

Relapsed or refractory Neuroblastoma (NBL) carries a dismal prognosis. Patients, who relapse after an upfront intensive treatment regimen, have an overall 3 year survival rate of < 10%. No treatment with a 'curative intent' is available for these groups of patients. Allogeneic HSCT, as a form of adoptive cellular therapy, has a high degree of efficacy in a number of malignancies. Engraftment of donor immune cells (i.e. T cells, NK cells) mediates Graft versus Tumor (GVT) effect.
NBL represents an excellent target for NK cells, as NK cells don't need expression of co-stimulatory molecules and surface expression of HLA Class I antigens to mediate their cyto-toxic effects. Recently, there has been an improved understanding of the role of Killer-Immunoglobulin-like Receptors (KIR) in regulating NK cell function in association with specific donor-recipient incompatibility in the Class I HLA alleles. KIR mis-matches between donor-recipient pairs during HSCT can activate NK cell to mediate GVT effects. Since, NK cell activation is critical to mediate anti-NBL effect, KIR mis-matches between donor-recipient pairs may be important to activate NK cells during HCST.
This Phase II pilot study is designed to confirm the feasibility of a RIC regimen (Fludarabine, Busulfan and rabbit ATG) for allogeneic HSCT for relapsed or refractory NBL patients. Toxicity, engraftment and incidence of severe GVHD will be monitored as statistical end-points. Class 1 mismatch donors will be preferred for the HSCT and the KIR mis-matches in the donor-recipient pairs will be analyzed, utilizing the 'receptor-ligand' model. The reconstituted donor NK cells repertoire will be followed by both the genotyping and phenotyping methods at multiple time-points post-transplant to understand the NK cell reconstitution and allo-reactivity against NBL. The cytotoxic effect of reconstituting donor NK cells on a panel of NBL cell lines will be studied to confirm the GVT effect.
The major aim of this pilot study is to generate preliminary clinical and laboratory data to confirm the feasibility of a RIC allogeneic HCST for heavily pre-treated NBL patients and elucidate the role of NK cells and KIR mis-matches in mediating the GVT effects against NBL.

Ganglioside metabolism, the tumor microenvironment, and tumor progression--Implications for Neuroblastoma

Stephan Ladisch

Children's National Medical Center, 111 Michigan Ave. NW, Washington, DC, 20010, USA
sladisch@cnmc.org

To attempt to better understand the pathogenesis of cancer, including human neuroblastoma, increasing attention has been directed to identifying interactions between the tumor cell and the surrounding in vivo tumor microenvironment. Certain key tumor cell membrane molecules, gangliosides, influence these tumor-host interactions. We hypothesized that by their synthesis and subsequent shedding, tumor cell gangliosides enhance tumor development and progression in vivo. Characteristically rapid tumor cell ganglioside synthesis and substantial shedding into the tumor microenvironment––which in humans was first observed in neuroblastoma and correlated with a poorer prognosis––results in transfer to surrounding normal cells and alters their function. Basic cellular mechanisms of this process in the tumor microenvironment (e.g., inhibition of the immune response, enhancement of stromal cell proliferation, and enhancement of the angiogenic response) will be illustrated. To probe the effect on tumor formation itself, we most recently created a genetically stable and specific murine model of tumor cell ganglioside depletion. Selective and complete depletion of tumor cell gangliosides resulted in strikingly inhibited tumor growth, with implications for understanding neuroblastoma pathogenesis and for developing novel experimental therapeutic strategies.

CP751,871

Sylvain Baruchel

There is remarkable heterogeneity in neuroblastoma genotype. This genetic diversity makes the development of new targeted therapies particularly challenging. VEGFR Tyrosine kinase inhibitors, mTor inhibitors and human monoclonal antibody directed against the IGF1R, CP751,871, displayed potential antineoplastic activity in some adult solid tumors. In the present study, by investigating the drug response to monoclonal antibody against IGF-1R, CP751,871, and mTOR inhibitors, rapamycin and RAD001,and VEGFR TKI , we developed new strategies targeting mTOR and IGF-1R/PI3K/AKT pathways based on the diversity of NB genotypes. Both NB cell lines and tumor initiating cells (TICs) from patient tumor samples were used in our in vitro and in vivo models for drug testing. We demonstrated that only NB overexpressing IGF-1R are reactive to CP751,871 treatment, and this anti-tumor effect was due to the suppression of tumor angiogenesis. We also observed the synergistic effects of CP751,871 and vinblastine in those IGF-1R overexpressed NB cells the drug response to Tyrosine kinase inhibitors Sunitinib , CP751,871 and mTOR inhibitors based on diversity of NB genotypes. Both NB cell lines SKNEB2 SKNBCE2 and tumor initiating cells (TICs) from patient tumor samples were used in our in vitro and in vivo models for these drug testing. In this study, we demonstrated that NB overexpressing of IGF-1R and VEGFRs are more sensitive to CP751,871 treatment, and this anti-tumor effect was due to the suppression of tumor angiogenesis. We also observed the synergistic effects of CP751,871 and vinblastine in those IGF-1R overexpressed NB cells. In vitro, as a single agent, vinblastine significantly inhibited NB cell proliferation in a concentration dependent manner. With the presence of CP751,871, vinblastine showed more significant inhibition on NB cell proliferation with decreased IC50 values by 2 - 3.4 times. In xenograft tumor models, both CP751,871 and vinblastine showed good antitumor activity in both locaolized and metastatic. Combined treatment has more significant antitumor activity. Surprisingly, CP751,871 TICs resistant NB cells are sensitive to the mTOR inhibitors, rapamycin or RAD001. Our Western Blot shows that those cells have lower expression level of mTOR. On the contrary, mTOR overexpressed NB cells are resistant to rapamycin treatment. This study suggests that VEGFR TKI , CP751,871 and mTOR are effective drugs for NB, but all them is not effective to all NB cell line or TICs populations. In order to make the right choice in future phase II clinical trials and better individualize drug response, screening for expression level of VEGFR2 , IGF-1R and mTOR in patient's tumor 's cells is necessary. This could result in major advances in the treatment and cure of neuroblastoma.

Antibody based immunotherapy of neuroblastoma: Ganglidiomab, a novel anti-idiotype of the ch14.18 anti-GD_2 antibody family

Holger N. Lode
University of Greifswald, Center for Pediatric and Adolescent Medicine, Soldmannstrasse 15, 17475 Greifswald, Germany

Targeting GD_2 continues to emerge as a valuable concept for anti-neuroblastoma immunotherapy. The combination of antibody and cytokine demonstrated efficacy in high risk neuroblastom patients in combination with 13cis retinoic acid. Here we report a concept for using ch14.18 long term infusion in combination with subcutaneous interleukin-2, aiming at the reduction of toxicity associated with this kind of immunotherapy. Furthermore, we report for the first time the generation and in vitro characterization of ganglidiomab, novel anti-idiotypic antibody of the ch14.18 anti-GD_2 antibody family. This antibody is a successor molecule of 1A7; it is available for production and may be used for active immunization against neuroblastoma.

MiRNA Involvement in the Pathogenesis of Neuroblastoma: Prospects for miRNA Mediated Therapeutics

I Bray [1,2], N Foley[1,2], K Bryan[1,2], P Buckley[1,2], L Alcock[1,2], P Mestadagh[3], J Vandesompele[3], F Speleman[3], W. London[4], M O'Sullivan[2], A O' Meara[2], **RL Stallings**[1,2]

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MiRNAs regulate gene expression at a post-transcriptional level and their dysregulation can play major roles in the pathogenesis of many different forms of cancer, including neuroblastoma. We have analyzed a set of neuroblastoma (n=145) that is broadly representative of the genetic subtypes of this disease for miRNA expression (430 loci by stem-loop RT qPCR) and for DNA copy number alterations (array CGH) to assess miRNA involvement in disease pathogenesis. The tumors were stratified and then randomly split into a training set (n=96) and a validation set (n=49) for data analysis. Thirty-seven miRNAs were significantly over or under-expressed in *MYCN* amplified tumors relative to *MYCN* single copy tumors, indicating a potential role for the MYCN transcription factor in either the direct or indirect dysregulation of these loci. There was also a highly significant correlation between miRNA expression levels and DNA copy number, indicating a role for large-scale genomic imbalances in the dysregulation of miRNA expression. In order to directly assess whether miRNA expression was predictive of clinical outcome, we used the Random Forest classifier to identify miRNAs that were most significantly associated with poor overall patient survival and developed a 15 miRNA signature that was predictive of overall survival with 72.7% sensitivity and 86.5% specificity in the validation set of tumors. We conclude that there is widespread dysregulation of miRNA expression in neuroblastoma tumors caused by both over-expression of the *MYCN* transcription factor and by large-scale chromosomal imbalances that has a significant impact upon the clinical behaviour of these tumors. To further assess the biological relevance of our expression profiling results, we have carried out functional analyses of miR-184, a miRNA that is significantly under-expressed in MYCN amplified tumors. Ectopic expression of miR-184 at physiological levels in neuroblastoma cell lines reduces cell proliferation through the activation of a caspase mediated apoptotic pathway. Conversely, knock-down of endogenous miR-184 increases cell proliferation and invasive capability, particularly in cells derived from non-MYCN amplified tumors that have relatively higher levels of miR-184. As a mechanism of miR-184 biological effects, we have identified and experimentally validated an mRNA target of miR-184 with high cancer relevance.

Optimising neuroblastoma assessment and targeted radiotherapy with MIBG

Katherine K. Matthay, MD

Professor Pediatrics
Chief Pediatric hematology-Oncology
Mildred V. Strouss Professor of Translational Research UCSF School of Medicine and UCSF Children's Hospital

Neuroblastoma is a tumor of the sympathetic nervous system and the most common pediatric extracranial solid cancer. Nearly half of patients have high risk disease due to metastases or unfavorable biology, with less than 40% long term survival despite intensive chemotherapy followed by myeloablative therapy and treatment of minimal residual disease. MIBG is actively transported into neuroblastoma cells by the norepinephrine transporter. This tumor is characterized by metaiodobenzylguanidine (MIBG) avidity in 90% of cases, prompting the use of radiolabeled MIBG for staging and response, as well as targeted radiotherapy in these tumors. Clinical studies of 131I-MIBG in patients with relapsed or refractory neuroblastoma have identified myelosuppression as the main acute dose-limiting toxicity, necessitating stem cell reinfusion at higher doses. Most studies report a response rate of 30-40% with single agent 131I-MIBG in this population. More recent studies in COG and NANT focus on the use of 131I-MIBG in combination with chemotherapy, radiosensitizers or myeloablative regimens. Initial studies of no-carrier added MIBG suggest the possibility of a higher therapeutic ratio. We will review preliminary recommendations and results on staging and risk stratification using MIBG scans, and on recently completed, current and planned studies of MIBG for neuroblastoma in the USA. Future studies, based on combinations of MIBG with other molecularly targeted therapies or radiosensitizers may further optimize the role of this targeted radionuclide for high risk neuroblastoma.

Aurora Kinase A (AURKA) as a therapeutic target for high-risk neuroblastoma

John M. Maris, MD

The Children's Hospital of Philadelphia and The Children's Oncology Group (COG)

Tumor genomics and unbiased screens hold promise for identifying oncogenic drivers of human malignancies. AURKA was identified as potential molecular target for high-risk neuroblastoma and acute lymphoblastic leukemia via a screen of the selective AURKA inhibitor MLN8237 by the Pediatric Preclinical Testing Program (PPTP: http://pptp.stjude.org). In this screen, there was broad in vitro cytotoxicity, and potent anti-tumor activity with complete regression of established xenografts observed in 3/6 cell line models, with significant growth delay in the others. AURKA functions as a mitotic kinase that is essential for the regulation of the G2-M checkpoint. While the

gene is amplified and over-expressed in several human malignancies, the locus is not amplified in a survey of over 650 primary neuroblastomas, and mutations were not discovered in a screen of 188 tumors. Based on the PPTP data, a phase 1 trial was activated in the COG in September 2008 (ADVL0812; Dr. Yael Mosse Chair) and completed recently. Preliminary toxicity data will be reported, and the Phase 2 component of the study focused on neuroblastoma and leukemia is ongoing. Furthermore, Otto and colleagues recently reported that AURKA also has a kinase-independent function of directly binding the MYCN oncoprotein and sequestering it away from proteolytic degradation (Cancer Cell, 2009). While it is unlikely that this newly discovered function for AURKA fully explains the activity of the MLN8237 in the PPTP screen, it clearly adds additional momentum to develop AURKA inhibition strategies for neuroblastoma. The COG plans to test MLN8237 in the setting of primary refractory disease, and ongoing work is focused on discovering proper combination chemotherapy approaches for this orally bioavailable cytotoxic compound.

Role of ALK activation in neuroblastoma and ALK inhibition by TAE684

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Background: Activating mutations of the Anaplastic Lymphoma Kinase (ALK) receptor could be responsible for most familial neuroblastoma cases and for up to 15% of somatic cases. The objective of the present study was to further investigate the role of ALK activation in neuroblastoma.
Methods: Tissue microarrays were constructed containing 101 primary tumors and 56 paired normal tissues. Sections were immunostained with anti-ALK or anti-P-ALK antibodies, and with antibodies directed against the ALK ligands: PTN (Pleiotrophin) or MDK (Midkine). The Wilcoxon signed rank test was applied for comparison of paired data. Associations with prognostic factors were analyzed using Student's t-tests. Efficacy of the ALK inhibitor TAE684 (Novartis) was evaluated in wild-type or mutated ALK neuroblastoma cell lines and xenografts.
Results: ALK was expressed in about 100% of tumors and normal tissues, while phospho-ALK was expressed in 5% of normal tissues and 50% of tumors. Sequencing of the tyrosine-kinase domain of ALK showed that its phosphorylation was largely independent of the presence of mutations and we found that MDK and PTN ligands were expressed in 66% and 50% of tumors, respectively. Interestingly, ALK, P-ALK and MDK were expressed at higher levels in tumors as compared with paired normal tissues ($p<0.0001$), while PTN showed an inverse tendency, being more expressed in normal tissues ($p=0.07$). In tumors, P-ALK was associated with good-prognosis factors, including favorable stages ($p=0.0085$), absence of MYCN amplification ($p=0.05$) and a younger age at diagnosis ($p=0.0263$). Targeting of ALK activity using TAE684 was efficient in all neuroblastoma cell lines, regardless of ALK status. TAE684 efficiently reduced ALK phosphorylation in tumors but failed to demonstrate antitumor activity in advanced stage neuroblastoma xenografts expressing either a wild-type or a F1174L mutated ALK. On the other hand, tumor regressions were observed in R1275Q mutated ALK xenografts-bearing mice.
Conclusion: ALK activation occurs during neuroblastoma oncogenesis, along with a concomitant switch between the expressions of PTN and MDK. The potential role of ALK as a relevant therapeutic target remains to be assessed since its activation was correlated with good prognosis factors and in vivo inhibition by TAE684 was limited to a sub-type of mutated xenograft.

ALK as a therapeutic target for high-risk neuroblastoma

Yael P Mosse, MD

The Children's Hospital of Philadelphia and The Children's Oncology Group (COG)

Neuroblastoma remains a leading cause of childhood cancer deaths even with recent dramatic increases in treatment intensity. We recently discovered activating mutations within the tyrosine kinase domain (TKD) of the ALK oncogene as the major cause of hereditary neuroblastoma, and that somatically acquired mutations and gene amplification events often drive the malignant process in a substantial subset of high-risk tumors. We report a comprehensive survey of ALK genomic status in all neuroblastoma phenotypic subsets and perform preclinical validation that targeted inhibition of activated ALK kinase is an effective therapeutic strategy. ALK mutations were detected in 8% of cases, were restricted to the tyrosine kinase domain and occured equally in all patient subsets. The most common germline mutation in familial cases (R1275Q) was also the most common somatic mutation (49% of tumors with a mutation). High-level amplification of ALK was detected in 2.4% of tumors and was associated with differentially increased ALK mRNA expression. ALK amplification is strongly associated with the high-risk subset ($P<0.001$) and MYCN amplification ($P<0.001$). Pharmacologic inhibition of ALK kinase activity with PF-02341066, a dual c-Met/ALK inhibitor, demonstrated in vitro cytotoxicity in an

ALK genomic status dependant manner. Cytotoxicity correlated with abrogation of constitutively activated ALK phosphorylation. Likewise, oral dosing of PF-02341066 in immunocompromised mice xenotransplanted with human neuroblastoma-derived cell lines resulted in differential anti-tumor activity in a genomic status-dependant manner. Xenografts harboring the R1275Q mutation showing complete regression, while those harboring an F1174L mutation showed significant tumor growth delay, and xenografts with wild-type ALK showed no response. These results suggest that ALK inhibition is a tractable therapeutic target in a subset of neuroblastomas, and provides the impetus for clinical development of ALK inhibition strategies.

Immunogenicity of Neuroblastoma – insights from experimental models

Shifra Ash, Isaac Yaniv

Continuous efforts are dedicated to develop immunotherapeutic approaches to neuroblastoma (NB), as the tumor relapses following chemotherapy and autologous bone marrow transplantation (BMT). Relapse is attributed to residual tumor cells resistant to radio-chemotherapy, which can in principle be attacked on an immunological basis.
Unlike the prevalent dogmas about Neuro-2a, the prevalent model of murine NB, we found: a) Neuro-2a cells express MHC antigens; b) Neuro-2a cells elicit cytolytic reactions as potent as immunogenic lymphoblastoma cell lines. Allogeneic BMT was found to reduce significantly NB growth rates in vivo as compared to syngeneic transplants. To evaluate the significance of MHC mismatch, we have performed a series of experiments and found: 1. T cell depleted donor BMC are as potent in inhibiting tumor growth as whole BMC transplanttion. In addition, cytolytic activity was proven to originate from bone marrow-derived lymphocytes, which are tolerant to host and tumor MHC. These data indicate that GVT and GVH reactivity are dissociated. 2. After haploidentical transplants, F1 lymphocytes anergic to host MHC are reactive against the tumor, suggesting that tumor associated antigens are the prime targets of GVT reactivity. 3. Anti-tumor immune reactivity is fostered by MHC disparity and by donor-type tumor-pulsed dendritic cells, indicating a likely role of antigen-presenting cells in initiation of GVT effects. 4. Transition to adaptive immunity by BMT is favorable to induction of GVT reactivity. Taken together, these data indicate provide evidence that anti-neuroblastoma reactivity by allogeneic and semi-allogeneic BMT is feasible and serves a good platform for development of effective immunotherapy.

Novel phage display-derived peptides for tumor and vascular targeted therapies against neuroblastoma

Fabio Pastorino[1,10], Monica Loi[1], Serena Marchiò[2], Daniela Di Paolo[1], Pamela Becherini[12], Flavio Curnis[3], Renato Longhi4[4], Beatrice Nico[5], Federico Bussolino[2], Domenico Ribatti[5], Michele Cilli[6], Claudio Gambini[7], Wadih Arap[8], Renata Pasqualini[8], Angelo Corti[3], Theresa M. Allen[9] and Mirco Ponzoni[1]
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Disseminated neuroblastoma (NB) is refractory to most current therapeutic regimens. Previously, we showed that the therapeutic index of anticancer drugs is increased by liposome encapsulation and further improvements have been obtained by coupling tumor-specific ligands to the surface of the lipidic envelop. Phage display technology was used as a powerful tool in discovering novel ligands specific to receptors on the surface of tumor epithelial and endothelial cells. The targeting of therapeutics to tumor blood vessels, using probes that bind to specific molecular addresses in the vasculature, combines blood vessel destruction with the expected anti-tumor activities of the drug, resulting in increased efficacy and reduced toxicity. Recently, we demonstrated that doxorubicin (DXR)-entrapped liposomes, targeting the endothelial tumor cell marker aminopeptidase N (APN), displayed enhanced anti-tumor effects and prolonged survival in NB-bearing mice. In this work, in vivo selection of phage display libraries was used to isolate peptides binding specifically to the tumor blood vessel address aminopeptidase A (APA), expressed on perivascular tumor cells. APA-targeted, liposomal DXR, displayed in vitro specific binding to APA-transfected cells. After having demonstrated by immunohistochemical (IHC) analyses the APA positiveness of cells within the vascular wall of orthotopically implanted NB tumors in mice, the novel APA-targeted formulation was validated for its anti-tumor effect in clinically relevant animal models of human NB. In these mice, the results indicate that APA-targeted liposomal DXR formulation led to an increase in life span compared to control mice, but less than that obtained by using APN-targeted formulation. These results were confirmed by IHC analyses performed in paraffin-embedded tumors derived from mice after 3 and 5 weeks of treatment, which revealed

the highest increase of TUNEL-positive tumor cells in mice treated with the formulation directed to APN. However, combined experiments using APN- and APA-targeted liposomes administered in a sequential manner following the same time schedule at half the dose of DXR for each formulation, led to a significant increase in life span compared to each treatment administered separately. TUNEL assay demonstrated statistically significant increased level of apoptosis in tumor of mice treated with the combination therapy and a pronounced destruction of the tumor vasculature with an almost total ablation of endothelial cells and pericytes.
Moreover, to find more specific NB-targeting moieties, we established a protocol for the isolation of heterogeneous cell populations by tissue fractionation of primary tumors and metastases from orthotopic NB-bearing mice and NB patient (Stage IV) specimens. Single cell suspensions constituted mainly by cancer cells, tumour endothelial cells, and ematopoietic cells will be subjected to screenings with phage-displayed peptide libraries. Indeed, the availability of novel ligands binding to additional tumor-associated antigens and to targets on both endothelial and perivascular tumor cells will allow to design more sophisticated liposomal targeted anticancer strategies that exhibit higher levels of selective toxicity for the cancer cells in a combined setting.

ALK mutation and expression in neuroblastoma

Gian Paolo Tonini
Translational Pediatric Oncology, National Cancer Research Institute, Genoa, Italy

ALK gene has been identified as the first neuroblastoma predisposition gene in familial cases. Indeed, several missense mutations have been discovered in the tyrosine kinase domain: the most frequent are F1174L and R1275Q. Recently, we identified a new Italian family carrying the R1192P mutation, which is peculiar of familial cases, in a healthy mother and her two affected children. A first degree relative, who also had neuroblastoma may potentially carry the same ALK mutation. ALK mutations have also been observed in sporadic neuroblastomas with a frequency ranging between 6% and 8%. We studied 114 sporadic neuroblastomas at different clinical stages and age and we found 6 out of 114 (5.3%) cases with ALK mutations. We identified a novel mutation (3509 T>G - I1170S) in a tumor of a patient at stage 3. Three of the 6 patients with ALK mutations died of disease progression but only one had high risk features (stage 4, over 1 year, protein mutation: R1275Q), whereas the other two were a stage 1 and a stage 4s, both with the mutation F1174L. We also investigated 28 human neuroblastoma cell lines for ALK mutations. The mutation 3522C>A (F1174L) was found in 7/28 (25%) cell lines (KELLY, LA1-5S, LAN-1, LAN-5, SH-SY5Y, SK-N-SH, SMS-KCNR), and only 1/28 (3.6%) NB cell line (UKF-NB3) showed the 3824G>A (R1275Q) mutation. To verify whether ALK abnormal expression could be dependent by mutations in the promoter region of ALK, we sequenced 2564 bases upstream the ATG starting codon in 29 NB tumors and 8 NB cell lines. However, no mutations have been found in the gene promoter, indicating that ALK expression would be altered by other mechanisms.
Since it has been demonstrated that cell proliferation can be impaired by siRNA induced silencing of ALK expression, ALK has been proposed as a druggable target and treatment by specific small molecule inhibitors has been tested. In collaboration with Dr. Lorena Passoni we used and tested CEP-14083 and CEP-14513 in order to observe an inhibition of ALK activity in 10 neuroblastoma cell lines. Our results show that CEP-14083 and CEP-14513 hampered neuroblastoma cell proliferation and induced cell death, strongly supporting a functional relevant oncogenic activity mediated by ALK. Noteworthy, the inhibitory effect of CEP compounds was observed in NB cell lines showing ALK overexpression and activation, harbouring either a mutated or a wild-type receptor but not in cells expressing low amounts of ALK, underlining the requirement of a critical threshold of protein expression to achieve oncogenic activity.

Supported by Italian Neuroblastoma Foundation

Overview on antibody developments in USA & Results of the COG ch14.18 randomised trial

Alice L. Yu

Aberrant glycosylation is a feature of cancer cells. The abnormally expressed cell surface glycoproteins or glycolipids may contribute to many aspects of the malignant phenotype. These tumor associated glycol-antigens are preferred targets for cancer immunotherapy. One of such targets for cancer therapy is a disialoganglioside, GD2, which is highly expressed in neuroblastoma, melanoma and small cell lung cancer. I have taken a chimeric anti-GD2, ch14.18, from initial IND filing to phase I and II trials which led to the ongoing international phase III study of the ch14.18 + cytokines in high-risk neuroblastoma. The latter is a randomized study comparing standard therapy with 13 cis-retinoid acid to immunotherapy with anti-GD2 antibody + GM-CSF/ IL2 + 13 cis-retinoid acid. The most common side effects in the immunotherapy group were pain (25% of immunotherapy courses), allergic reactions (15%), and vascular leak syndrome (8%). Other toxicities include transient thrombocytopenia, fever, hypokalemia, hyponatremia, liver dysfunctions, diarrahea and urticaria. Interim analysis in Spring of 2009 showed that immunotherapy significantly improves outcome for high-risk neuroblastoma patients in first response. The 2-year EFS estimates were 66% ±5% for immunotherapy group versus 46%±5% for standard therapy (p=0.0115). This is the first clinical trial to document that combination of anti-cancer monoclonal antibodies (mAbs) with cytokines is an effective anti-cancer therapy. This is also the first time an mAb targeting a glycolipid antigen is shown to be effective for cancer

immunotherapy since all therapeutic anti-cancer mAbs previously approved by FDA are directed against protein antigen. In addition, I have conducted the first clinical trial of a GD2-targeting anti-Id based vaccine in patients with high risk neuroblastoma in first or subsequent remission. This study demonstrated the efficacy of this vaccine in eliciting endogenous anti-GD2 with little toxicities. Overall, these findings establish immunotherapy as a cornerstone to high-risk neuroblastoma treatment.

International mIBG Scoring: European results

Val Lewington

Semi-quantitative analysis is required to standardise reporting of diagnostic 123I mIBG images in neuroblastoma. Pre-requisites for an appropriate method include low inter observer error and reproducibility across a broad disease spectrum. An international Expert Panel was formed to undertake a literature review and recommend a suitable method for use in the SIOPEN High Risk study.
A new semi-quantitative method based on patterns of abnormal skeletal 123I mIBG uptake was developed and tested by Panel members. 1968 data sets held electronically on the SIOPEN database were scored independently as unblinded pairs [pre and post induction chemotherapy images] and in random order as a blinded study. The score method proved reliable and reproducible across the expected disease spectrum with intraclass correlation coefficients in the range 0.95 – 0.99 for both blinded and unblinded studies. Skeletal score at diagnosis strongly predicted response to induction chemotherapy [p,0.007] and a threshold score of 45/72 at diagnosis predicted poor skeletal response. [p,0.002].
The method has been reviewed and accepted by the wider SIOPEN nuclear medicine committee and will be applied prospectively within the High Risk study. An educational reference atlas is in preparation and will be circulated by national representatives to European centres participating in the SIOPEN trial to ensure rapid adoption. National representatives will develop audit procedures to ensure consistent reporting standards.

Haploidentical SCT for high risk NBL combined with immunotherapy

Peter Lang, MD1, Jacek Toporski, MD2, I Müller, MD1, T. Klingebiel, MD3 W. Schwinger, MD4, Heiko-Manuel Teltschik MD1 Michael Albert, MD5 Peter Bader, MD3, Rupert Handgretinger, MD1
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Patients with relapsed high risk neuroblastoma have a poor prognosis and additional therapeutic strategies are warranted. We present preliminary results (Tuebingen, Lund, Frankfurt, Graz, Munich) with haploidentical stem cell transplantation in 26 pediatric patients with metastatic relapse after previous autologous stem cell transplantation as well as a proposal for posttransplant immunotherapy. 12/26 patients reached complete or very good partial remission after various chemotherapies, 14/26 patients reached partial remission or were not in remission prior to transplantation. The conditioning regimen comprised Flud, TT, Mel and OKT3, 13/26 patients received MIBG therapy 2-4 weeks prior to transplantation. MMF was given as GvHD prophylaxis. The CliniMACS® device was used for T and B cell depletion. After this procedure, the grafts comprised megadoses of stem cells as well as high amounts of NK-cells and monocytes/granulocytes with less than 1x10^5/kg residual CD3+ cells and <0.01% CD20+ cells. Primary engraftment occurred in all patients. Median time to reach >500 neutrophiles/µl was 11 days. Acute GvHD grade 0-1 occurred in 69%, 27% had GvHD grade II and 4% had grade III. Chronic GvHD occurred in 28%. No transplant related mortality (TRM) and, in particular, no lethal infections were observed. Over all survival after 2 years was 30%. Patients who received therapeutic MIBG prior to transplantation had a better outcome than patients without MIBG (60% vs. 10% EFS after two years; n.s., p=0.2). 11/26 patients are alive with a median follow up of 1.6 years. Some patients received interleukin 2 subcutaneously or DLIs posttransplant. Activity of natural killer cells from patients posttransplant was sufficient against the standard cell line K562 and rather low against neuroblastoma cell lines. However, in vitro stimulation with cytokines and/or use of chimeric antiGD2 antibodies markedly increased NK cell mediated lysis. Thus, we propose an open, prospective phase I/II study to evaluate the feasibility and safety of CH14.18/CHO administration in combination with low dose interleukin 2 after haploidentical transplantation. The study design comprises 6 cycles of mAb CH14.18/CHO (20mg/m2 infusion over 8 hours for 5 days; in cycles 4-6, 1x10E6 units /m2 aldesleukin will be given additionally on days 6, 8 and 10). Primary endpoint is "success of treatment" defined as a patient receiving the full protocol treatment, still alive 180 days after end of treatment without progression and without unacceptable toxicity and acute GvHD ú Grade III or extensive chronic GvHD. Secondary objectives are to evaluate the anti-tumour responses resulting from this immunotherapy regimen through clinical assessments, to evaluate pharmacokinetics of the ch14.18/CHO and to evaluate changes in NK cell activation and proliferation (immunological monitoring).
Conclusions: haploidentical transplantation and MIBG represent a therapeutic option for patients with relapsed high risk neuroblastoma and may be a basis for immunotherapeutic approaches. The

proposed study will adresss the question, whether the use of the monoclonal antibody CH14.18/CHO in combination with interleukin 2 will be feasible after this transplantation procedure.

Allogeneic stem cell transplantation from related or unrelated donor in high risk neuroblastoma. The italian AIEOP-HSCT experience in 21 pts.

E. Lanino, F. Fagioli, F. Locatelli, C. Favre, M. Rabusin, C. Messina, D. Caselli, A. Prete

Objectives: To evaluate the feasibility and efficacy of a reduced intensity conditioning regimen (RIC) followed by allogeneic stem cell transplantation (SCT) from HLA-matched related (MRD) or unrelated donor (MUD) in children with neuroblastoma (NB) who responded poorly to front line therapy or relapsed after autologous stem cell transplantation.
Methods: 21 patients (pts), aged 2-13 years, affected by resistant (5) or relapsed (16) NB were enrolled and submitted to an SCT after a RIC consisting of Thiotepa (TT) 15 mg/kg and Melphalan (PAM) 140 mg/sqm. The donor was an identical sibling in 11 cases or a MUD in 10. At time of transplant 15 pts were in any kind of remission of disease and 6 in progressive disease. Graft versus host disease (GVHD) prophylaxis consisted of CyA in recipient of MRD, CyA plus ATG and short-course MTX in recipients of MUDs. SC source was bone marrow in 17 cases and peripheral blood in 4.
Results. All patients engrafted and full donor chimerism was documented after 40 and 60 days in MRD and MUD recipients, respectively. Acute GVHD of grade 2-4 and 3-4 occurred in 11 and 5 cases, respectively. One pt who developped gr 4 aGVHD died of transplant-related causes 6 months after MUD-SCT. After a median follow-up of 23 (4-48) months, 11 pts relapsed (7 of them susequently died of disease progression and 4 are alive with persistent disease): the median interval between SCT and relapse was 9 (2-17) months.
Nine pts never relapsed after transplant: after a median time of 28 mos after allo-SCT, 6 are in continuous complete remission, whereas the 3 remaining pts present stable residual disease. All the pts transplanted with progressive disease experienced disease progression after SCT.
Conclusions: allogeneic-SCT after a TT-PAM based reduced-intensity regimen is feasible and the toxicity was very limited, also in children with a previous history of autologous SCT. The nearly 50% relapse-free survival at 2 yrs in our group of pts at very poor prognosis is encouraging and warrants further investigation in an earlier phase of disease.

Molecular Profiling in Neuroblastoma

Angelika Eggert, MD

This presentation will provide an overview of current molecular profiling approaches in neuroblastoma. The complexity of cancer phenotypes is characterized by multiple mutations and alterations in the cancer genome. A consequence of these alterations is the deregulation of various signaling pathways controlling cell function. Molecular profiling studies have the potential to describe this complexity, and provide an opportunity to link pathway deregulation with potential therapeutic strategies. In addition, the identification of novel molecular signatures using state-of-the-art high-throughput technologies can provide pediatric oncologists with advanced and more meaningful diagnostic tools. For neuroblastoma, it has been shown in numerous studies that molecular profiling allows new subclassifications of the tumor as well as a more accurate outcome prediction of the disease. Initially, single molecular technology platforms including microarrays and arrayCGH have been validated in comparison to classical cytogenetics in these studies. Current research strategies go beyond these approaches by integrating genomics and transcriptomics data obtained with the most advanced technologies to provide additional diagnostic panels. Such an approach requires the transnational integration of available resources and technologies.

Clinical Trials' Corner

EUNB

Treatment of children over the age of one year with unresectable localised neuroblastoma without MYC-N amplification

Janice Kohler

Opened (UK): January 2001
Closed: February 2007

This study closed in early 2007 and a major effort has been made to collect outstanding data during 2009. There are 166 eligible patients. There have been 17 deaths, all but one following relapse. One child died during surgical excision. There are 35 relapses giving an EFS of 72% and OS of 79% for the whole group. Of the relapses, two were distant, 12 combined, and the others local. Children less than 18 months old at diagnosis, and patients with favourable INPC have significantly better survival.

These results will be discussed in detail at the SIOPEN meeting.

INES 99.1

Hervé Rubie

Purpose: To evaluate the efficacy of low-dose chemotherapy in infants with localized and unresectable neuroblastoma (NB) without Myc-N amplification.
Patients and methods: All consecutive infants with localized NB and no MYCN amplification were eligible in the SIOPEN-INES 99.1 study. Primary tumour was deemed as unresectable according to imaging defined risk factors. Diagnostic procedures and staging were done according to INSS recommendations. For children having no threatening symptom, chemotherapy consisted in low-dose cyclophosphamide (5 mg/kg/d x 5 days) and vincristine (0.05 mg/kg at day 1) – CV and repeated 1 to 3 times every 2 weeks until surgical excision can be safely performed. No post operative treatment was to be given.
Results: Between December 1999 and December 2006, 120 were included in the INES 99.1. Among them 88 had no threatening symptom and 79 received CV. A second line chemotherapy was given to 48 infants because of insufficient response. Thirty two children had a threatening symptoms, of whom 26 received Carboplatine-Etoposide (CE). Surgery was attempted in 103 patients including 22 after CV alone, leading to complete resection in 81 and 19 after CV alone. Relapses occurred in 12 patients (9 local and 3 metastatic). Five year survival and event-free survival are 99% +/- 1% and 90% +/- 3% with a median follow up of 6.1 years (1.6-9.1).
Conclusion: Primary low-dose chemotherapy without anthracyclins is efficient in 62% of infants presenting with an unresectable NB and no MYCN amplification, allowing excellent survival rates without jeopardizing their long-term outcome.

LNESG2

Guidedlines for the treatment of patients with localized resectable neuroblastoma and analysis of prognostic factors

Maja Beck Popovic, Study coordinator

Study Committee	Maja Beck Popovic, Alessandro Jenkner, Adela Cañete, Anne-Sophie Defachelles, Bruno De Bernardi
Biology	Nicole Gross, Valérie Combaret
Pathology	Gabriele Amann, Klaus Beiske, Catherine Cullinane, Emanuele S.G. D'Amore, Claudio Gambini, Sam Navarro, Michel Peuchmaur
Surgery	Keith Holmes, Giovanni Cecchetto, Tom Monclair
Radiology	Claudio Granata
Statistics	Véronique Mosseri

Background. LNESG2 is the second European study on localized neuroblastoma. In the previous study the presence at diagnosis of high LDH serum level, deletion of 1p and unfavorable histology according to Shimada criteria were apparently associated with a greater propensity to relapse. However, a statistical value was not reached mainly due a great amount of missing data prohibiting justification of immediate treatment of these patients. A decision was taken to treat patients with unfavourable histology more aggressively following relapse.

Objectives. To maintain the good results in the cure of localized neuroblastoma without MYCN amplification by surgery alone, to improve surgical morbidity by respecting the presurgical risk evaluation and to define a subgroup of patients at higher risk of relapse.

Primary objective: to expand the information provided by LNESG1 on factors associated with clinical prognosis in localized neuroblastoma, especially preoperative LDH, 1p deletion and histology.
Secondary objectives

- to maintain or improve EFS and OS when compared to LNESG1,
- to improve the quality of management and data collection in patients with resectable localized neuroblastoma without MYCN amplification by
- a nationally centralized evaluation of the pathological and biological data with secure banking of material,
- improving data collection, with particular regard to LDH and 1p deletion
- to establish a uniform treatment for relapsed patients.

Update
Accrual. Patient accrual has started effectively in August 2005 and 237 pts have been registered until October 7th 2009: 161 INSS stage 1, 71 INSS stage 2 and 5 INSS stage 3 patients. This is lower than the expected accrual of 150 INSS stage 2 pts within 4 yours. Tumor sites are distributed as follows: cervical 11 pts (5.2%), thoracic 57(27.1%), adrenal 93 (44.3%), non-adrenal 46 (21.9%) and pelvic10 (4.8%). Seven pts present with tumor at double sites.

Data.
There are 100% results in the data base for preoperative LDH with only 3% being abnormal preoperatively; 100% for MYCN amplification with only 1.7% being amplified; 83.5% for local pathology, 77.2% for IDRF, 89.9% for surgery and 36.6% for surgical outcome. Thus, data availability improved by a few percents for all the items but surgical outcome which clearly decreased as no data has been entered for the last 12 months.
Biological analyses other than MYCN amplification are performed within the biology group and are still masked.

Relapses
There have been 18/237 (7.5%) relapses within 4.5 years: 8 INSS stage 1 and 10 INSS stage 2 patients. Among the 8 INSS stage 1 pts who relapsed, 2 had MYCN amplification. The other two with amplified tumors are doing well. No death has been reported.

Future
As accrual seems not to improve after the projected study duration of 4 years, study closure has to be discussed for data analysis, including the still masked biology results.

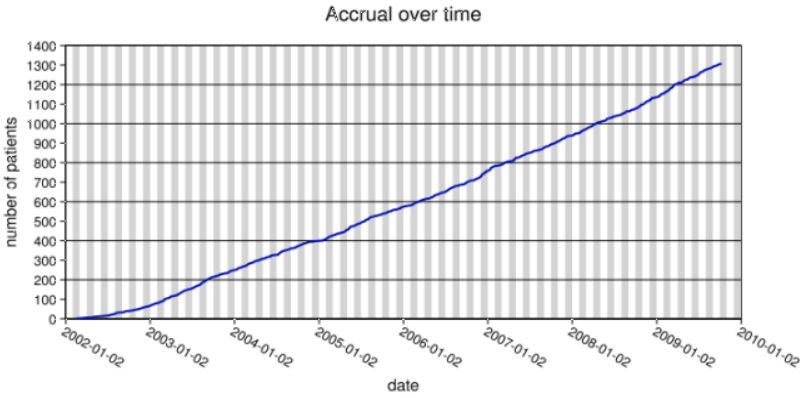
HR-NBL-1

High Risk Neuroblastoma Study

Ruth Ladenstein

Recruitment
The High-risk neuroblastoma trial is recruiting well and has now reached a total of 1308 patients.

An interim analysis and the amended study protocol have been submitted to the DMC in 2009.
The automated query system has been improved: over the next 6 months Alisa Alspach will follow queries thoroughly to improve our data quality and density of the HR-NBL1/SIOPEN study with special weight on HDT/SCT and toxicity profiles overall.

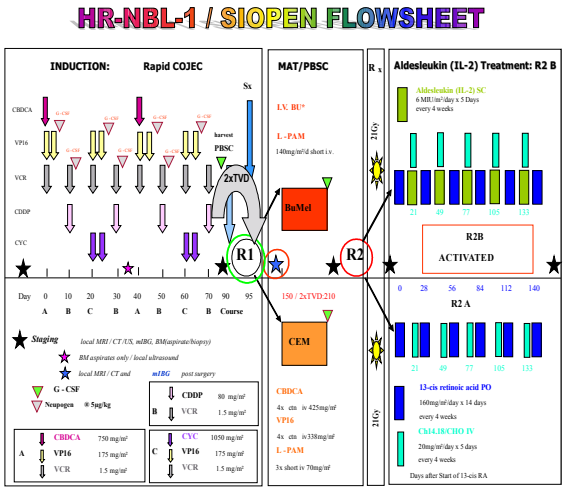


R0 Randomisation
The R0 supportive care question is answered and the article has been submitted to JCO. All patients are now getting G-CSF during induction.

R1 Randomisation
There are currently 519 patients randomised to R1. The R1 question should be effectively answered in approximately 2 years.

New R2 Randomisation
In spring and at the ASCO meeting 2009 we learnt about a major break through of immunotherapy when the COG was able to stop accrual into the immunotherapy arm in the COG Study ANBL0032 for early significance. This also had major implications for our group as we felt the need to adopt the immunotherapy arm (R2-randomisation) our current HR-NBL1/SIOPEN study. After intensive discussions and meetings the executive committee had approved the change towards a new SIOPEN immunotherapy strategy:
The amended version of the R2 randomisation (as of July 2009) compares the treatment with retinoic acid (RA) and antibody ch14.18/CHO versus ch14.18/CHO, RA and additional subcutaneous Interleukin 2 (Aldesleukin). The use of subcutaneous IL-2 in neuroblastoma patients has already been investigated and found to be of feasible and efficacious by a SIOPEN study (submitted to JCO). The amended protocol as of July 2009 has been approved by the Ethics Committee and the competent authorities in Austria.

The IMPD V.02 of the antibody ch.14.18 (distributed in May, 2009 and available upon request) contains additional virus safety and pharmacokinetic studies.
This, together with the amended protocol as of July 2009 (distributed on the 31st of August, 2009) should allow all national coordinators to approve the study in their countries.
The new eligibility criteria for R2 randomisation allow any patient receiving elective MAT of either BUMEL or CEM to be randomised. However the time from receiving the first day of induction treatment to ASCT must be less than 9 months. 400 patients are expected to be randomised within the next four years. A maximum accrual of 200 patients per year on trial is expected according to our previous experience.



Patients randomised to ch14.18/CHO alone will receive ch14.18/CHO at a dose of 20 mg/m²/day over 5 days every 4 weeks for 5 courses. The first course will start three weeks after initiation of 13-cis RA (week 4).

Arm 1: Treatment schedule for 13-cis RA with ch14.18/CHO

W_1	W_2	W_3	W_4	W_5	W_6	W_7	W_8	W_9	W_{10}	W_{11}	W_{12}
		rest				rest				rest	
RA	RA		GD_2	RA	RA		GD_2	RA	RA		GD_2

W_{13}	W_{14}	W_{15}	W_{16}	W_{17}	W_{18}	W_{19}	W_{20}	W_{21}	W_{22}
		rest				rest			
RA	RA		GD_2	RA	RA		GD_2	RA	RA

W: weeks related to start of 13-cis RA treatment
GD_2: ch14.18/CHO

Patients randomised to receive ch14.18/CHO and subcutaneous aldesleukin (IL-2) will start their immunotherapy with aldesleukin (IL-2) at week 3. Aldesleukin (IL-2) will be given according to the following administration schedule:

- During weeks 3, 7, 11, 15 and 19 aldesleukin (IL-2) will be given at a dose of 6 MIU/m²/day over five days subcutaneously (Monday-Friday).
- During weeks 4, 8, 12, 16 and 20 aldesleukin (IL-2) will be given two hours after the stop of the anti-body infusion at a dose of 6 MIU/ m²/day over five days subcutaneously (Monday-Friday).

Aldesleukin (IL-2) should be given after 6 p.m. with prophylactic paracetamol. The use of local anaesthetic cream (EMLA) 30 minutes prior to injection is highly recommended. In case of persistent Grade III or higher fever, a dose reduction of 50% is recommended.
Ch14.18/CHO will be given at a dose of 20 mg/m²/day over 5 days every 4 weeks for 5 courses, starting in week 4.
A detailed plan how to react to encountered toxicities is provided within the protocol.

Arm 2: Treatment schedule for 13-cis RA, ch14.18/CHO and aldesleukin (IL-2)

W_1	W_2	W_3	W_4	W_5	W_6	W_7	W_8	W_9	W_{10}	W_{11}	W_{12}
			GD_2				GD_2				GD_2
RA	RA	IL-2 sc	IL-2 sc	RA	RA	IL-2 sc	IL-2 sc	RA	RA	IL-2 sc	IL-2 sc

W_{13}	W_{14}	W_{15}	W_{16}	W_{17}	W_{18}	W_{19}	W_{20}	W_{21}	W_{22}
			GD_2				GD_2		
RA	RA	IL-2 sc	IL-2 sc	RA	RA	IL-2 sc	IL-2 sc	RA	RA

W: weeks related to start of 13-cis RA treatment
GD_2: ch14.18/CHO

The new R2 randomisation will be available on the data base as of November 1st 2009. R2 randomisation can only be performed after all necessary documents (approval of the ethics committee, competent authorities, insurance, and sponsor-ship agreement) have been provided.
A new GMP production run is ongoing for the new haplostudy (Peter Lang: Phase II Feasibility study) and the high risk study to have sufficient material available at the given time. This haplostudy aims to determine the safety and feasibility of the chimeric anti-GD2 monoclonal antibody ch14.18/CHO in combination with subcutaneous aldesleukin (IL-2, (Proleukin®)) after haploidentical stem cell transplantation in paediatric patients with relapsed neuroblastoma.
We are currently the only organisation in Europe with access to the antibody having it developed over the last 8 years. This is the time to acknowledge again the major funding efforts in 2001 and the fantastic support of Polymun Scientific being such a cooperative and generous partner throughout this project when undertaking the recloning and starting a new GMP production venture after a production failure elsewhere.

Low and Intermediate Risk Neuroblastoma Study LINES

from the LINES Writing Committee

The aim of this SIOPEN study is to have a clinical trial using a risk group stratification approach for the treatment of all non high risk neuroblastoma patients of any age who are MYCN non amplified. The risk groups will depend on age, stage, the presence or not of symptoms and the biological profile of the tumour.
There will be three well defined risk groups of patients included in this study: very low, low and intermediate.

- The **Very Low Risk Study** will be an observational study of Infants with adrenal masses, discovered antenatally or neonatally (ie suspected neuroblastoma). Our hypothesis is that these patients can be managed without initial surgery with close monitoring which does not jeopardise their outcome.

- The aim of treatment in the **Low Risk Study** will be to continue to reduce the amount of chemotherapy and surgery and therefore lessen the burden of treatment for all appropriate low risk patients, who in previous studies have been shown to have an excellent long term outcome (as in the SIOPEN 99.1-2 infant neuroblastoma studies when OS > 97%). This Low Risk study includes a randomisation for L2 patients >18 months of age, without segmental chromosomal aberrations and without life-threatening symptoms between 2 treatment arms either standard chemotherapy or observation with delayed chemotherapy if indicated clinically by the tumour progressing.

- The **Intermediate Risk Study** aims to unify treatment strategies to maintain the previously good EFS of > 80% and to intensify local treatment with radiotherapy for those intermediate risk patients with poorly differentiated or undifferentiated disease. A very small group of patients with L1, MYCN amplified tumours are included in this intermediate risk group .

The achievements of the LINES group in the last months are the following:

1. The writing committee has worked hard with weekly telephone conference calls to finalise this protocol. They are extremely grateful for the extra help provided by AA. She has been hugely helpful and has enabled the WC to finalize this task with her expertise including her knowledge of the EU regulations. The protocol has been distributed to National Coordinators prior to the Rome meeting for general review. Comments are welcome, but major modifications will not be accepted
2. The LINES WC has been working together for the last 3 years as a team and our purpose is to continue working in this way. There will be one sponsor for the whole protocol who will take the lead within Europe. After many discussions it has been decided that La Fe (Valencia) will be the main European sponsor. Although Spain will be the European leading sponsor (Dr. Adela Cañete Nieto, Unidad de Oncología Pediátrica, Hospital Infantil La Fe., Valencia.) The “spirit” of the team working of the LINES writing committee will continue
3. We are looking forward to finalizing this protocol after the October 2009 SIOPEN Rome meeting, so that the Eurdract number can be applied for prior to the protocol being launched next year 2010.
4. Much sustained and hard work has gone into this protocol and the writing committee would like to specifically thank Alisa Alspach for her brilliant work in "critically” going through the protocol as well as thanking all our colleagues from the different SIOPEN subcommittees that have collaborated with their contributions. There are also special thanks to our statistical team (Veronique Mosseri, Jose Bermudez and David Machin, in particular as he has just retired).

A COG – SIOPEN International Pilot Study for Newly Diagnosed Adolescents and Young Adults with Neuroblastoma

Isaac Yaniv

Background: Outcome of adolescents and young adults (AYA) with neuroblastoma is inferior as compared to children with the same clinical and known biological features. They have the lowest rate of enrollment on cancer clinical trials, lowest percent representation in tumor banks, and the lowest rate of improvement in outcome.

3% of patients with neuroblastoma are diagnosed above the age of 10 years. Neuroblastoma in AYAs is a rare orphan disease and we aim to improve outcome by a unique international collaboration effort involving the European and American groups.

Objectives: To assess the tolerability and feasibility of treating adolescents and young adults (AYA) with neuroblastoma with dose-intensive multi-agent induction chemotherapy followed by surgery, double high dose 131I-MIBG therapy, myeloablative consolidation therapy with autologous stem cell rescue, local radiation, and treatment of minimal residual disease with biologic agents.

1. To assess response after each phase of the treatment of adolescents and young adults with neuroblastoma with dose-intensive multi-agent induction chemotherapy followed by surgery, double high dose 131I-MIBG therapy, myeloablative consolidation therapy with autologous stem cell rescue, local radiation, and treatment of minimal residual disease with biologic agents.
2. We will specifically explore referral schemes as High dose MIBG can be delivered only in very few specialized centers.
3. Define clinical and biological characteristics of neuroblastoma in AYAs.

Hypothesis:

1. There are distinct clinical and biologic features of neuroblastoma in AYAs that may explain its unique clinical course and response to therapy.
2. Intensive multi modality therapy including double 131I-MIBG therapy followed by myeloablative therapy and autologous stem cell transplantation is feasible in AYAs with neuroblastoma in an international multi institiutional pilot study.
3. Intensive multi modality therapy in AYAs will result in similar outcome as compared to young children with neuroblastoma.

Study Design: This is a pilot study of 60 evaluable patients to assess the feasibility of a intensive induction regimen combined with double 131I-MIBG+Topotecan followed by myeloablative consolidation with BU – MEL and autologous stem cell rescue and minimal residual disease therapy with biologic agents in AYAs with newly diagnosed MIBG-positive high risk neuroblastoma. The induction regimen will utilize sequential administration of 7 cycles of multi-agent chemotherapy (CTX, TOPO, VP-16, CARBO, DOXO, IRINO, TEM. Filgrastim (G-CSF) will be administered with each cycle of Induction chemotherapy to minimize hematologic toxicity. Peripheral blood stem cell harvest and surgical resection of tumor will occur after the 2nd and 5th cycles of induction phase, respectively. Induction therapy will be followed by intensified consolidation with double 131I-MIBG +TOPO and MAT with BU-MEL and autologous stem cell transplant, which is based upon results from the European experience. External beam radiation will be given after recovery from ASCT at a dose of 2100 cGy, fractionated in 12 daily doses, to the pre-resection tumor volume with a boost up to 3600 cGy to residual tumor sites present after ASCT. After recovery from radiation therapy, patients will receive 6 months of cis-retinoic acid, and immunotherapy. Following the results of the COG study all patients will receive immunotherapy according to the COG or SIOPEN schedule.

During the Rome meeting the protocol writing committee will be established.

I would like to acknowledge Adam's Hat for the ongoing support enabling us to prepare this collaborative study.

OMS
Opsoclonus-Myoclonus Protocol

Gudrun Schleiermacher
Barbara Hero
For the Opsoclonus Myoclonus Collaboration Group

Opsoclonus-myoclonus syndrome (OMS) in childhood is a rare, severe neurological disorder occuring mainly in children aged under 3 years old, and is frequently assocated with neuroblastoma (NB). In order to improve the understanding of the underlying pathological mechanism and work towards a better neurological outcome, an international European collaborative group, consisting of SIOPEN (Société Internationale d'Oncologie Pédiatrique –Europe Neuroblastoma), GPOH (German Society of Pediatric Oncology and Hematology), and EPNS (European Pediatric Neurology Society) is working on a common protocol, to be opened shortly, aiming at common guidelines and collaboration for biological studies, oncological and neurological diagnostic procedures, and a common treatment proposition.

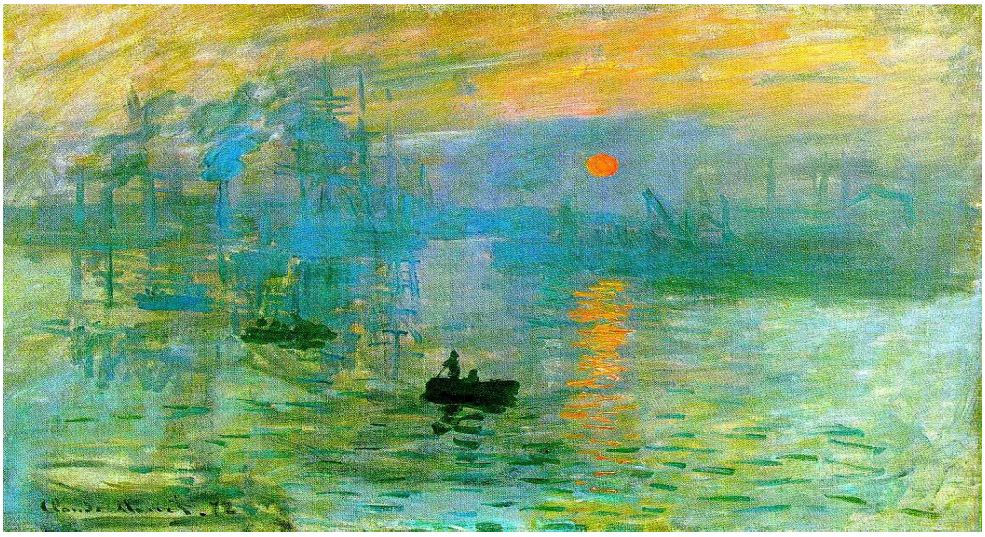
The planned study, entitled "Multinational European Trial for Children with the Opsoclonus Myoclonus Syndrome / Dancing Eye Syndrome", will include children (aged between 6 months and 8 years) with newly diagnosed OMS/DES, with or without neuroblastoma. Search for and staging of neuroblastoma must have been performed according to the guidelines of the study. The study proposes patient registration, sampling and storage of biological material, and immunosuppressive treatment: the first step will consist of a standard immunosuppressive treatment with monthly dexamethasone bolus (20 mg/m2 for 3 days, repeated at monthly intervals) for one year. In case of insufficient response after three months, immunosuppressive treatment will be increased and cyclophosphamide will be given (cyclophosphamide 750 mg/m2 at monthly intervals) for 6 cycles. In case of insufficient response despite this treatment, immunosuppressive treatment with dexamethasone and rituximab will then be given. Centers will be given the possibility to include patients during the first 3 months of the standard treatment using dexamethasone.

Primary endpoints of the study are to evaluate the response to treatment and the long term neuropsychological outcome of patients with OMS with or without NB.

Secondary endpoints are to determine the percentage of OMS-NBpos patients among all patients with OMS, the comparison of OMS-NBpos and OMS-NBneg in terms of presentation, severity and treatment response, the evaluation of the treatment burden and the evaluation of factors influencing long term outcome

Further objectives are to develop a European multidisciplinary network of specialists in the treatment of patients with OMS/DES, to develop a European biomaterial bank and a Europe-wide collaboration of scientist interested in OMS/DES, to evaluate incidence of OMS/DES in Europe and per country, and to investigate the biology of OMS/DES.

The study protocol is currently in the final stage of writing, and the CRFs are being completed. It has been proposed that Institut Curie (Paris, France) could be responsible for the international sponsorship, and the administrative steps are currently ongoing, the aim being to open the study beginning of 2010.



Committees' corner

Surgery

By Keith Holmes, Chair

Stefano Avanzini (Paediatric surgeon - Genoa, Italy), Piero Buffa (Paediatric surgeon - Genoa, Italy), Giovanni Cecchetto (Paediatric surgeon - Padua, Italy), Anna Maria Fagnani (Paediatric surgeon - Milan, Italy), Keith Holmes (Paediatric surgeon - London, UK), Jean-Marc Joseph (Paediatric surgeon - Lausanne, Switzerland), Dejan Kafka (Paediatric surgeon - Belgrade, Serbia), Carl-Magnus Kullendorff (Paediatric surgeon - Lund, Sweden), Tom Monclair (Paediatric surgeon - Oslo, Norway), Sabine Sarnacki (Paediatric surgeon - Paris, France), Roly Squire (Paediatric surgeon - Leeds, UK), Shifra Ash (Oncologist - Tel Aviv, Israel), Bruno De Bernardi (Oncologist - Genoa, Italy), Geneviéve Laureys (Oncologist - Gent, Belgium), Paula Pereira (Radiotherapist - Lisbon, Portugal), João C Silva (Paediatric radiologist - Lisbon, Portugal)

The group met last in Lausanne September 2008.
We continue close collaboration with the development of and recruitment of patients to International Treatment Protocols. This work particularly involves standardisation of operation strategies for each tumour type.
The outcome of all operations is recorded and all complications are examined carefully. There is an active advice network for difficult patient problems available constantly. These patients are then discussed with colleagues from all disciplines at the annual meeting.

Studies:

High risk HR-NBL-1/ESIOP (analysis in April 2009 study remains open)

Almost 800 operations - achieving 71% complete tumour excision. The complication rate is 10% in spite of operations of increasing complexity. There have been five deaths since the start of study.

Low risk LNESG1

The concept of Surgical Risk Factors (SRF) was developed based on images (CT MR) before operation. The presence of these factors predicted complete tumour removal and the risk of operation complication. (Published JCO 2005). SRF are incorporated in the International Neuroblastoma Risk Group (INRG).
The passage of time has allowed analysis of the influence of SRF on patient survival. Both SRF and operation complication have an adverse effect on survival. (Final draft is ready for publication).

LNESG2

This study opened in 2002 and develops the strategy of LNESG1.
The study is recruiting well and utilises the internet to allow real time International analysis of patient data.

Intermediate risk (unresectable localised)

This study ran from 2000 to 2008.
There are around 190 patients recruited and the comprehensive surgical and radiological data are now being analysed.

New studies

The surgery subcommittee is actively involved with the development of new studies for Infants and Intermediate Risk patients.
These will incorporate the structure of the International Neuroblastoma Risk Group (INRG)

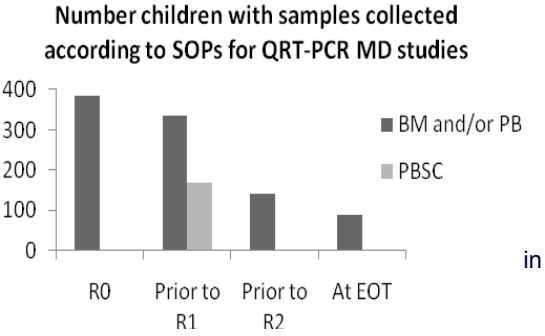
Molecular Monitoring Group

Sue Burchill, United Kingdom (chair), Maria-Valeria Corrias, Italy (vice-chair), Sandro Dallorso, Italy, Bertil Kagedal, Sweden, Katrien Swerts, Belgium, Andrei Tchirkov, France, Aleš Vicha, Czech Republic Virginie Viprey, United Kingdom (vice-chair)

Current Study Aims:
The minimal disease (MD) biological study in conjunction with the clinical trial HR-NBL1/ESIOP aims to determine the clinical significance of tumour cells detected by QRT-PCR for TH, DCX and Phox2B mRNA alone or in combination, in peripheral blood (PB), bone marrow (BM) and peripheral blood stem cell harvest (PBSC).

Recruitment:
Overall recruitment rate into MD studies at R0 within the UK and Italy is 55 and 64%, respectively. In other European reference countries entering smaller numbers of children into the clinical trial, recruitment is between 16 and 64%. UK acts as reference centre to receive samples from Greece (collection organised by Dr Vassilios Papadakis), Austria (Professor Peter Ambros) and Switzerland (Dr Nicole Gross). The Czech Republic is a new reference centre to receive samples from Slovakia. Recruitment into MD studies as children progress through the trial is reduced; this in part reflects the clinical course of children in the study but we are also working to improve this recruitment where possible. Recruitment of children from Spain into MD studies has not been possible as Spanish centres have been unable to collect samples according to standard procedures.

Analysis
Quality control samples for the specific and sensitive detection of circulating neuroblastoma cells using QRT-PCR for all three markers (TH, Phox2B, and DCX mRNA) have been prepared and successfully tested by the participating European reference laboratories. Additional fields have been implemented into the SIOPEN-R-NET QRT-PCR database to allow incorporation of results from all three markers. We have validated QRT-PCR results for TH and Phox2B mRNA at diagnosis and/or after induction chemotherapy from 341 patients across Europe (Italy, n=179; UK, n=108; France, n=19; Belgium, n=15; Czech Republic, n=13; Austria, n=4; Greece, n=2; Slovakia, n=1). These data are recorded, blinded on the SIOPEN database. End of induction therapy is a particularly interesting time to study minimal disease because at this point in treatment children may receive additional induction therapy prior to progression the trial; if the presence of disease detected by QRT-PCR predicts disease course after induction therapy this may influence subsequent treatment and potentially outcome.



in

The SIOPEN board has recently approved the release of clinical information for correlation with QRT-PCR data, to allow an interim analysis and accurately inform how many more children we need to recruit into different stages of the biological study. The clinical data is currently being cleaned and updated prior to this analysis, which will be prepared for submission to the Advances in Neuroblastoma Research Meeting, Stockholm, 2010.
Quantitative PCR data showed that TH and Phox2B mRNA levels in BM at diagnosis vary greatly between patients, ranging from -3 to +5 (Log10 transformed values). After induction therapy, levels of mRNA detected are up to 6.7 Log10 values lower than at diagnosis in some patients (median Log10 reduction is 2.7). The clinical significance of differences in frequency and levels of TH and Phox2B mRNA detection are to be evaluated in the proposed interim analysis.
Meetings: The group last met on Thursday 16th October 2008 in Lausanne, Switzerland. We will meet again on Tuesday 27th November 2009 in Rome, Italy.
Thank you to all the people who are contributing to the success of this study, the children, parents, health care professionals, members of the Molecular Monitoring Group, members of SIOPEN, SIOPEN-R-NET ……..and those who continue to fund our work.

CANCER RESEARCH UK

CCLG

The Neuroblastoma Society
Fighting Childhood Cancer
www.nsoc.co.uk

SIOPEN R NET

Kinderkankerfonds

Italian Neuroblastoma Foundation

Nuclear Medicine

by Val Lewington, Chair

2009 has been a productive year for the Nuclear Medicine Committee.

2 educational meetings for nuclear medicine specialists have been held in London [May 2009, sponsored by an educational grant from Adam's Hats] and at the European Association of Nuclear Medicine annual conference in Barcelona [October 2009].

At each meeting, the revised method for semi-quantitative mIBG analysis was presented by members of the Expert Review Panel to a broad spectrum of nuclear medicine specialists and technologists. The interactive format encouraged enthusiastic audience participation and was favourably received. The revised reporting method is now accessible on the SIOPEN R NET website and plans are underway to implement this by the end of the year. Teaching files and an image atlas are now completed.

Posters summarising the work of the committee have been presented this year at the Society of Nuclear Medicine Annual Meeting in Toronto, SIOP annual conference in Sao Paulo and at the EANM in Barcelona. A manuscript describing the semi-quantitative reporting method will be submitted for publication shortly.

On behalf of the Committee, I wish to thank Adam's Hats for providing unstinting encouragement and support for a successful project which has set a new standard for paediatric nuclear medicine.

on behalf of the Expert Review Panel and SIOPEN Nuclear Medicine Committee.

Radiology

by Marcus Hörmann, Chair

This has been partly and more detailed reported in Paris 2009, during the spring meeting (see last newsletter).

By initiative of the radiotherapy group and the study centre in Vienna (Ruth Ladenstein) a joint meeting with the radiology group last December was held in Vienna. The aim of this meeting was twofold:

1.Quality assurance of the radiotherapy as delivered to the patients treated in the study so far, based on the radiotherapy and radiology data electronically available in the database.

2. A funding from the government in Austria was to be justified first by a report, and further on by a publication of the radiotherapy and radiology group. The data collected during this meeting therefore will be used for publication of a paper.

In preparation of the meeting it was necessary to upload images of as many patients as possible. Karin Dieckmann was very successful in gathering data from the centres.
Unfortunately a lot of examinations were only available as hard prints, which have to be scanned. At the Medical University of Vienna a volunteer was found who will step by step scan hard copies and burn the images on a CD-Rom to further be disposable for upload on the data base (for future meetings).
In the future we will once again circulate calls in order to complete the data base.

The setting of the meeting and the technical equipment was as it is supposed to be in a central radiology review meeting.
During this joint meeting with the radiology and radiotherapy committees, we wanted to use the available data to test for the first time if this system indeed allows us to perform quality assurance with an electronic platform. In addition to reviewing the data stored on the system, we also had the opportunity to review data available on CD-ROM but not yet stored on the server.

Each person present at the meeting had a computer with internet access to explore the stored data. Two computers were equipped with beamers so that radiology and radiotherapy data could be projected side-by-side for direct comparison and measurement. We went through all the patients for whom we hoped we had complete data either on the system or on CD.

Several technical problems with the database were encountered:
The problems concerned upload and review of data sets. These facts have been presented at the spring meeting, however emphasizes some difficulties that many centres are dealing with, and partly explain why so many radiologists are refusing to participate in the study.

Imaging protocols were according to the study protocol for CT (Multislice CT) and MR and quality was sufficient for evaluation in almost all cases reviewed.

Image evaluation:
A team consisting of radiologists and radiotherapists in consensus description evaluated images.
In the end a draft of paper (report) was written, and to my knowledge accepted for justification of the funding by the Austrian government.

Summer activities:
During summertime we tried to keep in contact with the radiology members to persuade radiologists to support and participate. During several meetings and by personal contact we tried to get commitment of European radiologists to increase the number of uploaded data sets.
Dedicated studies, as the in the meeting of last December, to improve upload have not taken place.
I am still asking for substudies (of all disciplines) with which upload and completion of data sets could be acclompished.

Working on the role, mission and vision of radiology in a cluster of disciplines.

Biology

By Peter Ambros, Chair

Inge Ambros (Austria), Frank Speleman, Nadine Van Roy (Belgium), Ales Vicha (Czech Republic), Jean Benard, Valérie Combaret, Jérome Couturier, Olivier Delattre, Gudrun Schleiermacher, Alexander Valent (France), Raymond L. Stallings (Ireland), Marta Jeison (Israel), Raffaella Defferrari, Katia Mazzocco, Gian Paolo Tonini (Italy), Klaus Beiske (Norway), Barbara Marques (Portugal), Nicole Gross (Switzerland), Eva Villamón, Rosa Noguera (Spain), Tommy Martinsson (Sweden), Clare Bedwell, Nick Bown, John Lunec, Deb Tweddle (UK), Peter F. Ambros (Austria, Chairman).

Challenge LINES Study

We are facing a dramatic change in the genetic work up of neuroblastoma tumours. To remind you, the LINES protocol will implement the presence of segmental aberrations of the tumour genome as treatment stratifying element in L2 and Ms tumours. Despite the fact that the SIOPEN Biology group was the very first group in Europe to provide the basis for implementing genetic information in the treatment stratification process, the implementation of the presence of segmental aberrations into the decision making process is a big challenge to the SIOPEN Biology Group and requires a change in methods and tumour handling. All of you know the power of MYCN status. What is possibly not known is that the implementation of this genetic marker in the decision making process caused a completely new orientation of the geneticist working in basic research. It also urged standardizations and quality assessment efforts to enable high and uniform quality of the results of all Biology Reference Centres. To give you an idea on the pioneering work the SIOPEN Biology group has undertaken, be reminded that not even a definition what to call a MYCN amplification existed in the world literature when we started our work. But neuroblastoma research went on and worldwide efforts enabled the identification of a number of redurrent segmental aberrations. For most of these gains or losses of parts of chromosomes which we define as segmental aberrations, the prognostic power is described at least in small patient cohorts. However, some questions still remain open which should, however, be solved in the near future. For example, the impact of segmental aberrations on OS in the infant patient group is not fully understood. A certain segmental aberration may render a tumour aggressive in a child over 18 months, whereas the same aberration may have a weaker impact on OS in patients below 18 months.

The challenge for the Biology group is to provide quality controlled pan- or mulitgenomic data within a short time frame. Therefore, we decided to apply either array based or PCR based techniques applicable in all SIOPEN Biology Reference Centres to learn about all relevant genomic changes. To allow comparability of the results, commercialized platforms were found to be preferable. Commercialized array platforms would be ideal to apply, but are not available in all centres. Therefore, we worked out a multi-genomic test system, a so-called neuroblastoma specific MLPA kit (multiplex ligation-dependent probe amplification). After designing a neuroblastoma specific probe set together with MRCHolland we performed internal testings and a number of inter-technique and inter-laboratory comparisons. I-FISH, arrayCGH and SNParray data were compared with the MLPA results giving an excellent concordance of the results. Furthermore, to maximise the robustness of MLPA results, we have tested the performance and reproducibility of the newly developed kit in two international inter-laboratory ring trials which revealed a very high concordance rate. Together with the aCGH and SNParray the SIOPEN Biology Group has at its disposal tools to identify all relevant genomic aberrations seen in neuroblastomas as defined by the INRG. However, to be able to come to conclusive results it is of upmost importance that well preserved tumour tissue is sent to the National/Regional Reference Centre in due time. Be also aware of the genetic heterogeneity of neuroblastoma tumours and send in tumour tissue from all morphologically different looking areas (also when seen sonographically). Ideally, frozen tumour should be sent to the National/Regional Reference Centre together with non fixed touch slides and ideally also normal tissue (peripheral blood or non infiltrated bone marrow). The Biology Committee agreed to perform stringent quality control of the data and to undertake interlaboratory comparisons to guarantee the highest possible quality of the genetic data.

The SIOPEN Biology Speciality Committee came together three times this year. We met on the 1. and 2. April 2009 in Institut Gustave Roussy, Paris and two times in the CCRI, Vienna, on the 28. and 29. May and on the 16. and 17. October 2009. Main issues discussed in these meetings were: INES and LINES Studies, MLPA and arrayCGH inter-laboratory and inter-technique comparisons, 2p Study, Unresectable Study, LNESGI and LNESGII.

Parents' corner

Susan Hay
Adam's Hats (UK)
SIOPEN Advisory Board Charities representative

Calling all charities

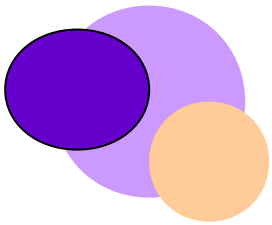
We are looking forward to seeing many of you at the special charities meeting, to be held alongside SIOPEN 09 AGM in Rome, on Tuesday 27 October.

This will be your chance to understand more about the charities involved with SIOPEN: what their priorities are, the way they work, and what they need from SIOPEN to release resources to contribute to its vital work.

It will also provide an opportunity to discuss the value of collaboration amongst charities, particularly through advocacy on a European, rather than an in-country platform, and to discuss how charities can help to raise the profile of SIOPEN within their own countries, encouraging others to join.

Charities will hear from SIOPEN about the difference it has made when it has had the support of charities, whether this was financial help, or channelling the voice of patients and families. And we will learn more about those projects that are most difficult to get off the ground, given the competition for European funds, and those that need charities' help most of all.

We look forward to welcoming you to the meeting. !



2009 S I O P E N Publications

- Ambros PF, Ambros IM, Brodeur GM, Haber M, Khan J, Nakagawara A,Schleiermacher G, Speleman F, Spitz R, London WB, Cohn SL, Pearson AD, Maris JM. International consensus for neuroblastoma molecular diagnostics: report from the International Neuroblastoma Risk Group (INRG) Biology Committee. Br J Cancer.2009 May 5;100(9):1471-82.
- Beiske K, Burchill SA, Cheung IY, Hiyama E, Seeger RC, Cohn SL, Pearson AD,Matthay KK; International neuroblastoma Risk Group Task Force. Consensus criteria for sensitive detection of minimal neuroblastoma cells in bone marrow, blood and stem cell preparations by immunocytology and QRT-PCR: recommendations by the International Neuroblastoma Risk Group Task Force. Br J Cancer. 2009 May 19;100(10):1627-37.
- Monclair T, Brodeur GM, Ambros PF, Brisse HJ, Cecchetto G, Holmes K, Kaneko M,London WB, Matthay KK, Nuchtern JG, von Schweinitz D, Simon T, Cohn SL, PearsonAD; INRG Task Force. The International Neuroblastoma Risk Group (INRG) staging system: an INRG Task Force report. J Clin Oncol. 2009 Jan 10;27(2):298-303.
- Cohn SL, Pearson AD, London WB, Monclair T, Ambros PF, Brodeur GM, Faldum A,Hero B, Iehara T, Machin D, Mosseri V, Simon T, Garaventa A, Castel V, MatthayKK; INRG Task Force. The International Neuroblastoma Risk Group (INRG)classification system: an INRG Task Force report. J Clin Oncol. 2009 Jan10;27(2):289-97
- Bagatell R, Beck-Popovic M, London WB, Zhang Y, Pearson AD, Matthay KK,Monclair T, Ambros PF, Cohn SL; International Neuroblastoma Risk Group. Significance of MYCN amplification in international neuroblastoma staging systemstage 1 and 2 neuroblastoma: a report from the International Neuroblastoma RiskGroup database. J Clin Oncol. 2009 Jan 20;27(3):365-70.
- Vermeulen J, De Preter K, Naranjo A, Vercruysse L, Van Roy N, Hellemans J,Swerts K, Bravo S, Scaruffi P, Tonini GP, De Bernardi B, Noguera R, Piqueras M,Cañete A, Castel V, Janoueix-Lerosey I, Delattre O, Schleiermacher G, Michon J, Combaret V, Fischer M, Oberthuer A, Ambros PF, Beiske K, Bénard J, Marques B,Rubie H, Kohler J, Pötschger U, Ladenstein R, Hogarty MD, McGrady P, London WB, Laureys G, Speleman F, Vandesompele J. Predicting outcomes for children with neuroblastoma using a multigene-expression signature: a retrospectiveSIOPEN/ COG/GPOH study. Lancet Oncol. 2009 Jul;10(7):663-71.
- Schreier G, Messmer J, Rauchegger G, Modre-Osprian R, Ladenstein R. A Web-based platform for interdisciplinary biomedical research. Front Biosci. 2009 Jan 1;14:2738-46.
- De Bernardi B, Gerrard M, Boni L, Rubie H, Cañete A, Di Cataldo A, Castel V, Forjaz de Lacerda A, Ladenstein R, Ruud E, Brichard B, Couturier J, Ellershaw C, Munzer C, Bruzzi P, Michon J, Pearson AD. Excellent outcome with reduced treatment for infants with disseminated neuroblastoma without MYCN gene amplification. J Clin Oncol. 27: 1034 -1040, 2009
- Canete A, Gerrard M, Rubie H, Castel V, Di Cataldo A, Munzer C, Ladenstein R, Brichard B, Bermúdez JD, Couturier J, de Bernardi B, Pearson AJ, Michon J. Poor survival for infants with MYCN-amplified metastatic neuroblastoma despite intensified treatment: the International Society of Paediatric Oncology European Neuroblastoma Experience. J Clin Oncol, 27:1014-1019, 2009

Hallmark on other Publications

Gigliotti AR, Di Cataldo A, Sorrentino S, Parodi S, Risso A, Buffa P, Granata C, Sementa AR, Fagnani AM, Provenzi M, Prete A, D'Ippolito C, Clerico A, Castellano A,Tonini GP, Conte M, Garaventa A, De Bernardi B. **Neuroblastoma in the Newborn. A study of the Italian Neuroblastoma Registry.**Eur J Cancer, 2009 Sept 18
CONCLUSION. The outcome of neuroblastoma in newborns is excellent. In localised tumours, surgery-related deaths outnumbered deaths due to disease. Symptomatic stage 4S patients were at greater risk of dying.

Garaventa A, Parodi S, De Bernardi B, Dau D, Manzitti C, Conte M, Casale F, Viscardi E, Bianchi M, D'Angelo P, Zanazzo GA, Luksch R, Favre C, Tamburini A, Haupt R. **Outcome of children with neuroblastoma after progression or relapse. A retrospective study of the Italian neuroblastoma registry.** Eur J Cancer. 2009 Jul 16
CONCLUSION. Survival of children with recurrent neuroblastoma is very poor. A small cohort of patients, mainly represented by children with stages 1 and 2 who underwent local recurrence or developed late relapse may still benefit from further conventional treatment. For the remaining larger proportion of patients, experimental therapies should be proposed.

Passoni L, Longo L, Collini P, Coluccia AM, Bozzi F, Podda M, Gregorio A, Gambini C, Garaventa A, Pistoia V, Del Grosso F, Tonini GP, Cheng M, Gambacorti-Passerini C, Anichini A, Fossati-Bellani F, Di Nicola M, Luksch R. **Mutation-independent anaplastic lymphoma kinase overexpression in poor prognosis neuroblastoma patients.** Cancer Res. 2009 Sep 15;69(18):7338-46.

Revet I, Huizenga G, Koster J, Volckmann R, van Sluis P, Versteeg R, Geerts D. **MSX1 induces the Wnt pathway antagonist genes DKK1, DKK2, DKK3, and SFRP1 in neuroblastoma cells, but does not block Wnt3 and Wnt5A signalling to DVL3**. Cancer Lett. 2009 Oct 6.

Combaret V, Hogarty MD, London WB, McGrady P, Iacono I, Brejon S, Swerts K, Noguera R, Gross N, Rousseau R, Puisieux A. **Influence of neuroblastoma stage on serum-based detection of MYCN amplification.** Pediatr Blood Cancer. 2009 Sep;53(3):329-31.

Ambros PF, Ambros IM. Free DNA in the blood serum can unmask MYCN amplified tumors. Pediatr Blood Cancer. 2009 Sep;53(3):306-7.

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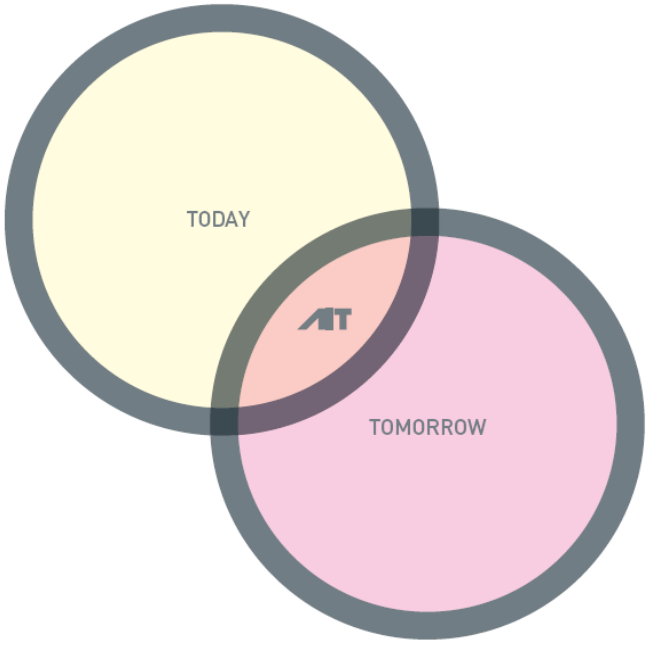

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