haematologica
the hematology journal

s1

ISSN 0390-6078
Official Organ of the European Hematology Association
Published by the Ferrata-Storti Foundation, Pavia, Italy
Volume 94, supplement no. 1, April 2009

www.haematologica.org

V International Congress of the European Working Group on Myelodysplastic Syndromes and Bone Marrow Failures

Rotterdam, The Netherlands, April 22-24, 2009

Guest Editors
M.M. van den Heuvel-Eibrink, C.M. Zwaan

Official Organ of the
European Hematology Association

haematologica
the hematology journal

The origin of a name that reflects Europe's cultural roots.

Ancient Greek

αἷμα [haima] = blood
αἵματος [haimatos] = of blood
λόγος [logos]= reasoning

Scientific Latin

haematologicus (adjective) = related to blood

Scientific Latin

haematologica (adjective, plural and neuter, used as a noun) = hematological subjects

Modern English

the hematology journal
2007 JCR® Impact Factor = 5.516

Haematologica/The Hematology Journal, as the official organ of the European Hematology Association (EHA), aims not only to serve the scientific community, but also to promote European cultural identity.

Official Organ of the European Hematology Association

haematologica
the hematology journal

Owned & published by the Ferrata Storti Foundation, Pavia, Italy

Affiliated Scientific Societies

SIE (Italian Society of Hematology, *www.siematologia.it*)
AEHH (Spanish Association of Hematology and Hemotherapy, *www.aehh.org*)
SETH (Spanish Society of Thrombosis and Hemostasis, *www.seth.es*)
SIES (Italian Society of Experimental Hematology, *www.sies.ws*)
AIEOP (Italian Association of Pediatric Hematology/Oncology, *www.aieop.org*)
EAHP (European Association for Haematopathology, *www.socforheme.org/eahp*)
SIdEM (Italian Society of Hemapheresis and Cellular Manipulation, *www.emaferesi.it*)

Official Organ of the
European Hematology Association

haematologica
the hematology journal

European Hematology Association (EHA)

EHA is a scientific society aiming to support research, education and clinical practice in hematology.
Its main objective is to be useful to scientific researchers, clinicians, medical students,
as well as all those working in other fields but who are interested in hematology.

The European Hematology Association was founded in June 1992.
Today, EHA – with over 3000 active members from 95 countries – is a consolidated organization
that pursues a large and growing number of projects and programs.

EHA aims to promote

- Exchange and dissemination of knowledge and scientific information in the field of hematology.
- Education and training in hematology.
- Medical practice in the area of hematology and the position of hematology as medical discipline.
- Scientific research in hematology.
- Exchange of information for all European doctors, scientists and other professionals interested in hematology.
- A unified European training program in hematology in collaboration with European National Societies of Hematology.

In order to achieve these goals, EHA

- Maintains regular contacts and organizes meetings with all European National Societies of Hematology.
- Holds an annual scientific and educational congress in a major European city; European Cooperative Groups and Networks are encouraged to take advantage of this major event to gather.
- Disseminates medical research, both basic and clinic, through the new journal Haematologica/The Hematology Journal.
- Has established a link with European National Societies of Hematology and other organizations such as the European Group for Bone Marrow Transplantation, European Association for Hematopathology, European Society of Medical Oncology, and American Society of Hematology.
- Provides postgraduate education through the annual congresses, seminars, courses, workshops and meetings organized in collaboration with the European School of Haematology.
- Has a Fellowship/Grants Program to promote research in hematology.
- Accredits scientific meetings and provides CME accounts in collaboration with the European National Societies for hematology.

If you recognize the need for a strong European Hematology Association
and would like to take advantage of the various activities of the Association,
you may wish to become a member of the EHA and contribute to its objectives.

Benefits of EHA Membership

- Subscription to Haematologica/ The Hematology Journal, including on-line access
- Reduced registration fee for EHA Annual Congresses
- Eligible to EHA Research Fellowships & Grants
- EHA Newsletter
- Access to EHA membership database
- Access to webcast sessions of the EHA Annual Congress

www.ehaweb.org

Official Organ of the European Hematology Association

haematologica
the hematology journal

supplement 1 - April 2009

Table of Contents

V International Congress of the European Working Group (EWOG) on Myelodysplastic Syndromes and Bone Marrow Failures

Rotterdam, The Netherlands, April 22-24, 2009

Guest Editors
M.M. van den Heuvel-Eibrink, C.M. Zwaan

Oral Communications

Posters

Official Organ of the European Hematology Association

haematologica
the hematology journal

Information for readers, authors and subscribers

Haematologica/The Hematology Journal (print edition, pISSN 0390-6078, eISSN 1592-8721) publishes peer-reviewed papers on all areas of experimental and clinical hematology. The journal is owned by a non-profit organization, the Ferrata Storti Foundation, and serves the scientific community strictly following the World Association of Medical Editors (WAME) recommendations on publication ethics policies for medical journals (www.wame.org/pubethicrecom.htm).

Haematologica/The Hematology Journal publishes editorials, perspectives, research papers, decision making & problem solving papers, review articles, brief reports and scientific letters. Manuscripts should be prepared according to the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, prepared by the International Committee of Medical Journal Editors (ICMJE) and fully available online (http://www.icmje.org). Additional papers may be considered for the purely online journal (Haematologica/The Hematology Journal on Internet, eISSN 1592-8721). Because there are no space constraints online, Haematologica/The Hematology Journal will publish several items deemed by peer review to be scientifically sound and mainly useful as educational papers. These will include case reports, irreplaceable images, educational material from scientific meetings, meeting abstracts, and letters to the Editor.

Papers should be submitted online at http://www.haematologica.org/.

Conflict of interests. According to the International Committee of Medical Journal Editors (http://www.icmje.org/#conflicts), "Public trust in the peer review process and the credibility of published articles depend in part on how well conflict of interest is handled during writing, peer review, and editorial decision making". The ad hoc journal's policy is reported in detail online (http://www.haematologica.org/misc/about.dtl).

Galley Proofs and Reprints. Galley proofs should be corrected and returned by email, fax or express delivery within 48 hours. Minor corrections or reasonable additions are permitted; however, excessive alterations will require editorial re-evaluation and will be possibly charged to the authors. Papers accepted for publication will be printed without cost. The cost of printing color figures will be communicated upon request. Reprints may be ordered at cost by returning the appropriate form sent by the Publisher.

Transfer of Copyright and Permission to Reproduce Parts of Published Papers. Authors will grant copyright of their articles to the Ferrata Storti Foundation. No formal permission will be required to reproduce parts (tables or illustrations) of published papers, provided the source is quoted appropriately and reproduction has no commercial intent. Reproductions with commercial intent will require written permission and payment of royalties.

Haematologica/The Hematology Journal is published in two printed editions: International (worldwide except Italy, Spain, Portugal and Latin America), and Spanish (Spain, Portugal and Latin America). Detailed information about subscriptions is available online at URL http://www.haematologica.org. While access to the online journal is free, online access to some additional items available on http://www.haematologica.org may require either institutional or personal subscription.

Rates of the International edition for the year 2009 are as following:

	Institutional	*Personal*
Print edition (including full access to the online CME for personal subscribers)	*Euro 400*	*Euro 150*
Full access to the online CME only		*Euro 75*

To subscribe to the International edition, please visit our web site http://www.haematologica.org/misc/subscribe.dtl or contact: Haematologica Office, via Giuseppe Belli 4, 27100 Pavia, Italy (phone +39.0382.27129, fax +39.0382.394705, E-mail: office@haematologica.org).

Advertisements. Contact the Advertising Manager, Haematologica Office, via Giuseppe Belli 4, 27100 Pavia, Italy (phone +39.0382.27129, fax +39.0382.394705, e-mail: info@haematologica.org).

Direttore responsabile: Prof. Edoardo Ascari; Autorizzazione del Tribunale di Pavia n. 63 del 5 marzo 1955.
Editing: MEDIT Medical Editions, via G. Belli 4, Pavia, Italy
Printing: Tipografia PI-ME, via Vigentina 136, Pavia, Italy. Printed in April 2009.

V International Congress of the European Working Group on Myelodysplastic Syndromes and Bone Marrow Failures

Rotterdam, April 22-24, 2009

Childhood myelodysplastic syndromes

CO-01

MYELODYSPLASTIC SYNDROME AND MYELOPROLIFERATIVE DISEASE IN CHILDREN: A PROSPECTIVE REGISTRATION OF 222 CASES

A Manabe, S Hirabayashi, S Watanabe, Y Zaike, M Tsuchida, A Masunaga, A Yoshimi, M Ito, A Kikuchi, K Tsuji, A Ohara, S Kojima, T Nakahata

St. Luke's International Hospital, Tokyo, Japan and the MDS Committee of the Japanese Society of Pediatric Hematology, Tokyo, Japan

Objectives. Myelodysplastic syndrome and myeloproliferative disease are rare in children, however, they are important because diagnosis is difficult and the prognosis is generally poor. We conducted a prospective registration of patients suspected of having MDS employing pathological central review through the MDS Committee of the Japanese Society of Pediatric Hematology. *Results*. Of total, 222 children were enrolled between 1999 and 2006: 96 cases of primary MDS (RA 52, RAEB 25, RAEB-T 19, by FAB classification; RA 28, RCMD 24, RAEB-1 11, RAEB-2 28, AML 5 by WHO classification), 71 cases of JMML (excluding patients with Noonan syndrome and NF1), 1 case of CMML, 19 cases of therapy-related MDS (t-MDS), and 35 cases with constitutional predisposition to MDS. The median age at diagnosis was 4.5 years (range, 0.0-19.2 years. The median follow up for 144 surviving patients was 39 months (range 1 to 118 months) after diagnosis. The 4-year overall survival (OS) for primary MDS, JMML, t-MDS and constitutional MDS was 69%, 57%, 70% and 58% respectively. In primary MDS classified by FAB, OS for RA, RAEB and RAEB-T was 90%, 37% and 40% respectively, while in primary MDS classified by WHO, OS for RA, RCMD, RAEB-1 and RAEB-2 was 95%, 85%, 41% and 35%. Thus, the WHO system could also be employed in children. The data of karyotype were available in 214 of 222 patients (96%). Cytogenetic abnormality was noticed in 55% of primary MDS. In primary MDS, OS for -7/7q-, complex, others and normal were 42%, 25%, 80% and 78%. Thus, the cytogenetic classification defined by the IPSS was useful to predict outcome in childhood primary MDS while it was not the case in patients with JMML. *Discussion.* Although we obtained important information employing a prospective central reviewing, we could not define an optimal management for patients in each category. Since MDS and MPD in childhood are rare, an international cooperation is urgently needed to establish a better classification and develop novel treatments.

CO-02

MYELODYSPLASTIC SYNDROMES IN ADOLESCENTS: CHARACTERISTICS AND CLINICAL OUTCOME

M Zecca, P Noellke, E Bergsträßer, B De Moerloose, H Hasle, S Matthens-Martin, L Sainati, J Stary, B Strahm, MM van den Heuvel-Eibrink, D Wójcik, CM Niemeyer, F Locatelli

On behalf of EWOG-MDS, Pavia, Italy

Myelodysplastic syndromes (MDS) are a rare disorder in children, representing only 4-5% of childhood haematological malignancies. Specific information on adolescents (defined as patients with age between 15 and 19 years) is extremely scarce. For this reason, we analysed the data of patients affected by MDS other than JMML, prospectively enrolled in the EWOG-MDS 98 protocol and reported to the EWOG-MDS registry, in order to evaluate the characteristics of MDS in the adolescent population and the clinical outcome of this group of patients. A total of 482 patients fulfilling the inclusion criteria were analyzed: 383 (79%) were younger than 15 years and were defined children and 99 (21%) had an age between 15 and 19 yrs and were considered adolescents. No significant difference in terms of gender distribution, WHO MDS classification, primary vs. secondary MDS, secondary subtype form and marrow cellularity was observed between the two groups of patients. On the contrary, a significantly higher percentage of complex karyotypes was observed in adolescents as compared to children (15% *vs.* 6%), while monosomy 7 was more frequent in children than in adolescents (27% *vs.* 15%, $p<0.05$). Administered treatment was similar in the 2 groups, with nearly 70% of patients who received an allogeneic SCT (from a matched unrelated donor in 55% of cases) in both groups. Overall survival from diagnosis was 62% for adolescents and 70% for children (p=N.S.). Considering patients with refractory cytopenia (RC), EFS after HSCT was 88% for adolescents and 76% for children (p=N.S.), with children showing a higher TRM (17% *vs.* 4%, p=N.S). No difference in outcome was observed, also in advanced MDS patients (RAEB, RAEB-T and MDS-related AML): EFS being 48% for adolescents and 54% for children (p=N.S.). Nevertheless, TRM cumulative incidence was significantly higher in adolescents as compared to children (42% *vs.* 16%, $p<0.05$), while relapse cumulative incidence was higher in children (30% *vs.* 10%, p=N.S.). Adolescents with a complex karyotype had a significantly worse prognosis, while no difference was observed between primary and secondary MDS patients. In conclusion, our data show that the outcome of adolescents with MDS treated in pediatric institutions is similar to that of children. However, adolescents with advanced MDS showed a very high TRM. A specific, less toxic, conditioning regimen and a more effective GVHD prophylaxis are needed for this subgroup of patients.

IS-01

IMMUNOPHENOTYPING OF MYELODYSPLASTIC SYNDROMES

VHJ van der Velden,[1] A van de Loosdrecht[2]

[1]*Department of Immunology, Erasmus MC, Rotterdam;* [2] *Department of Hematology, VUmc, Amsterdam, The Netherlands*

The diagnosis of myelodysplastic syndromes (MDS) is mainly based on morphology and cytogenetic analysis. However, recent studies indicate that flowcytometric immunophenotyping can identify abnormalities in the maturation pathways of erythroid, monocytic, and myeloid cell lineages in MDS patients. In addition, immunophenotyping allows the detailed characterization of abnormalities in the CD34-positive progenitor compartment. It has been shown that immunophenotypic abnormalities may contribute to the diagnosis of MDS, especially in cases in which morphology and cytogentic analysis are inconclusive. Furthermore, the presence of abnormal myeloid blasts (e.g. expression of CD7)

is related to clinical features and the prognosis of the patient. Immunophenotyping has been proposed as a diagnostic and prognostic criteria for MDS. Interpretation of flowcytometric data is however complex. It will therefore be necessary to develop standardized antibody panels and procedures for immunophenotypic analysis of MDS patients; this is currently being done in several international networks. In addition, a uniform 'flow score system' should be developed and evaluated for its diagnostic and prognostic significance. This may be facilitated by new software for flowcytometric data analysis, like Infinicyt.

CO-03

PHENOTYPIC CHARACTERIZATION OF PEDIATRIC MDS-RELATED-ACUTE MYELOID LEUKEMIA

M Veltroni,[1,2] L Sainati,[1] M Zecca,[3] S Fenu,[4] G Tridello,[1] A Maria Testi,[5] A Di Cesare Merlone,[3] B Buldini,[1] A Leszl,[1] L Lo Nigro,[6] D Longoni,[7] G Bernini,[2] A Pession,[8] F Locatelli[3] G Basso[1]

[1]Laboratory of Pediatric Onco-Hematology, Department of Pediatrics, University Hospital of Padua, Padua; [2]Department of Pediatrics, University of Florence, Florence; [3]Pediatric Hematology/Oncology, Fondazione IRCCS Policlinico San Matteo, Pavia; [4]Department of Hematology, Azienda Ospedaliera San Giovanni-Addolorata, Roma; [5]Cattedra di Ematologia La Sapienza, Roma; [6]Centro di Riferimento Regionale di Ematologia ed Oncologia Pediatrica, Università di Catania, Catania; [7]Clinica Pediatrica, Università di Milano-Bicocca, Ospedale San Gerardo, Monza; [8]Pediatric Oncology and Hematology, University of Bologna, Bologna, Italy

MDR-AMLs shares many clinical and biological features with MDS and have a worse prognosis than *de novo* AML, showing resistance to chemotherapy. The WHO classification considers MDS and MDR-AML as a single entity. If no obvious history of MDS is known, the distinction between MDR-AML and *de novo* AML can be difficult. The diagnosis of MDS is based on morphology and cytogenetics; nevertheless, clonal abnormalities can be identified in a minority of cases. Recently, several attempts to analyse MDS by flow cytometry (FC) have been reported in adult patients. We previously reported that pediatric MDS blast cells displayed an immature myeloid phenotype ($CD34^+$, $CD117^+$, $CD38^+$, $HLA\text{-}DR^+$, $CD33^+$) with a significant correlation between CD7 expression and poor outcome. The aim of our study was to evaluate the blast phenotype in children with MDR-AML, in comparison with data obtained from MDS and *de novo* AML pediatric cases. We evaluated by multiparametric FC 11 MDR-AML cases, median age 11.5 years (range: 2.6-17.9) and compared the results with a) 26 MDS cases, median age 13.8 years (range: 2.3-18), b) 145 *de novo* AML cases, median age 7 years (range: 0.1-18). Diagnoses were made according to the FAB criteria. As control group, 12 healthy age-matched donors for allogenic bone marrow transplantation were also studied. MDR-AML expressed early myeloid antigens (CD34, CD117) in a high percentage (82% and 100% respectively), as MDS patients, and maturation markers (CD11b, CD64, CD15) as *de novo* AML; CD7 was more frequently expressed in MDR-AML (64%), than *de novo* AML (40%) and MDS (50%). AML occurring after a history of MDS seems to present a distinct phenotype, co-expressing phenotypic features of MDS and *de novo* AML, together with a strong expression of CD7. Even though confirmation in a larger setting is needed, these data suggest that MDR-AML has a distinct phenotype and this could have a diagnostic impact and provide future directions for biologic and therapeutic studies.

CO-04

IMMUNOPHENOTYPIC FINDINGS IN CHILDREN WITH MYELODYSPLASTIC SYNDROME/JUVENILE MYELOMONOCYTIC LEUKEMIA AND CYTOGENETICALLY PROVEN MONOSOMY 7 AS A SOLE ABNORMITY OR AS A PART OF OTHER GENETIC ABNORMALITIES

E Mejstříková,[1] V Pelková,[1] M Suková,[1] Z Zemanová,[2] K Michalová,[2] E Vodičková,[3] D Pospíšilová,[4] V Mihál,[4] J Šterba,[5] Y Jabali,[6] J Hak,[7] K Toušovská,[7] Z Černá,[8] T Votava,[8] P Sedláček,[1] R Formánková,[1] J Trka,[1] J Stary,[1] O Hrušák[1]

[1]Department of Pediatric Hematology and Oncology, 2nd Medical School and University Hospital Motol, Prague; [2]Institute of Clinical Biochemistry and Laboratory Diagnostics, First Faculty of Medicine, Prague; [3]Department of Clinical Hematology, University Hospital Motol, Prague; [4]Department of Pediatrics, University Hospital Olomouc; [5]Department of Pediatric Hematology and Oncology, University Hospital Brno Bohunice; [6]Department of Pediatrics, Hospital eské Bud jovice; [7]Department of Pediatrics, University Hospital Hradec Kralove; [8]Department of Pediatrics, University Hospital Pilsen, Czech Republic

Monosomy 7 or partial loss in the long arm of this chromosome is a recurrent nonrandom abnormality in MDS and AML. Flow cytometry (FC) is an established method for complex evaluation of various cellular subsets and estimation of maturation abnormalities. Role of FC in the diagnostic process of childhood MDS is less clear. Part of genetic abnormalities especially in acute leukemias has specific immunophenotypic pattern with a potential usefulness for the genotype prediction. Monosomy 7 as a sole abnormity or as a part of complex karyotype was identified in 15 pts (2 pts with complex karyotype, 1 pt with additional trisomy 8) out of 46 Czech patients with available information cytogenetic data and who were reported into the EWOG DS database between years 1998-2008. Patients with advanced forms of MDS were analyzed using more extensive panel (in MDS RC at least lymphocyte subsets together with CD34 and/or CD117 percentage were analyzed). Patients were grouped into the following MDS subtypes: refractory cytopenia (RC) 5, RAEB/RAEB-t 7 and JMML 3. Atypical population by FC was defined as an increase of immature blasts with or without aberrant expression of antigens from other lineages or with an atypical pattern of granularity according to Ssc parameter. Patients with RC monosomy 7 do not differ in percentage of immature cells/blasts CD34pos from remaining RC patients. The following individual abnormalities were identified in RC patients and monosomy 7: excess of double positive CD4&CD8 T cells in PB and BM first detected more than 2 years before diagnosis (1 pt), low to negative CD38 on CD34pos blasts (1 pt), high CD45 on granulocytic lineage (1 pt), remaining 2 pts were without detectable abnormalities. Advanced forms can be characterized: 1) by an increased percentage of immature CD34pos myeloid blasts (typically with low to negative CD38) (4 pts), or 2) by the presence of blasts with megakaryocytic differentiation (2 pts). In addition, in 1 patient we did not observe any significant abnormalities; JMML patients had typical findings similar to the other JMML patients: atypical pattern of maturation and/or increased percentage of B cells and/or, increase percentage of CD34 and CD117pos. *Conclusion.* Immunophenotypic findings in patients with monosomy 7 are heterogeneous and reflect disease stage. Advanced forms of MDS are characterized either by very immature phenotype or by megakaryocytic differentiation. No abnormality identified in patients with monosomy 7 is specific for this subgroup and was identified also in other MDS patients without monosomy 7. Supported by M MT VZ MSM0021620813, MZO 00064203 VZ FNM, IGA NR/9531-3.

Biology and molecular background of childhood myelodysplastic syndromes

IS-02

FINDING NEW TRANSLOCATION PARTNERS IN MYELODYSPLASTIC SYNDROME

C Mecucci

Hematology, University of Perugia, Italy

Conventional cytogenetics is a powerful tool to classify MDS. However more than 50% of cases show a normal karyotype and significant advances were obtained by the help of additional technologies such as FISH, expression profiling, microarray-CGH, SNPs. In our laboratory we developed a strategy which integrates chromosome walking by FISH with PCR, to identify genes at chromosomal breakpoints and to investigate partners of fusion genes originating from translocations, inversions, deletions, insertions. Important contributions of this strategy in MDS are represented by discovery of multiple translocations involving promiscuous genes, such as PDGFRB, ETV6, FGFR1 in cases with clinical features overlapping MDS and Myeloproliferative Disorders (MDS/MPD). Another gene which behaves as a promiscuous gene is NUP98, encoding for a nucleoporin with an important role in nucleus-cytoplasm trafficking. NUP98 is usually rearranged in high risk patients and we and others identified at least two categories of partner genes, with or without homeodomains. This approach is also helpful to understand the molecular counterpart of rare reciprocal and complex rearrangements.

IS-03

CRITICAL APPRAISAL OF NOVEL GENETIC AND MOLECULAR TOOLS FOR CHILDHOOD MDS AND BONE MARROW FAILURE

HB Beverloo

Department of Cytochemistry and Cytometry, University of Leiden, Leiden, The Netherlands

Chromosome analysis is an important diagnostic tool for the detection of chromosomal abnormalities in hematological disorders. These abnormalities have proven to be relevant for diagnosis and prognosis, and the presence of some of these is related to the response to chemotherapy. The WHO classification for AML also incorporates cytogenetic data as a component of the classification system. In addition unraveling of e.g. the translocations and inversions has led to the identification of the involved genes through which insight in normal hematopoiesis and leukemogenesis was obtained. In the recent years the cytogenetic field has evolved rapidly through the use of microarray-based comparative genomic hybridization (array CGH) or the use of SNP-arrays. Small, submicroscopic, deletions and amplicifications were identified in several hematological subtypes. In addition SNP arrays enabled the detection of copy neutral loss of heterozygosity, in principle allowing tumor suppressor gene detection. These techniques have successfully been applied in several studies on acute leukemia where in general there is a high percentage of aberrant cells. If these techniques are applicable for diseases with lower amounts of abnormal cells, then it is expected that much knowledge will be obtained through these techniques. A great challenge is in the determination of the relevance of these abnormalities for diagnosis, prognosis and treatment stratification. The use of the array and other recently developed techniques will be discussed with respect to application in MDS and bone marrow failure.

CO-05

GENE EXPRESSION PROFILE ANALYSIS OF PAEDIATRIC MDS PATIENTS CORRELATES WITH FAB CLASSIFICATION AND HAS PROGNOSTIC RELEVANCE

S Bresolin,[1] L Trentin,[1] G te Kronnie,[1] M Zecca,[2] L Sainati,[1] F Locatelli,[2] G Basso[1]

[1]Laboratory of Oncohematology, Department of Paediatrics, University of Padova; [2]Laboratory of Oncohematology, Department of Paediatrics, IRCSS, Policlinico San Matteo, Pavia, Italy

Introduction and objectives. Myelodysplastic syndromes (MDS) are a rare, heterogeneous disorder characterized by ineffective hematopoiesis and a varying propensity to transform into acute myeloid leukaemia (AML). We analyzed the gene expression profile (GEP) of a cohort of paediatric MDS and AML patients with the aim of identifying a distinctive signature for these two diseases. Moreover, we investigated whether GEP analysis correlated with FAB classification and if it might have prognostic relevance. *Methods.* We performed gene expression analysis of 21 paediatric MDS (8 RC, 7 RAEB and 4 RAEB-t), of 50 *de novo* AML patients and of 6 normal bone marrow (BM) aspirates. We used Affymetrix HG U133 Plus 2.0 oligonucleotides microarray platforms. Analysis was performed using Partek Genomic Suite Software, Ingenuity Pathway Analysis (IPA) and Leukemia Classifier version 7 (LCver7). *Results*. Unsupervised hierarchical clustering analysis of all patients separated MDS from *de novo* AML and placed the normal BM into the MDS cluster. Remarkably, all MDS cases that evolved into AML within one year, except one, clustered together inside the *de novo* AML group. Furthermore, we classified the MDS samples using the LCver7 classifier, an algorithm developed during the international MILE (Microarray Innovation in LEukemia) study that gives an overall cross-validation accuracy of >95% for distinct sub-classes of paediatric and adult leukemias classification using GEPs. Fifty-five % of MDS patients had an AML-like signature, while 45% had a non-AML like signature. Remarkably, 90.9% of MDS samples that were characterized by an AML-like signature are RAEB/RAEB-t at diagnosis, while 77.8% of MDS specimens with a non-AML like signature are RC ($p<0.01$). One sample had a no call classification. So, we observed here for the first time among paediatric MDS samples a statistical correlation between FAB classification and the gene expression signatures. Moreover, we could also detect a correlation between the classifier call and evolution into AML: 86% of AML-like MDS progressed to AML while only 29% of non-AML like MDS showed a progression ($p<0.05$). Interestingly, several RAEB/ RAEB-t samples showed an up-regulation of genes involved in AML, including HOX genes, FLT3, KIT, WT1 and MEIS1. *Discussion.* Gene expression technology can not only distinguish between MDS, *de novo* AML and normal BM, but can also identify an AML-like signature with a higher risk of AML evolution.

IS-04

IMPAIRED HEME SYNTHESIS IN MYELODYSPLASTIC SYNDROMES?

N Gattermann

Dept. of Hematology/Oncology, Heinrich-Heine-University, Düsseldorf, Germany

It is by no means obvious that myelodysplastic syndromes are associated with impaired heme synthesis. In fact, one would expect microcytic hypochromic anemia as a consequence of impaired heme synthesis, whereas patients with MDS usually present with moderately elevated MCV. However, MDS pathophysiology may involve overlapping derangements of several metabolic pathways, thereby rendering interpretation of MCV difficult. A strong indication of impaired heme synthesis is found in one of the MDS types, namely refractory anemia with ring sideroblasts (RARS). Here, mitochondrial iron accumulation suggests that iron imported into mitochondria is not properly used to make heme. Since there is no deficiency of the penultimate product of the heme biosynthetic pathway, protoporphyrin IX, the defect can be narrowed down to the last step, i.e. incorporation of iron by ferrochelatase. However, no mutations have been found in this enzyme. Ferrochelatase processes ferrous iron (Fe^{2+}) but cannot utilize ferric iron (Fe^{3+}). In ring sideroblasts, mitochondrial iron accumulates as ferric iron (Fe^{3+}), mainly bound to mitochondrial ferritin, not readily available for heme synthesis. Our working hypothesis postulates that the problem may be attributable to a failure of the respiratory chain (RC) to effectively remove oxygen from the mitochondrial matrix. Increased O_2 in erythroblast mitochondria could turn imported Fe^{2+} into Fe^{3+} which will then be rejected by ferrochelatase and therefore accumulate in the mitochondrial matrix. Since mitochondrial respiratory chain defects can be caused by mutations of mitochondrial DNA, we performed a comprehensive analysis of the mitochondrial genome in 104 patients with MDS (Wulfert *et al.,* Exp Hematol, 2008), identifying acquired, clonally expanded mtDNA point mutations in 56% of patients. However, the functional consequences of these mutations are difficult to assess. Recently, research on disturbed cellular iron metabolism has focused on impaired mitochondrial iron sulfur cluster biosynthesis. Such a defect can mimick iron deficiency by turning cytoplasmic aconitase, an Fe/S cluster protein, into an iron regulatory protein which induces increased cellular iron uptake. In erythroblasts, the flux of incoming iron is directed to the mitochondria where it may cause mitochondrial iron overload.

CO-06

PEDIATRIC MYELODYSPLASTIC SYNDROME IN A FAMILY WITH MITOCHONDRIAL DNA MUTATIONS

ACH de Vries,[1] CM Zwaan,[1] IFM de Coo,[2] D Hellebrekers,[3] ATM Hendrickx,[3] HJM Smeets,[3] HB Beverloo,[4] MM van den Heuvel-Eibrink[1]

[1]Department of Pediatric Oncology/Hematology, Erasmus Medical Center, Sophia Children's Hospital, Rotterdam; [2]Department of Pediatric neurology, Erasmus Medical Center, Sophia Children's Hospital, Rotterdam , The Netherlands; [3]Laoboratory of cytogenetics, DNA diagnostics, University Hospital Maastricht, The Netherlands; [4]Department of Cytogenetics, Erasmus Medical Center, Rotterdam, The Netherlands

Introduction. Until now only scarce information is available on the pathophysiology and molecular background of childhood myelodysplastic syndrome (MDS). Mutations in the mitochondrial DNA (mtDNA) have been found in 56% of adult MDS patients, with an increasing frequency in more advanced MDS and with rising age. In general, cells contain different amounts of mitochondria and can simultaneously harbour wildtype and mutated mtDNA. Co-existence of normal and mutant mtDNA is called heteroplasmy, whereas the existence of only mutated mtDNA is called homoplasmic. No reports are yet available on the role of mtDNA mutations in pediatric MDS. *Cases.* We describe a family with three daughters of which a 15-year-old girl, one of a monozygotic twin, presented with RAEB. Cytogenetics demonstrated a der(1;7)(q10;p10), +11 (case 1). Evaluation of the twin sister showed asymptomatic MDS-refractory cytopenia (RC, harbouring +8 (case 2). An older sister, 17 years old, was known with a visual handicap due to a disorder in the oxidative phosphorylation caused by decreased enzyme activities in complex I of the respiratory chain. Two homoplasmic mtDNA mutations (m.13528A>G and m.13565C>T) that might be involved in this mitochondrial phenotype were identified in blood, fibroblasts, bone marrow and muscle tissue (case 3). Case 1 also showed both mtDNA mutations in blood, urine, fibroblasts and bone marrow. The m.13528A>G mutation was homoplasmic (71%) in the bone marrow and homoplasmic in the other tissues. Case 2 revealed both homoplasmic mutations in blood, fibroblasts and urine. Functional studies demonstrated a relatively decreased enzyme activity in complex I of the respiratory chain in case 1 and 3. The enzyme activity in case 2 was normal (all measured in fibroblasts). *Discussion.* We describe the first case childhood MDS with (germline) mtDNA mutations. We hypothesize in our patient mtDNA mutations, catalysed by ATP deficiency, resulted in previously described genetic instability of the stem cells and facilitate the event that initiates an MDS clone. Moreover this may indicate that mtDNA mutations may play a role in the pathogenesis of (subgroups of) MDS, especially in familial cases, although more functional studies are needed to further investigate the exact role of the mtDNA mutations in MDS.

Molecular aberrations and new therapy approaches

IS-05

FUTURE DEVELOPMENT OF NOVEL THERAPIES IN MDS; LESSONS FROM ADULT MYELODYSPLASTIC SYNDROME

E Hellström Lindberg

Division of Hematology, Karolinska University Hospital, Karolinska University Hospital Huddinge, Stockholm, Sweden

The WHO classification from 2008 should now be used to divide myelodysplastic syndrome (MDS) in refractory cytopenia with unilineage dysplasia, refractory anemia with ring sideroblasts (RARS), refractory cytopenia with multilineage dysplasia (RCMD±RS), refractory anemia with excess of blasts (RAEB-1 and RAEB-2), MDS with <5% blasts and an isolated deletion of 5q, and unclassifiable MDS. Prognosis of MDS has hitherto been based on the International Prognostic Scoring System (IPSS) from 1997, but new systems taking the WHO classification and the transfusion dependency into account (WPSS) probably add valuable information. The decision about treatment of adult MDS is based on MDS subtype, age and risk profile and ranges from watchful waiting to allogeneic stem cell transplantation. In low-risk MDS the main aim of treatment is to improve blood values and quality of life. Erythropoietin± the addition of G-CSF is useful in patients with a moderate transfusion need, in particular in RARS. Treatment has recently been shown to be associated with improved survival. Thrombopoietin derivates are in clinical trials, and may constitute a hope for patients with severe thrombocytopenia. Antithymocyte globulin may be of use in younger (<65y) patients with hypo and normoplastic MDS, no blast increase and no ringsideroblasts. Lenalidomide for low-risk patients with del(5q) is highly efficacious for the anemia and is approved in the US, however disapproved by EMEA, who has not been able to exclude a treatment-related risk for leukemic transformation. The major breakthrough during the last years is the results of azacytidine in high-risk MDS, which has been shown to significantly prolong survival and time to leukemic transformation. Azacytidine is now emerging as a new first-line treatment for this MDS Group. Allogeneic SCT is the only potentially curative treatment for MDS, but results are still impaired by a high TRM and a high relapse rate. New concept to improve cure in MDS most likely involve the combination of SCT, demethylating and conventional cytostatic drugs, reinforced immune therapy and targeted therapy.

CO-07

TELOMERE/CENTROMERE FLUORESCENCE *IN SITU* HYBRIDIZATION IN PATIENTS WITH DIFFERENT SUBTYPES OF MYELODYSPLASTIC SYNDROME

K Lange,[1] W Hofmann,[1] H Kreipe,[2] A Ganser,[3] B Schlegelberger,[1] G Göhring[1]

[1]Institute of Cell and Molecular Pathology, Hannover Medical School, Hannover; [2]Institute of Pathology, Hannover Medical School, Hannover; [3]Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany

Objective. Telomere shortening and genomic instability play an important role in the development of myeloid neoplasia. Published data are only available on the average telomere length in myelodysplastic syndrome (MDS) and AML but not on the telomere length of individual chromosomes. *Methods.* Using Telomere/Centromere Fluorescence *in situ* Hybridization (T/C-FISH), a centromere-calibrated method generating fluorescence signals proportional to the number of telomere repeats, the telomere length of each chromosome can be measured quantitatively. After validation, T/C-FISH was established following R-banding of the same metaphase. This was performed on 18 adult patients with secondary AML after MDS with a complex karyotype as well as on 3 cohorts (10 patients each) with either isolated monosomy 7, isolated deletion in 5q or normal karyotype. The control cohort comprised 18 age- and gender-matched healthy individuals. *Results.* In line with previous results, telomeres of patients with complex karyotypes, i.e. the mean telomere length of a metaphase (7.5 kb), as well as the average value of each chromosome arm are significantly shorter than those of the healthy controls (9.6 kb) (p<0.005). Irrespective of the subtype, the mean telomere length was shorter in patients with MDS than in healthy individuals. However, there was

no significant difference within the cytogenetic subgroups of MDS. Although telomeres of some chromosome arms were particularly short and neo-telomeres have been found in some patients with complex karyotypes, there was no correlation between telomere length and specific aberrations. Rearrangement of the cohorts according to the WHO subtype of MDS, showed a correlation of short telomere lengths and MDS subtypes associated with an impaired prognosis. *Discussion.* Thus, telomere erosion may play a role in the development of complex aberrant karyotypes in MDS. An upregulation of telomerase and thus elongation of telomeres in this stage of MDS could be one possible explanation for these observations.

CO-08

TELOMERASE GENE MUTATION SCREENING AND TELOMERE LENGTH DETECTION IN CHINESE PATIENTS WITH BONE MARROW FAILURE SYNDROME

B Han, B Liu, W Cui, Y Zhao

Hematology, Peking Union Medical Collegue Hospital, Beijing, China

Acquired bone marrow failure syndrome (BMF) is a group of diseases including Aplastic anemia(AA), Melodysplastic syndrome (MDS) and paraoxymal nocturnal hemoglobinuria (PNH). Some BMF patients have short telomeres in their peripheral nucleated cells. The length of telomere is maintained by a group of enzymes called telomerase complex. The core components of this complex are a RNA template and a reverse transcriptase, called TERC and TERT, respectively. Recently several studies in the west and Japan have disclosed the presence of telomerase complex gene mutation in a small group of patients with acquired bone marrow failure. They speculated that this small group of patients might represent a subset of cryptogenic DKC, in which the premature exhaustion of hematopoietic reservoir is caused by mutations in the telomerase gene. This group of patients, though very small in number, would benefit from early bone marrow transplantation instead of traditional immunosuppressive therapy. The incidence of aplastic anemia in China people is relatively high compared with that in the western country. But there has so far been no study in China about the incidence of telomerase gene mutation in acquired bone marrow failure and its relationship with telomere length. *Objectives*. To study the incidence of telomerase gene (namely TERC and TERT) mutation in Chinese patients with acquired bone marrow failure and explore its relationship with telomere shortening. *Methods*. Blood samples from 90 patients with AA, MDS, and PNH in northern China were collected and performed TERC and TERT mutation analysis. Telomere length is measured by Southern blotting and compared with their normal counterparts. Results 2 TERC mutations and 2 TERT mutations were identified in 90 BMF patients. Among them, 3 mutations are reported first time. 1 patient with TERT mutation, however, was finally diagnosed as DKC instead of AA. The incidence of telomerase gene mutation in Chinese people with acquired bone marrow failure is 3.4%, similar to that of the western people. Southern Blot analysis showed the small group of patients carrying TERC and TERT mutations has very short telomeres, compared both with normal controls and with their aplastic counterparts. They represent a group of cryptic congenital bone marrow failure patients and would benefit from early transplantation instead of traditional therapy. *Conclusions.* The incidence of telomerase gene mutation in Chinese people with acquired bone marrow failure is 3.4%, similar to that of the western people. This small group of patients has very short telomeres, it is thus clinically important to screen for this small group of patients.

CO-09

LOSS-OF-FUNCTION ANALYSIS IDENTIFIES MLL5 AS A CRITICAL MEDIATOR OF HEMATOPOIETIC STEM CELL AND NEUTROPHIL FUNCTION

M Heuser,[1]* D Yap,[2,3]* M Leung,[1] T Ruiz de Algara,[2] A Tafech,[2] S McKinney,[2] J Dixon,[4] R Thresher,[4] B Colledge,[5] M Carlton,[4] K Humphries,[1,6+] S Aparicio[2,3+]

*[1]Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver, British Columbia, Canada; [2]Department of Molecular Oncology, British Columbia Cancer Research Centre, Vancouver, BC, Canada; [3]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada; [4]Takeda Pharmaceuticals Cambridge Ltd Cambridge, UK; [5]Physiological Laboratory, Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge UK, [6]Department of Medicine, University of British Columbia, Vancouver, BC, Canada *denotes equal contribution*

MLL5 is a divergent member of the Drosophila Trithorax-related SET and PHD domain-containing chromatin regulators that are involved in the regulation of transcriptional "memory" during differentiation. Human MLL5 is located on chromosome 7q22, that is frequently deleted in myelodysplastic syndromes and myeloid leukemias, suggesting a possible role in hematopoiesis. To address this question, we generated a loss of function allele (Mll5tm1Apa) in the murine Mll5 locus. Unlike other Mll genes, Mll5tm1Apa homozygous mice are viable but display defects in immunity and hematopoiesis. First, Mll5tm1Apa homozygous mice show increased susceptibility to spontaneous eye infections, associated with a cell-autonomous impairment of neutrophil function. Second, Mll5tm1Apa/tm1Apa mice exhibit a mild impairment of erythropoiesis. Third, Mll5tm1Apa/tm1Apa hematopoietic stem cells (HSCs) have impaired competitive repopulating capacity both under normal conditions and when subjected to self-renewal stimulation by NUP98-HOXA10. Fourth, Mll5tm1Apa homozygous HSCs show a dramatic sensitivity to DNA demethylation-induced differentiation (5-Azadeoxycytidine). Several of these features are reminiscent of myelodysplasia. Taken together our data show that MLL5 is involved in terminal myeloid differentiation and the regulation of HSC self-renewal by a mechanism that involves DNA methylation. Interestingly, AML and MDS patients with deletion of chromosome 7 benefit most from treatment with DNA methyltransferase inhibitors and heterozygous loss of MLL5 may be the critical mediator of this response. Taken together, our data identify MLL5 as a key regulator of normal hematopoiesis.

IS-06

DRUG DEVELOPMENT TRIALS FOR CHILDREN WITH CANCER IN EUROPE

CM Zwaan

Sophia Children's Hospital/Erasmus MC Rotterdam, Netherlands

Overall survival from childhood malignancies has dramatically improved, with survival rates now reaching over 70%. Nevertheless, some types of childhood cancer remain a difficult challenge, and for those who survive the burden of treatment can be considerable. This specifically includes relatively rare diseases such as high-grade MDS and/or JMLL, where stem-cell transplant is the only treatment option, with subsequent relapse rates approximating 25% for high-grade MDS and 35% for JMML (data from EWOG-MDS studies). New therapies are therefore urgently needed. Historically, drug development was focused on adult cancers, and the potential efficacy in childhood malignancies was not considered. In adult MDS, recently several new compounds have greatly enhanced the therapeutic potential, including the demethylating agent azacytidine, and the immune-modulatory drug lenalidomide for MDS cases characterized by a chromosome 5q deletion. Attempts should be made to develop these agents for pediatric MDS as well, although 5q- is a rare event in children. The current paradigm for novel anti-cancer therapies is to increase our knowledge of the molecular basis of carcinogenesis, followed by the development of cancer-cell specific therapies. The increasing knowledge on JMLL and RAS-pathway activation though various mechanisms could be a prime examples in this respect. Recently, a European academic phase I/II consortium was established, namely 'Innovative Therapies for Children with Cancer' (ITCC, www.itcc-consortium.org), to address pediatric drug development. This initiative is focused on the evaluation of novel agents in specific pediatric cancer pre-clinical models (including cell-line screening and xenograft models), and early clinical development of promising new drugs. Until recently, clinical development of new drugs in childhood cancer was restricted by the limited accessibility of such agents. Recent changes in EU legislation oblige pharmaceutical companies to provide pediatric clinical data for all new drugs relevant to children, including anti-cancer drugs. Pediatric consortiums like ITCC have established networks of expertise with the specific aim of evaluating new drugs for the treatment of childhood cancers. Moreover, increasing collaboration with disease specific collaborative groups is realized in order to select the right compounds for further development including prospects for phase III studies, once early development is successful. Through proper evaluation in collaborative clinical trials we will learn how best to use these new therapeutic approaches and improve the survival rates and reduce toxicity for children with cancer.

Bone marrow failure in children

IS-07

FUTURE STEPS IN THE TREATMENT OF SEVERE APLASTIC ANEMIA

S Kojima

Department of Pediatrics, Nagoya University Graduate School of Medicine Japan Childhood Aplastic Anemia Study Group, Japan

The major treatment option for children with aplastic anemia (AA) include allogeneic stem cell transplantation (SCT) and immunosuppressive therapy (IST). The Japan Childhood AA Study Group previously conducted two studies, Childhood AA-92 and Childhood AA-97. In Childhood AA-92 Study, we randomized children with severe AA & moderate AA into G-CSF$^+$ and G-CSF$^-$ groups and evaluated the role of G-CSF. There were no significant differences between the 2 groups with respect to the response rate and probability of survival. Now, median observation time of surviving patients in this cohort is longer than 11 years. There is no statistically significant difference in overall survival (OS) and failure free survival (FFS) between 2 groups. A significant proportion of SAA children are refractory to IST. In AA-97 Study, we prospectively compared the efficacy of repeated IST with SCT from an alternative donor in non-responders to initial course of IST. FFS was much better in the SCT group compared with IST group, which suggest that SCT from an alternative donor offers a better chance of FFS than a second IST in children not responding to an initial IST. In the previous studies, horse antithymocyte globulin (ATG) was administered combined with cyclosporine. However, horse ATG became unavailable and replaced by rabbit ATG in Japan. Rabbit ATG (Thymoglobulin) has been approved to use for the treatment of AA. The reported dose of Thymoglobulin ranged from 2.5 mg/kg/day to 3.75 mg/kg/day. There is no study to evaluate the effectiveness between the lower dose of Thymoglobulin and the higher dose for treatment of AA. Also, there is some concern that prolonged T cell suppression and more active EBV reactivation caused by IST with rabbit ATG might cause a higher incidence of Epstein – Barr virus related lymphoproliferative disorder. Based on these backgrounds, we have planned to conduct a prospective randomized study to compare the 2 dose of Thymoglobulin for the treatment of childhood AA in the next trial.

IS-08

PAEDIATRIC SEVERE ACQUIRED APLASTIC ANAEMIA – TOWARDS A EUROPEAN APPROACH

M Füehrer for the German/Austrian Paediatric Aplastic Anemia Working Group

Dr. von Haunersches Kinderspital, University of Munich, Germany

Childhood severe acquired aplastic anaemia (SAA) is a very rare and still life-threatening disease. Long term results for both treatment options – bone marrow transplantation (BMT) and immunosuppressive therapy (IST) – improved significantly during the last decade. In the German SAA 94 cohort 213 patients were treated either with BMT from a matched sibling donor (n=67) or with combined IST with ALG and cyclosporin A (CSA) (n=146). Overall survival after 8 years was 89% after BMT and 81% after IST, respectively. Even survival after BMT from matched unrelated donors has recently improved to 70% in selected cohorts. However, about 50% of responders show incomplete response, half of them relapse, and CSA dependency and the risk of clonal progression have a significant impact on the patients' quality of life. So far only disease severity has been identified as a predictor for IST response and survival, with a significantly different long term survival of 92% in vSAA patients and 65% in SAA patients, respectively. Because of the long latency period of IST a better understanding of the underlying pathomechanisms is needed to assign patients early during the course of their disease to the appropriate treatment. However, since SAA is a very rare disease in childhood with an incidence of 0.2/100.000 children per year a co-operative European and international effort is needed to answer main questions on basic disease mechanisms and therapeutic decisions. In a first step a European-Japanese collaboration was recently started to define and evaluate common criteria and time points for IST response. For future comparisons of different cohorts agreement on common diagnostic criteria for histopathology and cytogenetics is very important to differentiate between aplastic anemia and hypoplastic MDS. Therapy guidelines for IST and BMT and standardized data collection could then provide the basis to correlate treatment response and the results of molecular or immunological research.

CO-10

APPLICATION OF WHOLE GENOME SNP ARRAYS FOR MOLECULAR KARYOTYPING IN ACQUIRED BONE MARROW FAILURE

M Wlodarski,[1] L Gondek,[2] J Huh,[2] S Mohan, C O'Keefe, C Flotho,[1] C Niemeyer,[1] J Maciejewski[2]

[1]Division of Pediatric Hematology and Oncology, Department of Pediatric and Adolescent Medicine, University of Freiburg, Freiburg, Germany; [2]Experimental Hematology, Cleveland Clinic, Cleveland, USA

Hypocellular bone marrow failure (hBMF), including aplastic anemia (AA) and refractory cytopenia (RC) is characterized by pancytopenia due to contraction of the vital stem cell pool. Clonal malignant evolution occurs in a significant proportion of patients and the inability to early identify this subgroup constitutes a significant clinical problem. Metaphase cytogenetics (MC) is often non-informative in hBMF. We hypothesized that karyotyping using SNP-arrays (SNPA) that allows for whole genome analysis from archived DNA samples, will improve detection of genomic lesions in hBMF. We applied Affymetrix 250K/6.0 chips to study whole genomes of hBMF patients (AA, N=102; RC, N=34) and detected genomic gains, losses and copy number neutral LOH. The overall diagnostic yield for the detection of genomic aberrations nearly doubled when MC was complemented by SNPA karyotyping. Clonal evolution was observed in 13 AA patients, and remarkably in 38% of evolving AA patients, numerical aberrations were detected earlier by SNPA than MC. Lesions of acquired uniparental disomy were found in 4 cases and were not present in nonmyeloid lineage. Interestingly, these somatic aberrations disappeared in 2 patients after therapy and 2 years later newly recruited clones with mono7 dominated as a sole abnormality by SNPA and MC. Our study demonstrates that SNPA is a powerful tool for molecular karyotyping and can greatly complement MC in the detection of previously cryptic genomic aberrations.

IS-09

TREATMENT OF APLASTIC ANEMIA: IMPACT OF MOLECULAR PATHOGENESIS

NS Young

Hematology Branch, National Heart, Lung, and Blood Institute, NIH, Bethesda MD, USA

Most patients with severe aplastic anemia respond to immunosuppressive therapy, usually as a combination of anti-thymocyte globulin and cyclosporine. An immune mechanism is implicated by clinical observations, and in laboratory studies, including animal models, type I T cells appear to be the proximate cause of hematopoietic stem and progenitor cell destruction. Analysis of T cell clones by flow cytometry and spectratyping of the T cell receptor β chain suggest that pathogenic oligoclones are not eliminated by current ATG regimens. In addition, horse and rabbit ATG do no appear to be equivalent *in vitro* in altering the T cell transcriptome and immune function. For target cells, telomere abnormalities, discovered in children with dyskeratosis congenita, have been found also in adults with apparently acquired aplastic anemia. Hypomorphic mutations in the telomerase gene *TERT* affect hematopoietic cell number and regenerative capacity. Chromosomes with short-for-age telomeres are susceptible to genomic instability. TERT mutant patients have T cell oligoclones, and aplastic anemia patients with short telomeres respond to immunosuppression at the same rate as those with normal telomeres. However, their relapse rate is doubled, and virtually all clonal evolution to myelodysplasia/acute leukemia occurs in patients with the shortest telomeres. In complementary studies, we have detected constitutional *TERT* mutations in adults with acute myeloid leukemia. Mutations in *TERT* also occur in pulmonary fibrosis and in *TERT* and *TERC* in hepatic cirrhosis. Accelerated telomere shortening due to genetic lesions, but also likely after chemotherapy and physiologically with aging, may underlie both diseases of defective organ regeneration and also malignant transformation to cancer originating from inflammatory conditions.

Congenital bone marrow failures

IS-10

FANCONI ANEMIA: RECENT ADVANCES AND PERSPECTIVES

H Joenje,[1] JC Dorsman,[1] Q Waisfisz,[1] JP de Winter,[1] M Bierings[2]

[1]Department of Clinical Genetics, VU University Medical Center, Amsterdam; [2]Department of Pediatric Hematology/Oncology, UMC Utrecht, The Netherlands

Fanconi anemia (FA) is a recessively inherited disease characterized by congenital abnormalities, progressive bone marrow failure, and an elevated risk of malignancies. The diagnosis and treatment of FA patients is unusually complex. A national protocol has recently been approved by the Dutch Childhood Oncology Group and is currently being discussed internationally. Life expectancy of FA patients is strongly reduced (average 20-25 years) due to aplastic anemia, AML and/or solid tumors. Cells from FA patients feature spontaneous chromosomal instability (breaks and rearrangements) and excessive sensitivity to the chromosome-breaking and cytostatic effects of DNA cross-linking agents, such as cisplatin and mitomycin C. The latter feature is the basis for the diagnosis 'FA'. FA is genetically heterogeneous: mutations in one of the following 13 genes causes the disease: *FANCA, -B, -C, -D1, -D2, -E, -F, -G, -I, -J, -L, -M,* and *-N*. The genes are localized on the autosomes, except for *FANCB*, which is on the X-chromosome. Patients with mutations in *BRCA2* (subtype D1) or *PALB2* (subtype N) seem to be clinically distinct from the other subtypes, since they have a particularly severe phenotype with a life expectancy of 5 years, mainly due to childhood malignancies (AML, medulloblastoma, Wilms tumor). In addition, female heterozygous carriers of mutations in *BRCA2*, *PALB2* as well as *FANCJ/BRIP1* have an increased risk to develop breast cancer. The proteins encoded by the FA genes act together in a molecular pathway that is supposed to be essential for error-free DNA replication. Thus, elucidation of this 'FA/BRCA' genomic maintenance pathway will be important to better understand the mechanisms underlying bone marrow failure, leukemogenesis and (breast) carcinogenesis.

IS-11

CANCER IN INHERITED BONE MARROW FAILURE SYNDROMES

BP Alter,[1] N Giri,[1] SA Savage,[1] PS Rosenberg[2]

[1]Clinical Genetics Branch and [2]Biostatistics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD, USA

The major rare inherited bone marrow failure syndromes (IBMFS) include Fanconi Anemia (FA), dyskeratosis congenita (DC), Diamond Blackfan anemia (DBA), Shwachman Diamond Syndrome (SDS), severe congenital neutropenia (SCN), amegakaryocytic thrombocytopenia (Amega), and thrombocytopenia absent radii (TAR). Information with regard to cancer in these disorders is derived from literature reports and from analyses of our National Cancer Institute (NCI) IBMFS cohort. All of these are at high risk of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), while the first 3 have high risks of solid tumors (head and neck squamous cell carcinoma [SCC] in FA and DC, and osteogenic sarcoma in DBA). Almost 20% of the close to 2000 cases of FA in the literature developed cancer, with a median age of below 30 years. In several FA cohorts including ours the relative risk of cancer compared with the general population was around 50-fold, and was in the hundreds-fold to thousands-fold for head and neck or vulvar SCC, AML, and MDS. For DC, the crude cancer rate in close to 500 reported cases was 10%. In the NCI DC cohort, the risk of any cancer was 10-fold the general population, with tongue SCC, AML, and MDS resembling the high risks in FA. Cancers were reported in 3% of 900 patients with DBA, and were primarily AML and osteogenic sarcoma. Literature reports of leukemia in SDS comprised 7% of more than 500 patients. Data for patients with SCN come from the SCN International Registry; many of the cases with MDS/AML occurred in patients whose neutropenia did not respond to high doses of G-CSF. There were small numbers of cases of leukemia in Amega or TAR reported in the literature. An IBMFS (particularly FA or DC) should be considered in patients with atypical presentations of the characteristic cancers, and the appropriate screening tests should be performed, including chromosome breakage for FA, and telomere length in DC. Overall, patients with any of the IBMFS have inordinately high risks of MDS, leukemia, and solid tumors.

CO-11

DIAGNOSIS OF FANCONI ANEMIA IN A COHORT OF 87 PATIENTS WITH BONE MARROW FAILURE

FO Pinto, T Leblanc, D Chamousset, B Brethon, B Cassinat, J Larghero, J-P de Villartay, D Stoppa-Lyonnet, A Baruchel, G Socie, E Gluckman, J Soulier

Hematology Laboratory APHP, Hopital Saint-Louis, Paris; INSERM U728 and UMR944, Hopital Saint-Louis, Paris; Universite Paris Diderot, Paris; Bone Marrow Transplant Unit, Hopital Saint-Louis, Paris; Pediatric Hematology Department, Hopital Saint-Louis, Paris; AP-HP, Cell Biology Unit, Hopital Saint-Louis, Paris; APHP, Cell Therapy Unit, Hopital Saint-Louis, Paris; INSERM U768, Hopital Necker, Paris; and Department of Genetics, Curie Institute, Paris, France

Background. Patients with bone marrow failure (BMF) and undiagnosed underlying Fanconi anemia (FA) may experience major toxicity if given standard-dose conditioning regimens for hematopoietic stem cell transplant (HSCT). Due to clinical variability and/or potential emergence of genetic reversion with hematopoietic somatic mosaicism, a straightforward FA diagnosis can be difficult to make, and diagnostic strategies combining different assays in addition to classical breakage tests in blood may be needed. *Materials and Methods.* We evaluated FA diagnosis on blood lymphocytes and skin fibroblasts from 87 BMF patients (55 children, 32 adults) with no obvious full clinical picture of FA. A combination of FA tests was performed, which included chromosomal breakage tests, FANCD2-monoubiquitination assays, a new flow cytometry-based mitomycin C (MMC) sensitivity test in fibroblasts, and, when FA was diagnosed, complementation group and mutation analyses. The MMC sensitivity test in fibroblasts was validated on control FA and non-FA samples, including other chromosomal instability disorders. *Results.* When this diagnosis strategy was applied to the cohort of BMF patients, seven FA patients were found (3 children and 4 adults). Classical chromosomal breakage tests in blood detected 4, but analyses on fibroblast were necessary to diagnose 3 more patients with hematopoietic somatic mosaicism. Importantly, FA was excluded in all the other patients fully evaluated. *Conclusions.* In this large cohort of patients with BMF our results confirmed that when any clinical/biological suspicion of FA remains after chromosome breakage tests in blood, based on physical exam, history or inconclusive results, then further evaluation including fibroblast analysis should be done. For that purpose, the flow-based MMC sensitivity test here described proved to be a reliable alternative method to evaluate FA phenotype in fibroblasts. This global strategy allowed early and accurate confirmation or rejection of FA diagnosis with immediate clinical impact for those who underwent HSCT.

CO-12

GENETIC ANALYSIS OF INHERITED BONE MARROW FAILURE SYNDROMES FROM ONE COMPREHENSIVE COHORT OF PATIENTS

E Tsangaris,[1] R Klaassen,[2] CV Fernandez,[3] R Yanofsky,[4] E Scherek,[5] J Champagne,[6] M Silva,[7] JH Lipton,[8] J Brossard,[9] Y Samson,[10] S Abish,[11] M Steele,[12] K Ali,[13] N Dower,[14] U Athale,[15] L Jardine,[16] JP Hand,[17] S Stephen,[18] J Beyene,[18] C Allen,[1] I Odame,[1] Y Dror[1]

From the [1]Marrow Failure and Myelodysplasia Program, Division of Haematology/Oncology and Cell Biology Program, Research Institute, The Hospital for Sick Children and the University of Toronto, Toronto, Ontario; [2]Children's Hospital of Eastern Ontario, Ottawa, Ontario; [3]Isaak Walton Killam Hospital for Children, Halifax, Nova Scotia; [4]CancerCare Manitoba, Winnipeg, Manitoba; [5]British Columbia Children's Hospital, Vancouver, British Columbia; [6]Hôpital Ste.Justine, Montréal, Québec; [7]Queen's University, Kingston, Ontario; [8]Princess Margaret Hospital, Toronto, Ontario; [9]Centre U Sante de l'Estrie-Fleur, Sherbrooke, Quebec; [10]Centre Hospital University Quebec-Pav CHUL, Sainte-Foy, Quebec; [11]Montreal Children's Hospital, Montreal, Québec; [12]Alberta Children's Hospital, Calgary, Alberta; [13]University of Saskatchewan, Saskatoon, Saskatchewan; [14]University of Alberta/Health Sciences Centre, Edmonton, Alberta; McMaster Children's Hospital/McMaster University Health Sciences Centre, Hamilton, Ontario; [15]McMaster University Health Sciences Centre, Hamilton, Ontario; [16]Children's Hospital of Western Ontario, London, Ontario; [17]Janeway Child Health Centre, St. John's, Newfoundland; [18]Population Health Sciences, Research Institute, The Hospital For Sick Children, Toronto, Ontario, Canada

Background and Objective. Inherited bone marrow failure syndromes (IBMFSs) are heterogeneous genetic disorders characterized by hematolog-

ical and non-hematological manifestations. Due to considerable phenotypic overlap among these conditions, genotyping has become critical in establishing a diagnosis and classifying these disorders. Many groups have analyzed specific genes in individual IBMFSs; however, there is no data for mutations in one cohort which comprises all IMFSs. The Canadian Inherited Marrow Failure Registry (CIMFR) is a prospective study for all patients with IBMFSs. The aim of this present study was to characterize the genetic backgrounds of IBMFSs from one comprehensive cohort of patients. *Methods*. Clinical and genetic information was extracted from the CIMFR database and analyzed. *Results*. As of August 2008, 232 patients were enrolled on the CIMFR. Significantly, the largest cohort on our registry consists of unclassifiable patients followed by Diamond Blackfan anemia (DBA), Shwachman Diamond syndrome (SDS), Fanconi anemia (FA), Kostmann neutropenia/Severe congenital neutropenia (KN/SCN), and Dyskeratosis Congenita (DC). A total of 28.3% of the patients had a relevant family history. Genetic testing was conducted on 117 (52.7%); of them patients with only 27 (23%) were positive. The most prevalent mutated genes included SBDS, RPS19, ELA2, and FANC genes. In addition, several patients had syndromes which had not been previously known to be associated with bone marrow failure and 3 patients had chromosomal abnormalities which potentially harbor novel IMFS genes. The mutated genes in our cohort have been suggested to be involved in 14 different cellular pathways. Many of them are postulated to be multifunctional and in most genes have been shown to lead to accelerated apoptosis. *Conclusion*. Despite the identification of about 50 IBMF-causing genes, most patients cannot be genotyped. However, data on recently-identified IBMF genes done in research laboratories was not available for this study and might have slightly increased the percentage of positive testing. The most common causes for negative genotyping or no testing include low likelihood of positive results using testing for the current list of IMFSs, an unclassifiable syndrome or unknown genetic background for the particular condition. The IBMF genes seem to function in pathways that lead to either cell cycle arrest or apoptosis, thereby slowing down cell expansion capacity.

CO-13

ANALYSIS OF RIBOSOMAL PROTEIN GENES IN DIAMOND-BLACKFAN ANEMIA PATIENTS FROM THE CZECH NATIONAL DBA REGISTRY

D Pospisilova,[1] J Cmejlova,[2] J Stary,[3] Z Cerna,[4] R Cmejla[2]

[1]Department of Pediatrics, Palacky University, Olomouc; [2]Department of Cell Physiology, Institute of Hematology and Blood Transfusion, Prague; [3]Department of Pediatric Hematology and Oncology, Charles University, 2nd Medical School, Prague; [4]Department of Pediatrics, Charles University Hospital, Pilsen, Czech Republic

Objective. Diamond-Blackfan anemia (DBA) is a congenital red cell aplasia that is also associated with various physical anomalies in 40% of patients. Haploinsufficiency of ribosomal protein (RP) production due to mutations is believed to be the cause of DBA pathogenesis though the precise mechanism remains unknown. Here we report a summary of the mutations in RPs identified in Czech DBA patients and provide genotype-phenotype correlations. *Methods.* The Czech Registry currently contains 33 patients from 29 families. Most cases (25) are sporadic, 8 patients are from 4 families. PCR and direct sequencing was used to identify mutations in the genes coding for the following 10 RPs of the small ribosomal subunit: RPS2, RPS3, RPS3a, RPS13, RPS14, RPS16, RPS17, RPS19, RPS24 and RPS30; and 5 RPs of the large subunit: RPL5, RPL11, RPL13, RPL35a and RPL23. *Results.* In total, mutations were identified in 4 different RPs in 20 patients (60.1%) from 16 families (55.1%). A mutation in RPS17 was found in one patient (3.4%); in RPS19 in 9 patients from 7 families (24.1%); in RPL5 in 8 patients from 6 families (20.7%); and in RPL11 in 2 patients from 2 families (6.9%). Patients with RPL5 and RPL11 mutations were generally born small for gestational age compared with the RPS19-mutated group. All patients with either an RPS17, RPL5 or RPL11 mutation exhibited one or more physical anomalies; specifically, thumb anomalies were always present, while no such anomaly was observed in patients with an RPS19 mutation. *Discussion.* To date, a mutation in RP has been discovered in 60% of all Czech DBA patients. Mutations identified so far are evenly distributed between RPs of the small and large ribosomal subunit (8 each), indicating that malfunction of the ribosome as a whole rather than a specific role of subunits is the mechanism leading to DBA phenotype. Our results also suggest that mutations in at least RPL5 seem to generally have a more profound impact on fetal development than mutations in RPS19.

Acquired bone marrow failure

CO-14

PROPOSAL FOR THE RESPONSE CRITERIA OF IMMUNOSUPPRESSIVE THERAPY IN CHILDREN WITH APLASTIC ANAEMIA

A Yoshimi,[1] S Kojima,[2] A Ohara,[3] C Niemeyer,[1] M Fuehrer[4]

[1]Division of Pediatric Hematology and Oncology, Department of Pediatrics, University of Freiburg, Freiburg, Germany; [2]Department of Pediatrics, Nagoya Graduated School of Medicine, Nayoga, Japan; [3]First Department of Pediatrics, Toho University, Tokyo, Japan; [4]Dr. von Haunersches Kinderspital, University of Munich, Germany

Immunosuppressive therapy (IST) with anti-thymocyte globulin (ATG) and cyclosporine A (CSA) is standard therapy for young patients with severe acquired aplastic anaemia (SAA), if an HLA matched sibling donor is not available. Pediatric trials in SAA have reported a response rate to IST between 60% and 80%. However, these trials have used various criteria to assess results, making cross study comparisons difficult. Therefore, paediatric haematologists from Europe and Japan aimed to achieve a consensus on response criteria. Here, we propose standardized criteria for evaluating the response to IST in children with SAA. Response to treatment is defined as complete response (CR) or partial response (PR). CR indicates a normal blood count; all criteria below have to be fulfilled: ANC >1.5×10^9/L, Hb > age-adjusted cut-off value (0.5-2 years: 10.5 g/dL, 2-14 years: 11.5 g/dL, and 15-18 years: 12 g/dL (girls), 13.0 g/dL (boys)), platelet count > 150×10^9/L. In the absence of CR, PR is defined when all following criteria are fulfilled: ANC> 0.5×10^9/L, no platelet or red cell transfusion, platelet count >20×10^9/L. No response (NR) is defined when neither PR nor CR is reached. The date of CR/PR is the date of the first blood count indicating CR/PR which is at least 28 days after the last platelet or red cell transfusion and 14 days after the last G-CSF dose. PR and CR should sustain for a minimum of 3 consecutive blood counts over a period of at least 28 days. The response should be evaluated at least at day 90,120, and 180. Failures are defined by death, NR at day 180, relapse, secondary MDS, second IST, and HSCT. Relapse is defined by conversion to NR from PR/CR. Applying these criteria, we retrospectively evaluated the results of IST at 6 months in a German and Japanese cohort of SAA and very severe AA (VSAA) patients. In the German study SAA 94 (n=170), response to IST was observed in 76% (CR 22%, PR 54%) of the VSAA (n=111) and 68% (CR 22%, PR 46%) of SAA (n=49) patients. In patients treated according to the Japanese AA-97 study (n=235), the response rate was 55% (CR 6.5%, PR 48.5%) in VSAA (n=138) and 62% (CR 7%, PR 55%) in SAA (n=97). The relevance of these response criteria will have to be validated prospectively in future clinical trials.

CO-15

RELAPSE OF CHILDREN WITH APLASTIC ANEMIA AFTER IMMUNOSUPPRESSIVE THERAPY

T Kamio,[1] E Ito,[1] A Ohara,[2] Y Kosaka,[3] M Tsuchida,[4] H Mugishima,[5] H Yabe,[6] A Morimoto,[7] S Ohga,[8] H Yagasaki,[9] F Bessho,[10] T Nakahata,[11] S Kojima[9]

[1]Department of Pediatrics, Hirosaki University Graduate School of Medicine, Hirosaki; [2]Department of Pediatrics, Toho University, Tokyo, Japan; [3]Department of Hematology and Oncology, Kobe Children's Hospital, Kobe; [4]Department of Pediatrics, Ibaraki Children's Hospital, Mito; [5]Department of Pediatrics, Nihon University School of Medicine, Tokyo; [6]Department of Cell Transplantation, Tokai University School of Medicine, Isehara; [7]Department of Pediatrics, Kyoto Prefectural University of Medicine, Graduate School of Medical Science, Kyoto; [8]Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, Maidashi, Fukuoka; [9]Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya; [10]Department of Pediatrics, Kyorin University School of Medicine, Tokyo; [11]Department of Pediatrics, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Although the therapeutic outcome for acquired aplastic anemia (AA) has improved markedly with the introduction of immunosuppressive therapy (IST) with antithymocyte globulin (ATG) and cyclosporine A (CyA), a significant portion of patients treated with IST subsequently relapse and require second-line therapy. However, detailed analysis of the relapse cases has not been reported. In this study, we assessed the

relapse rate, response to second-line treatment after relapse and prognosis. From November 1992 to July 2007, 441 newly diagnosed AA children (196 female, 245 male) entered two consecutive prospective studies (AA-92 and AA-97). The median age was 8 years, ranging from 1 to 18 years. Patients with very severe (n=210), severe (n=149) and non-severe disease (n=82) received initial IST consisting of ATG and CyA. At six months after the initial IST, 261 patients (59.2%) achieved CR (n=90) or PR (n=171). Relapse is defined by conversion to no response (NR) from partial or complete response (PR/CR). Among the 264 patients who responded to IST, 42 (9.5%) relapsed. The cumulative incidence of relapse was 12.7% at 10 years and the median time to relapse was 24 months, ranging 6 to 138 months. Multivariate analysis showed that relapse risk was significantly associated with non-severe aplastic anemia and treatment with danazol. Among the 42 patients who relapsed, 28 received a second round of IST with ATG and/or CyA. Fifteen patients (53.6%) responded to the second round. However, two relapsed, and two developed paroxysmal nocturnal hemoglobinuria (PNH). Seven patients received CyA after relapse. Three patients responded to CyA, but one relapsed subsequently. Hematopoietic stem cell transplantation (HSCT) was attempted in 23 patients who relapsed after initial responses. Before HSCT, nine received a second course of IST with ATG and/or CyA and two received CyA. Twelve patients progressed directly to HSCT. Bone marrow transplantation (BMT) from an HLA-matched sibling (n=5), HLA-matched family member (n=1), or HLA-mismatched family member (n=2) was performed in 9 patients, and eight are presently alive. BMT from an unrelated donor was attempted in 12 patients, and four died of complications related to BMT. Cord blood transplantation from an unrelated donor was attempted in two patients and two patients are alive. Of the 23 patients who received HSCT, 18 are alive and well, 41 to 162 months (median 119 months) following transplantation. There were seven deaths out of total 42 who initially relapsed. The causes of death were HSCT-related complications (n=5), MDS/acute myelogenous leukemia (n=1), bacteremia (n=1). Eight of 11 patients who received HSCT following a second round of IST are alive and well. The present study suggests that a second round of IST should be offered to the patients who relapse, and HSCT should be considered for the patients who fail to respond to the second round of IST.

CO-16

DECREASED INCIDENCE OF CLONAL EVOLUTION TO MYELODYSPLASTIC SYNDROME/ACUTE MYELOID LEUKEMIA WITH MONOSOMY 7 IN CHILDREN WITH APLASTIC ANEMIA FOLLOWING REDUCED USE OF G-CSF

Y Takahashi,[1] A Ohara,[2] R Kobayashi,[3] H Yabe,[4] M Tsuchida,[5] H Mugishima,[6] A Morimoto,[7] S Ohga,[8] F Bessho,[9] N Yoshida,[1] I Tsukimoto,[10] T Nakahata,[11] S Kojima[1] for the Japan Childhood Aplastic Anemia Study Group

[1]Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Aichi; [2]Department of Transfusion, Toho University School of Medicine, Tokyo; [3]Department of Pediatrics, Sapporo Hokuyu Hospital, Hokkaido; [4]Specialized Clinical Science, Pediatrics, Tokai University School of Medicine, Isehara, Kanagawa; [5]Department of Paediatrics, Ibaraki Children's Hospital, Mito, Ibaraki; [6]Department of Pediatrics, Nihon University School of Medicine, Tokyo; [7]Department of Pediatrics, Jichi Medical School, Tochigi; [8]Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, Fukuoka; [9]Department of Pediatrics, Kyorin University School of Medicine, Tokyo; [10]Department of Pediatrics, Toho University School of Medicine, Tokyo; [11]Department of Pediatrics, Kyoto University Graduate School of Medicine, Kyoto, Japan

A serious complication of aplastic anemia (AA) is evolution to myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). In a previous nationwide study conducted between 1988 and 1992 in Japan, 11 of 67 patients treated with immunosuppressive therapy (IST) and G-CSF developed MDS/AML with monosomy 7, giving a cumulative incidence of 47±17% over 6 years. In the study, all but one of the patients who developed MDS/AML had received G-CSF for more than 12 months. We prospectively examined the relationship between the development of MDS/AML with monosomy 7 and the use of G-CSF in newly diagnosed AA patients. From 1993 to 2006, 387 newly diagnosed AA children who received antithymocyte globulin (ATG) and cyclosporine with or without G-CSF entered the two consecutive prospective studies. The median age of the 366 evaluable patients was 9 years, ranging from 1 to 18 years. The median follow up period of the surviving patients was 60 months (range: 1-167 months). The cumulative durations of G-CSF therapy were as follows: 0 days (n=111), 1-30 days (n=66), 31-60 days (n=70), 61-90 days (n=43), 91-180 days (n=52), 181-365 days (n=17), over 366 days (n=4), and unknown (n=3). Twenty-one of the 366 analyzed patients developed clonal cytogenetic abnormalities between 6 and 62 months (median: 18 months) after the time of diagnosis, giving a cumulative incidence of 7.1±1.5% at 6 years. Cytogenetic analysis revealed monosomy 7 (10 patients), trisomy 8 (6 patients), others (5 patients) at the time of clonal evolution. Notably, although only 4 of the 366 patients received G-CSF over 12 months, three of them developed MDS/AML with monosomy 7. We observed a drastic decrease in the cumulative incidence of MDS/AML with monosomy7 in AA patients treated with IST and G-CSF. The current study confirms the finding of our previous report, which suggested a close relationship between long-term use of G-CSF and secondary MDS/AML with monosomy 7 in AA patients.

Stem cell transplantation for childhood myelodysplastic syndromes, juvenile myelomonocytic leukemia and bone marrow failure

IS-12

THE ROLE OF ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION IN CHILDREN WITH MYELODYSPLASTIC SYNDROMES

F Locatelli

Oncoematologia Pediatrica, Fondazione IRCCS Policlinico San Matteo, Università di Pavia, Italy

Allogeneic haematopoietic stem cell transplantation (HSCT) is the only curative approach for children with juvenile myelo-monocytic leukemia (JMML) and it is routinely offered also to all children with refractory anaemia with excess of blasts (RAEB) and RAEB in transformation (RAEB-t), to pediatric patients with MDS secondary to chemo-radiotherapy, and to those with refractory cytopenia (RC) associated with cytogenetic anomalies or transfusion dependence. In children with JMML given an allogeneic HSCT from either a histocompatible relative or from an HLA-matched/1-antigen disparate donor, the probability of disease-free survival (DFS) is approximately 55%. Leukemia recurrence represents the main cause of treatment failure, relapse rate being as high as 30-40%. In the more recent years, using an unrelated donor offers minimal or possibly no significant disadvantage as compared to employing an HLA-identical sibling. Age greater than 4 years at diagnosis predicts poorer outcome in children with JMML, while there is no difference in terms of DFS probability in children who do or do not have cytogenetic anomalies. Splenectomy before HSCT does not appear to have an impact on post-transplantation outcome of children with JMML. Available data indicate that umbilical cord blood transplantation (UCBT) is a suitable option for children with JMML lacking an HLA-compatible relative and that the search for an unrelated cord blood unit should be initiated at the same time as that for an unrelated bone marrow donor. For children with JMML experiencing leukemia relapse after HSCT, infusion of donor lymphocytes is largely ineffective, while a second allograft, from either the same or a different donor is able to rescue about one third of the patients. Results on HSCT in children with advanced MDS other than JMML are scanty, the reported DFS being in the order of 60% when the donor is an HLA identical sibling. The need for pre-HSCT remission induction chemotherapy remains a debated question in pediatric patients with RAEB and RAEB-t. The outcome of children with MDS secondary to previous cytotoxic or radiant treatment remains still poor, for both a high risk of disease recurrence and transplantation-related mortality. Patients with RC must be considered for an early allograft from either a related or an unrelated donor if they have cyogenetic abnormalities, in particular monosomy 7. As the risk of disease recurrence after the allograft in patients with RC is low, there is a great interest in testing the safety and efficacy of reduced intensity regimens in this setting.

CO-17

ACCEPTABLE HLA-MISMATCHING OF UNRELATED DONOR BONE MARROW TRANSPLANTATION FOR PATIENTS WITH APLASTIC ANEMIA BASED ON MOLECULAR TYPING OF 10 HLA LOCI: AN ANALYSIS OF 301 PATIENTS THROUGH THE JAPAN MARROW DONOR PROGRAM

H Yagasaki,[1] S Kojima,[1] Y Takahashi,[1] N Yoshida,[1] S Kato,[2] K Kato,[3] H Yabe,[4] H Sakamaki,[5] T Kawase,[6] M Tsuchida,[7] H Kigasawa,[8] Y Morishima,[6] Y Kodera[9]

[1]Pediatrics, Nagoya University, Nagoya; [2]Tokai University, Isehara; [3]Pediatric Hematology/Oncology, Japanese Red Cross Nagoya First Hospital, Nagoya; [4]Cell Transplantation, Tokai University, Isehara; [5]Department of Hematology, Tokyo Metropolitan Komagome Hospital, Tokyo; [6]Aichi cancer center, Nagoya; [7]Ibaraki childrens hospital, Mito; [8]Kanagawa childrens hospital, Yokohama; [9]Japanese Red Cross Nagoya First Hospital, Nagoya, Japan

We retrospectively analyzed the impact of HLA mismatching (HLA-A, -B, -C, -DQB1, -DRB1) on the transplant outcome for 301 patients with acquired severe aplastic anemia who received unrelated bone marrow transplants (URD-BMT) between 1993 and 2005 through the Japan Marrow Donor Program. HLA typing was determined by DNA sequencing. Additional impact of HLA-DPB1 was analyzed for 10/10 or 9/10 matched pairs (n=169). The probability of overall survival (OS) at 5 years was comparable among complete matched pairs, HLA 1 locus (A or B) mismatched pairs, HLA 1 locus (C or DQB1 or DRB1) mismatch pairs, and 2 or more mismatched (C, DQB1 and DRB1) pairs (75.2%, 72.7%, 60.5% and 69.7%, respectively). In contrast, OS in transplants from 2 or more loci mismatched donor including HLA- A or -B was significantly worse (46.8%) (p=0.003). The incidence of II-IV acute GVHD was significantly lower in 10/10 matched pairs among these 5 groups, while the incidence of graft rejection and chronic GVHD was comparable. HLA-DPB1 mismatching did not provide any additional effect on survival, acute GVHD, chronic GVHD and rejection in the setting of 10/10 and 9/10 matched URD-BMT. In conclusion, 10/10 matched unrelated donor is optimal, but 9/10 (any locus) matched unrelated donor and 8 or less matched unrelated donor (mismatched loci restricted to C, DQB1, DRB1) are also acceptable for patients with SAA.

CO-18

TARGETED IMMUNOTHERAPY THERAPY, GEMTUZUMAB OZOGAMICIN, DURING MYELOABLATIVE CONDITIONING FOLLOWED BY ALLOGENEIC STEM CELL TRANSPLANTATION IN CHILDREN WITH POOR-RISK MYELODYSPLASTIC SYNDROME AND ACUTE MYELOGENOUS LEUKEMIA

P Satwani,[1] V Moore,[1] B Tallamy,[1] M Bhatia,[1] J Garvin,[1] D George,[1] J Schwartz,[2] MB Bradley,[1] C van de Ven,[1] E Morris,[1] MS Cairo[1,2,3]

[1]Departments of Pediatrics, [2]Pathology, and [3]Medicine, Columbia University, New York, NY, USA

Objective. Outcome following poor-risk myelodysplastic syndrome (MDS) and AML (CR3, induction failure [IF], refractory disease [RD]) is dismal (10% overall survival [OS]). CD33, the target of gemtuzumab ozogamicin (GO), is expressed in 90% poor-risk myelogenous leukemia (AML) and MDS with excess blasts. We sought to determine the safety and maximum tolerated dose (MTD) of GO as part of a myeloablative conditioning (MAC) regimen (immunochemotherapy [IC]) and event-free survival (EFS)/OS followed by allogeneic stem cell transplantation (AlloSCT) in children with poor-risk MDS/AML. *Methods.* Children with CD33 expressing poor-risk MDS/AML received GO Day -14 at doses of 3.0, 4.5, 6.0, 7.5 mg/m^2, and Busulfan (Bu) Day -8, -7, -6, -5 at doses 12.8-16.0 mg/kg and Cyclophosphamide (CY) Day -4, -3 at doses 120 mg/kg followed by AlloSCT. GVHD prophylaxis consisted of tacrolimus/ mycophenolate mofetil (Osunkwo/Cairo *et al.* BBMT, 2004). *Results.* Twelve patients, med age 4 (0.75-17yr), M/F 5/7, 7 IF, 4 RD, 1 CR3 received 9 umbilical cord blood transplants (UCBT) (3 each: 6/6, 5/6, 4/6), 2 MUD 10/10, and 1 Sib 6/6, (GO: 3 each, 3.0, 4.5, 6.0, 7.5 mg/m^2). There have been no dose-limiting toxicities secondary to GO, MTD and tolerable dose is 7.5 mg/m^2 of GO. Median ANC and platelet recovery is 24 and 54 days, respectively. Mean day +30 donor chimerism is 98%. Median follow-up 22 months, 58% are alive and disease free. *Discussion*. GO, up to 7.5 mg/m^2, combined with Bu/CY as IC MAC and AlloSCT is well tolerated in children with poor-risk MDS/AML and associated with improved EFS/OS compared to historical controls.

CO-19

TARGETING TO OPTIMAL BUSULFAN EXPOSURE IS ASSOCIATED WITH A LOW RELAPSE RATE AND HIGH ACUTE-GVHD 2-4 RATES IN MDS AND JMML PATIENTS

IH Bartelink,[1] RGM Bredius,[2] M Bierings,[1] CAJ Knibbe,[3,4] M Egeler,[2] AC Lankester,[2] ACG Egberts[1,5] J Zwaveling,[2] JJ Boelens[1]

Departments of Hematology and Immunology and Clinical Pharmacy of [1]University Medical Center Utrecht, [2]Leiden University Medical Center, [3]St. Antonius Hospital, Nieuwegein, [4]Leiden/Amsterdam Center for Drug Research, Division of Pharmacology, Leiden University, Leiden, The Netherlands

Background. Busulfan combined with therapeutic drug monitoring and guided dosing is associated with higher event free survival (EFS) rates due to less graft-failure/relapses and lower toxicity in haematological stem cell transplantation. We previously demonstrated an optimal AUC between 74-82 mg*h/L in a group of children with (non)-malignant indications. Therapeutic drug monitoring of busulfan is still controversial. MDS and JMML patients usually receive a busulfan containing conditioning regimen that also includes mephalan and cyclophosphamide. The optimal target AUC in these children is not known. Historically intravenous busulfan has been used in the two Dutch pediatric stem cell transplantation centers in various myelo-ablative regimens with different targets for the area under the curve (AUC) and different dosing

regimens of busulfan. We retrospectively analyzed the association between busulfan exposure (AUC) and clinical outcomes. *Methods.* All children, transplanted for MDS and JMML between 2001-2008, receiving intravenous busulfan as part of a myeloablative regimen, were included. 34 patients received busulfan, cyclophosphamide 120 mg/kg, and melphalan 140 mg/m^2. Based on the findings of these patients (n=3) the most recent transplantations were performed using busulfan and cyclophosphamide 120 mg/kg only (AUC of >74 mg*h/L).The association between an AUC below or above the previously found lower limit of the optimum of 74 mg*h/L and the main endpoints; relapse rate, overall survival (OS) and event free survival (EFS) and the toxicity endpoints: acute-Graft-versus-Host Disease (aGvHD) grade 2-4; and Veno-occlusive Disease (VOD), were tested using Cox regression analysis and log-rank statistics. *Results.* 38 patients (27 MDS, 11 JMML) were transplanted (1 patient was excluded because of unpredictable pharmacokinetics). Median follow up time was 2.2 (range 1-18) years. EFS in the <74 mg*h/L and >74 mg*h/L was 58% and 82% (p=0.13), resp. OS rates were respectively 51% and 82% for these AUCs. EFS and OS were negatively influenced by higher rates of graft-failures/relapses in the <74 mg*h/L group (38% *vs.* 0%, p=0.047). Regarding the toxicity endpoints, busulfan exposure > 74 mg*h/L was associated with more aGvHD grade 2-4: 20% *vs.* 58% (p=0.037), and showed a trend to a higher VOD-rate: 12% *vs.* 34%, (p=0.105). *Conclusion.* Higher busulfan exposure (>74 mg*h/L) in combination with melphalan/cyclophosphamide was associated with higher EFS/OS rates due to lower rates of graft-failure/relapses. A concern however is the incidence of toxicity (aGvHD grade 2-4 and VOD) in the higher exposure group in patients receiving a combination of busulfan, cyclophosphamide and melphalan. More precise targeting (defining the upper limit for AUC) might further optimize the outcomes in SCT for MDS/JMML.

CO-20

HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR MYELODYSPLASTIC SYNDROME IN CHILDREN: PRELIMINARY RESULTS OF A TREOSULFAN-BASED CONDITIONING REGIMEN

B Strahm, C Niemeyer, P Sedlacek, A Fischer, M Zecca, F Locatelli

Pediatric Hematology and Oncology, Center of Pediatrics and Adolescent Medicine, University of Freiburg, Germany; Department of Pediatric Hematology and Oncology, University Hospital Motol, Prague, Czech Republic; Fondazione IRCCS Policlinico San Matteo, Pediatric Hematology/Oncology, University of Pavia, Italy

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative treatment in children with MDS. In the study EWOG MDS 98, a preparative regimen including busulfan, cyclophophamide and melphalan has been recommended for children transplanted for MDS. Transplantation-related mortality (TRM) has been a major cause of treatment failure especially in pts aged 12 or older and in those with secondary MDS. Therefore, we tested the safety and efficacy of a treosulfan-based conditioning regimen, which proved to be well tolerated and effective in pts with thalassemia major. Thirteen pts (median age 13.3 (5.5-19.7) yrs) with MDS (5 RC, 3 advanced MDS, 5 secondary MDS) were transplanted in 3 centres (Pavia 8, Freiburg 4, Prague 1). Cytogenetics revealed monosomy 7 (5), deletion 5q (1) or a normal karyotype (7). The preparative regimen consisted of thiotepa (8 mg/Kg), treosulfan (3x14 g/m^2) and fludarabine (4x40 mg/m^2). Seven pts were grafted with bone marrow from a matched unrelated (6) or sibling donor (1), while 6 pts received T-cell depleted peripheral stem cells from an HLA-haploidentical parent. Median time to neutrophil and platelet engraftment was 18 (11-31) and 21 (8-35) days, respectively. 4/12 pts developed acute GVHD II-III, while moderate chronic GvHD was observed in 2/10 pts. After a median follow up of 9.5 (5.8-31.5) mo 10/13 pts are alive and disease free. One child with advanced MDS relapsed 12 mo after haploidentical HSCT and is alive and disease-free after second HSCT. Three pts with secondary MDS died of treatment related complications: 1 CNS haemorrhage, 1 liver adenovirus infection, 1 disseminated thrombosis. These preliminary results show that HSCT with a treosulfan based preparative regimen is able to offer a remarkable event free survival with acceptable TRM for these high risk MDS patients. Larger number of pts treated and longer follow up are needed to confirm the results and evaluate the long-term efficacy and side effects of this regimen.

Juvenile myelomonocytic leukemia and the global interaction of patients, parents, scientists and physicians

IS-13

JUVENILE MYELOMONOCYTIC LEUKEMIA, PAST, PRESENT AND FUTURE PERSPECTIVES

MM van den Heuvel-Eibrink

On behalf of the EWOG-MDS group

JMML is a specific and rare subtype of leukemia which is characterised by young age, skin infiltration, hepatosplenomegaly, lymphadenopathy, elevated HbF, low platelet counts and the presence of abnormal myeloid precursors as well as monocytosis in the peripheral blood. In the past, in addition, in vitro GM-CSF hypersensitivity has been shown JMMLs hallmark. Until recently, the diagnosis was mainly based on the clinical picture and because of its rarity very often delayed by the absence of diagnostic criteria and classification. Currently, the diagnostic process of JMML has improved based on gained knowledge of the disease by systematic registration by several international groups. Moreover, studies on the biology of the disease have as yet unravelled molecular mechanisms which are involved in over 80% of all JMML cases. The biology of JMML has proven to be due to a continuous activation of the RAS-RAF-MEK-ERK signalling pathway. Recent studies have shown that mutations of the *RAS* and the *PTPN11* genes (in respectively 25 and 35% of the JMML cases) are responsible for this hyperproliferative activation. Another gene involved in this pathway is the neurofibromatosis (*NF1*) gene, i.e. JMML has shown to be associated with clinical neurofibromatosis type 1 (in 11-15 % of the cases). Moreover, JMML cases without clinical signs of NF1 can harbour NF1 gene mutations. Interestingly, in JMML the gene mutations involved in the RAS pathway occur mutually exclusive. Recently, an upstream gene, i.e. the son-of-sevenless (SOS1) was shown to be involved in a single case of JMML. So far, the only curative option for children with JMML is allogeneous stem cell transplantation. This treatment with its attendant short and long term toxicity, results in the curation of over 50-60% of all JMML patients. Hence, further future studies are underway in order to reach a better understanding of the disease and to develop novel, preferably targeted treatment strategies, which increase survival and diminish short and long term side effects.

IS-14

PERSPECTIVE OF A NORTH AMERICAN JUVENILE MYELOMONOCYTIC LEUKEMIA PARENT

TL Harris

JMML Foundation

Our family's journey with JMML began in late 2005 when our son, Evan, suffered from recurrent infections. By early 2006, we were on the emotionally wrenching and delay ridden path to a JMML diagnosis. My wife, Lisa, and I restructured our lives around the necessities of being parents to a child with cancer; she left a career that she loved to care for Evan full-time. Daycare was no longer an option, nor was it desired, our time together as a family had become infinitely more precious. Evan was among the first North American patients transplanted using the EWOG-MDS protocol. His leukemia was "cooled off" with daily oral 6-MP, from which he received only benefit and no side-effects. A donor search provided a 9-of-10 match in a kind and generous man named David. Evan was transplanted in summer 2006 at age 14 months and soldiered through VOD, then GvHD of the skin and gut. He was hospitalized for three months. Evan is now the picture of health, remains 100%-donor, requires no medication, and has normal development. The ordeal of those months struggling through diagnosis, transplant, and recovery brought out the best in us and those who rallied to our side. Lisa and I leveraged our complimentary strengths, forming a team stronger than the sum of its parts. We also leveraged technology, blogging to keep in contact with family and friends, and enlisting an army of helpers using web-based tools. Throughout, Evan never failed to surprise with his resilience and delight with his irrepressible personality. He gave us strength. Our journey continues beyond Evan's struggle. We joined the Board of Directors of The JMML Foundation, the only organization dedicated to the mission to cure JMML and to improve the quality of life of JMML patients and families through research, education, advocacy, and

charity. I plan to convey not only my perspective and that of The Foundation, but also what I can glean from blogs, and informal surveys and interviews of other North American JMML parents.

IS-15

PERSPECTIVE OF A EUROPEAN PARENT OF A CHILD WITH JUVENILE MYELOMONOCYTIC LEUKEMIA

NMJ Guequierre

JMML Foundation

March 2003, our 1,5 year old daughter Rosa was diagnosed with JMML. A day we will never forget. We were pregnant of our second child which turned out to be a perfect donor for Rosa. In the summer of 2003 Rosa was transplanted (EWOG-MDS protocol) and September 4th we were back home. Looking back, it was a relatively easy transplant, considering what other children had to go through. However, our struggle came soon afterwards when a month later Rosa's own cells returned.

From a doctors perspective, Rosa must have a second transplant including all risks and with an uncertain outcome. From the parents perspective we had no sick child. JMML was not detected, only forecasted. In these uncertain times we became real experts. About living between hope and fear, between emotions and rational considerations, between being a parent of Rosa and looking from a doctors perspective. We have been there all. Later, when everything turned out well for Rosa and our family, we got involved from the sidelines. Often parents of children with JMML came to us because of our comprehensively website about Rosa's illness. I took part in the JMML Foundation as a front office employee and website maintainer. And I was asked to help setting up a parents organization that raises money for research on child cancer at the Erasmus MC in Rotterdam the Netherlands. During these last 6 years that JMML played a role in our lives, we stayed interested in JMML and thanks to our doctor Marry van den Heuvel-Eibrink we were updated on, and even have been part of scientific research.

Refractory cytopenia

CO-21

IS IT POSSIBLE TO DISTINGUISH PEDIATRIC HEMATOLOGICAL DISORDERS DISPLAYING BONE MARROW DYSPLASIA?

RJ Leguit, MCA Bruin, MB Bierings, JG van den Tweel

University Medical Centre Utrecht, Netherlands, Depts of Pathology and Paediatrics, The Netherlands

In this study we investigated all bone marrow biopsies performed for cytopenia in one or more cell lines in children up to 18 years of age, from 1990 until the end of 2006. This group comprises 156 children, among which the following clinical diagnoses: definitely myelodysplastic syndrome (MDS): 16 patients, possible MDS: 6 patients, Down syndrome related MDS: 6 patients, - aplastic anemia (AA): 15 patients, - inherited or congenital bone marrow failure syndromes: 22 patients. Of the 16 MDS patients, 13 received allogenic stem cell transplantation (SCT), of which 6 patients died on average at 89 months after the first BMB. The surviving transplanted patients have a mean follow-up of 79 months. Of the 3 non-transplanted MDS patients, 2 had refractory cytopenia (RC) and were still alive at follow-up (mean 45 months). The one patient that died was known with Revesz syndrome and could not be transplanted because of managing problems. In the group of possible MDS, none was transplanted and none died (mean survival 81 months). In the AA group, 6 patients received SCT (one after developing MDS after 13 years of AA) and 3 patients (all without SCT) died, with an overall survival of 125 months. Reviewing the original first pathology reports, the bone marrow biopsy (BMB) findings in the MDS group were in 13/16 patients consistent with MDS. Most cases were hypocellular for the age of the patients and showed multilineage dysplasia. In the remaining 3 patients the bone marrow findings were not diagnostic of MDS, either because the dysplastic features were only mild and not diagnostic (2 patients), or because the biopsy was insufficient for assessment (1 patient). Of the 6 patients with possible MDS, all had BMB findings consistent with MDS (trilinear dyshematopoiesis). In the AA group, one case showed significant trilinear dysplastic features in the BMB. This was however a complicated case of a boy with Revesz syndrome and monosomy 7. All other cases of AA were hypocellular with only mild or no dysplastic features in the residual cell lines. In the remaining subgroups, dysplasia was also seen in some degree in various cases, and based on BMB findings MDS was suggested in some of them. In conclusion we can state that it is possible to suggest a diagnosis of MDS or AA on a bone biopsy in the majority of patients with cytopenias. However it is extremely difficult to distinguish with certainty, MDS from transient forms of dysplasia due to external factors. Also in the other subgroups of pediatric cytopenias, dysplastic features can be found, which makes dysplasia a difficult and non pathognomonic feature in bone marrow biopsies.

CO-22

MUTATIONAL SCREENING IN REFRACTORY CYTOPENIA - IN SEARCH OF CONGENITAL BONE MARROW FAILURE

A Karow,[1] M Fliegauf,[1] E Bergsträsser,[2] H Hasle,[3] M van den Heuvel-Eibrink,[4] F Locatelli,[5] S Matthes-Martin,[6] BM De Moerloose,[7] J Stary,[8] D Wójcik,[9] M Zecca,[5] I Furlan,[1] CM Niemeyer[1]

[1]Division of Pediatric Hematology and Oncology, Department of Pediatric and Adolescent Medicine, University of Freiburg, Freiburg, Germany; [2]Kinderspital Zürich, Steinwiesstr, Zürich, Switzerland; [3]Department of Pediatrics, Aarhus University Hospital Skejby, Aarhus N, Denmark; [4]Sophia Children´s Hospital, Dr. Molewaterplein, Rotterdam, The Netherlands; [5]Department of Pediatrics, University of Pavia, IRCCS Policlinico San Matteo Pavia, Italy; [6]St. Anna Kinderspital, BMT Unit, Wien, Austria; [7]Dept. of Pediatric Hematology-Oncology, Ghent University Hospital, Ghent, Belgium; [8]Dept. of Pediatric Hematology and Oncology, University Hospital Motol, Prague, Czech Republic; [9]Department of Pediatric Hematology/Oncology and Bone Marrow Transplantation, Wroclaw, Poland

Childhood-MDS is a rare hematological disorder. Especially the subgroup of patients with refractory cytopenia (RC) often reveals clinical parallels to inherited bone marrow failure syndromes (IBMF) such as congenital neutropenias or congenital dysceratosis. In order to elucidate the number of cases with IBMF diagnosed as refractory cytopenia, we

are screening the cohort of patients with RC included in the study EWOG-MDS-98 for gene mutations typically underlying IBMF including the SBDS-gene and genes of the telomerase complex. Focusing on children diagnosed through EWOG-MDS-98 as primary hypocellular RC without caryotypic abnormalities or myelofibrosis and exclusion of Fanconi Anemia (FA) our screen comprises a total of roughly 200 patients. Here we present our first data on the incidence of gene mutations underlying IBMF in RC patients revealing only sporadic cases. Our findings speak in favour of the diagnostic criteria applied in childhood-MDS.

CO-23

UPDATE ON IMMUNOSUPPRESSIVE THERAPY FOR CHILDREN WITH REFRACTORY CYTOPENIA

A Yoshimi,[1] AMJ Peters,[1] I Baumann,[2] B Strahm,[1] I Furlan,[1] E Bergsträsser,[3] A Fischer,[1] F Locatelli,[4] MM van den Heuvel-Eibrink,[5] M Führer,[6] CM Niemeyer[1]

[1]Department of Pediatrics and Adolescent Medicine, Division of Pediatric Hematology and Oncology, University of Freiburg, Germany; [2]Institute of Pathology, Medical Hospital, Bayreuth, Germany; [3]Kinderspital, Zürich, Switzerland; [4]Department of Pediatrics; University of Pavia, IRCCS Policlinico San Matteo, Italy; [5]Erasmus Medical Center, Sophia Children's Hospital, Rotterdam, The Netherlands; [6]Dr. von Haunersches Kinderspital, University of Munich, Germany.

Refractory cytopenia (RC) of childhood is the most common subtype of MDS in childhood accounting for about half of the cases, which is characterized by persistent cytopenia with less than 5% blasts in the bone marrow and less than 2% blasts in the blood. Because early bone marrow failure can in part be mediated by T-cell immunosuppression of haematopoiesis, immunosuppressive therapy (IST) can be a successful therapy strategy for some children with RC. We have previously published the results of IST with antithymocyte globuline (ATG) and cyclosporine for childhood RC based on the recommendations for severe aplastic anemia (SAA) (Yoshimi A, *et al.* Haematologica 2007, 92:397). Here, we present the results of longer-term follow-up (2.7-7.8 years, mean 4.4 years) of the described cohort of 31 patients. The median age at diagnosis was 10.1 (1.8-17.2) years. The patients were all transfusion dependent for platelets and/or red cells. Bone marrow cellularity was decreased in all patients. Cytogenetic analysis revealed a normal karyotype in 13 patients, no results in 17 patients, and abnormal karyotype in one patient, which was no longer detected after IST. At 6 months following IST, 2 patients (6%) were in complete response (CR) and 20 (65%) in partial response (PR). Of these 22 responders 10 patients were in CR, 5 patients in PR at the last follow-up, and 7 patients underwent hematopoietic stem cell transplantation (HSCT) for relapse (n=5) and/or clonal evolution (n=4). In total 5 patients developed clonal evolution. One patient with non response at 6 months received second IST and responded. In total 10 patients underwent HSCT. Three patients died; one toxic death during IST and 2 transplant related mortality. Defining toxic death, no response at 6 months, relapse, HSCT and clonal evolution as failure, 15/31 patients remained failure-free. The estimated overall and failure-free survivals at 5 years were 86% (71%-100%) and 44% (22-66%). Although the results showed the effectiveness of IST in some patients with RC, the rates of late relapse and clonal evolution were relatively high. Since the results of HSCT from matched unrelated donors in RC indicate a cure rate of approximately 70% to 80%, it is reasonable to recommend that, in the presence of a suitable alternative donor, non-responders and relapsed patients should proceed to HSCT early in the course of their disease.

CO-24

DNA DAMAGE IN THE HEMATOPOIETIC SYSTEM: BONE MARROW FAILURE AND THE TRANSFORMATION TO LEUKEMIA

JR Haanstra,[1] J Verhagen-Oldenampsen,[1] P van Strien,[1] M Valkhof,[1] B Schumacher,[2] M von Lindern,[1] I Touw[1]

[1]Department of Hematology, Erasmus MC, Rotterdam; [2]Department of Genetics, Erasmus MC, Rotterdam, The Netherlands

Objective. DNA damage can cause transforming mutations, but may initially result in cellular senescence and tissue hypoplasia. Bone marrow cells escaping senescence are easily selected in such a growth factor-rich, empty environment, and senescent tissue may be a pro-leukemic environment. Therapy-related myelodysplasia (tMDS) and Fanconi anemia (FA) are examples of bone marrow failure with a high risk of leukemic transformation. The aim of our studies is to understand the molecular mechanisms of leukemogenesis in a genotoxic background. *Methods*. FA is characterized by extreme sensitivity to DNA cross-linking, resulting in stalled replication. The DNA repair protein Ercc1 is functionally involved in the FA pathway and is required to remove interstrand cross-links. We employ Ercc1-deficient mice as a model for FA to study the effects of DNA damage on the hematopoietic system. Hematopoietic organs were analysed by FACS and the potential of progenitors was tested in colony assays. To investigate whether bone marrow senescence depends on the tumor suppressor genes Cdkn2a (p16/Ink4A) or Trp53 we generated Ercc1-/- mice deficient for these genes. *Results.* Although Ercc1-/- mice have normal peripheral blood counts, myeloid progenitors are markedly reduced, while lymphoid progenitors are hardly affected. The lower number of myeloid progenitors coincides with reduced *in vitro* myeloid colony formation of total bone marrow. Loss of Ercc1 also results in reduced stem cell numbers, but the earliest stem cells are least affected. Interestingly, loss of Trp53 but not loss of cdkn2a can restore stem cell levels and the presence of colony forming cells. *Discussion*. Replication fork stalling caused by Ercc1 deficiency results in a strong p53-dependent bone marrow senescence. We are currently testing the *in vivo* reconstitution potential of Ercc1-deficient cells in transplantation experiments. We have observed the first leukemias after transplantation and this may provide a potent model to study leukemic transformation following DNA damage induced hypoplasia.

Juvenile myelomonocytic leukemia

IS-16

GENETICS OF JUVENILE MYELOMONOCYTIC LEUKEMIA IN CLINICAL PRACTICE

C Flotho

University of Freiburg, Germany

Juvenile myelomonocytic leukemia (JMML) is a clonal malignant disorder of the myeloid bone marrow compartment characterized by excessive proliferation of monocytes, granulocytes and myeloid precursors. The intracellular RAS signaling pathway is a key modulator of deregulated proliferation in JMML. RAS signaling is disturbed by deficiency of the neurofibromatosis-1 (*NF1*) tumor suppressor gene (11% of patients), mutations in the NRAS or *KRAS* genes (25% of patients), or *PTPN11* mutations (35% of patients). Another candidate gene is lately being investigated. This presentation will focus on how the advanced understanding of molecular genetic lesions in JMML contributes to the clinical management of this notoriously challenging disease. Due to the lack of pathognomonic bone marrow morphology, JMML is inherently difficult to diagnose. Clinical, hematologic and cytogenetic criteria were internationally agreed on to facilitate and standardize the diagnostic process. The guidelines will be reviewed, integrating recommendations on how the improved knowledge of genetic lesions in JMML leads, in the majority of cases, to a streamlined and more accurate diagnosis of this disease. Although the contribution of various RAS pathway gene mutations to myeloid hyperproliferation has been well established, it is less clear (and, indeed, doubtful) whether the contribution of each gene is functionally equivalent to that of the others. It is therefore of clinical interest in JMML to study possible correlations between genotype and disease phenotype or prognosis. The EWOG-MDS patient data, and that of other groups, will be reviewed from this perspective. Lastly, JMML gene mutations make for useful disease markers. Measuring the "mutational burden" in the bone marrow or peripheral blood has the clinical benefit of being able to quantitate disease response to treatment, or to detect disease recurrence after stem cell transplantation. In summary, state-of-the-art clinical management of JMML involves careful genetic work-up, which translates into the diagnostic process, into making therapy decisions, and into efficient monitoring of the disease.

IS-17

IN VIVO MODELS AND MOLECULAR MECHANISMS OF JUVENILE MYELOMONOCYTIC LEUKEMIA

Z Yang, RJ Chan

Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN, USA

Juvenile myelomonocytic leukemia (JMML) is a lethal myeloproliferative disease (MPD) of young childhood characterized clinically by overproduction of myelomonocytic cells and by the in vitro phenotype of hematopoietic progenitor hypersensitivity to granulocyte-macrophage colony-stimulating factor (GM-CSF). Despite the use of multiple chemotherapeutic agents, the only curative therapy is allogeneic stem cell transplantation. Approximately 70% of JMML patients bear loss-of-function mutations in *NF1* (~15-20%), gain-of-function mutations in *KRAS* or *NRAS* (~15-20%), or gain-of-function mutations in *PTPN11* (~35%). Definition of key mutated genes in JMML has permitted basic researchers to develop *in vitro* and *in vivo* models of JMML. Hematopoietic progenitors from *Nf1*$^{-/-}$, *Kras*^{G12D}, and *Shp2*^{D61Y} mice demonstrate elevated mitogen activated protein kinase signaling, hypersensitivity to GM-CSF, and produce MPD *in vivo*. In addition to myeloid progenitor hyperproliferation, the composition of progenitor colonies from JMML patients is almost exclusively macrophages and monocytes, indicating that JMML is a disease distinguished phenotypically by shunting of hematopoietic differentiation toward the monocytic pathway. Considering this unique phenotypic characteristic of JMML, the central role of Ras deregulation in JMML, and previous studies demonstrating mutant Ras-induced skewed myelomonocytic differentiation, we hypothesized that activating *PTPN11* mutations promote commitment to the myelomonocytic lineage by altering expression of hematopoietic-specific transcription factors and transcription factor interactions. We present a novel molecular model underlying mutant Shp2-enhanced monocytic differentiation and propose that Ras hyperactivation leads to constitutive JNK activation, elevated c-Jun expression, and increased interaction between c-Jun and PU.1, thus promoting skewed monocytic differentiation.

CO-25

EPIDEMIOLOGY AND OUTCOME OF PEDIATRIC MYELODYSPLASTIC SYNDROME AND JUVENILE MYELOMONOCYTIC LEUKEMIA: A NATION-WIDE, RETROSPECTIVE, CLINICAL NETWORKING STUDY IN KOREA

H Kook, DK Han, TJ Hwang, NG Chung, B Cho, DC Jeong, HK Kim, KH Yoo, HH Koo, HJ Im, HJ Kang, HY Shin, HS Ahn, JK Park, SY Lee, YT Lim, JE Park, ES You, KH Ryu, SY Kim, HJ Park, KS Lee, CJ Lyu, JY Lim, KC Lee, MJ Lee, JY Kim, YH Lee, JO Hah, SG Park, JJ Seo

Chonnam National University, Catholic University, Sungkyunkwan University, Ulsan University, Seoul National University, Inje University, Pusan National University, Ajou University, Ewha Womans University, Chungnam National University, National Cancer Center, Kyungpook National University, Yonsei University, Gyeoungsang National University, Korea University, Dankook University, Chungpook University, Hanyang University, Youngnam University, Korea

Recent advances in the diagnosis, molecular pathogenesis, classification and therapy have been made in the field of childhood myelodysplastic syndrome (MDS) and juvenile myelomonocytic leukemia. We report a nation-wide retrospective analysis of children with MDS and JMML diagnosed between Jan. 2001 and Jun. 2008 in Korea. In total, 148 patients were enrolled from 21 major hospitals with pediatric hematology oncology clinics: MDS, 103 (primary MDS, 80; constitutional anomalies with MDS, 15; treatment-related MDS, 8) and JMML, 45. The incidence of MDS/JMML was 19.7/year, which comprised about 6% of childhood leukemias. Various classification systems including FAB, WHO, IPSS, CCC system, WPSS and pediatric adjustment of the WHO classification were applied. The median ages at diagnosis were 78 and 12 months in MDS, and JMML, respectively. Males dominated in JMML. Cytogenetic abnormalities were observed in 44% of MDS (monosomy 7, 8; trisomy 8, 11) and in 13% of JMML. Treatment was chosen by each institute's preference: 34 patients with MDS received AML-type intensive chemotherapy, with complete remission rate of 68.0%. The 5-year Kaplan-Meier overall survival rate was 56% and 55% for MDS and JMML, respectively. Survival of MDS patients was influenced by the marrow blast % (p=0.001) and disease category (p=0.000). Stem cell transplantations (SCT) were undertaken in 64 patients (MDS, 32; JMML, 32). The sources of stem cells were as follows: bone marrow (BM), 34; peripheral blood (PB) + umbilical cord (UC), 12; PB, 11; UC, 5; BM + UC, 2. Matched related transplants were done in 12 cases. Conditioning regimens were various, but BuCy-based regimen was used in 59.4%. Acute GvHD ≥ Grade II was found in 46.9% and chronic GvHD in 40.6%. The 5-year Kaplan-Meier overall survival rate was 57% for MDS, and 60% for JMML. Survival after unrelated transplant was comparable with that of matched related transplants. This networking study will serve as a platform for nation-wide, prospective study in Korea, encompassing morphologic study by a central pathology review board, epidemiologic study, molecular pathophysiologic study, and therapeutic trials incorporating SCTs, or new drugs.

CO-26

IMPACT OF GM-CSF SIGNALING PATHWAY-RELATED GENES ON CLINICAL OUTCOME OF CHILDREN WITH JUVENILE MYELOMONOCYTIC LEUKEMIA

N Yoshida,[1] H Yagasaki,[2] Y Xu,[2] K Matsuda,[3] A Yoshimi,[4] Y Takahashi,[2] K Matsumoto,[1] K Kato,[1] J Ueyama,[5] H Inada,[6] H Goto,[7] M Yabe,[8] K Kudo,[9] J Mimaya,[10] A Kikuchi,[11] K Koike,[12] A Manabe,[13] S Kojima[2]

[1]Department of Hematology and Oncology, Children's Medical Center, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan; [2]Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan; [3]Department of Laboratory Medicine, Shinshu University Hospital, Matsumoto, Japan; [4]Division of Pediatric Hematology and Oncology, University of Freiburg, Freiburg, Germany; [5]Division of Pediatrics & Perinatology, Faculty of Medicine, Tottori University, Yonago, Japan; [6]Department of Pediatrics, Kurume University School of Medicine, Kurume, Japan; [7]Department of Pediatrics, Yokohama City University Hospital, Yokohama, Japan; [8]Department of Cell Transplantation, Tokai University School of Medicine, Isehara, Japan; [9]Department of Pediatrics, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Japan; [10]Department of Hematology and Oncology, Shizuoka Children's Hospital, Shizuoka, Japan; [11]Division of Hematology / Oncology, Saitama Children's Medical Center, Saitama, Japan; [12]Department of Pediatrics, Shinshu University School of Medicine, Matsumoto,Japan; [13]Department of Pediatrics, St.Luke's International Hospital, Tokyo, Japan

Background. Juvenile myelomonocytic leukemia (JMML) is characterized by hypersensitivity to GM-CSF *in vitro*. Mutations in RAS, NF1 or PTPN11 positioned in the GM-CSF signal pathway are thought to be involved in the pathogenesis of JMML. *Objective.* To assess the impacts of these mutations on clinical features and outcome of JMML. Methods: We tested 71 Japanese children with JMML for NRAS, KRAS and PTPN11 mutations and evaluated their clinical significance. *Results.* Of the 71, PTPN11 and N/KRAS mutations were found in 32 (45%) and 13 (18%) patients, respectively. For 3 patients, a clinical diagnosis of neurofibromatosis type 1 (NF1) was confirmed. Neither PTPN11 nor RAS mutations nor NF1 were present in 23 (32%) patients. Compared with patients with RAS mutation or without any aberrations, patients with PTPN11 mutation were significantly older at diagnosis and had higher HbF level, both of which have been recognized as poor prognostic factors. As was expected, overall survival (OS) was lower for patients with PTPN11 mutation than for those without (25% *vs.* 64%; p=0.0029). Age older than 24 months (p=0.003) and abnormal karyotype (p=0.012) were also associated with poor survival. Presently, stem cell transplantation (SCT) is the only curative treatment; however, disease recurrence remains the major cause of treatment failure. In an analysis of 48 patients who received SCT, PTPN11 mutation was the most significantly associated factor with OS and the only unfavorable factor for relapse after SCT (p=0.001). Notably, all 12 patients who died of relapse had PTPN11 mutation. Of particular interest cytogenetically is the fact that all 7 patients with an abnormal karyotype other than monosomy 7 died, and all had a PTPN11 mutation. *Conclusions.* JMML with PTPN11 mutation might be a distinct subgroup with specific clinical characteristics and poor outcome. In these patients, early SCT should be considered and better strategies to lower the risk of relapse are warranted.

CO-27

ABERRANT GM-CSF SIGNAL TRANSDUCTION PATHWAY IN JUVENILE MYELOMONOCYTIC LEUKEMIA BY MULTIPARAMETRIC FLOW CYTOMETRY: RELIABILITY OF A RETROSPECTIVE STUDY

G Gaipa,[1] C Bugarin,[1] E Giarin,[2] D Longoni,[1] M Zecca,[3] G Basso,[2] A Biondi[1]

[1]Centro Ricerca M. Tettamanti, Clinica Pediatrica Università Milano-Bicocca, Ospedale San Gerardo, Monza (MI); [2]Laboratorio di Oncoematologia Pediatrica, Dipartimento di Pediatria, Università, Padova; [3]Oncoematologia Pediatrica, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

Juvenile myelomonocytic leukemia (JMML) is a rare clonal myeloproliferative disorder of infancy and early childhood characterized by overproduction of myeloid cells (Aricò *et al.,* Blood, 1997) and a selective hypersensitivity of the hematopoietic precursor cells to GM-CSF (Emanuel PD *et al.,* Blood, 1991). Multiparametric flow cytometry analysis has demonstrated new potentialities for assaying intracellular levels of phosphorylated proteins (Irish JM *et al.*, Cell, 2004). We and others have reported that *in vitro* response of JMML cells demonstrated a greater increase in the % of STAT5-phosphorylated cells, in single a cell profiling assay. (Gaipa G *et al.,* Leukemia, 2008; Kotecha N et al., Cancer Cell, 2008). *Objective and methods.* Here we analyzed the STAT5-phosphorylated cells in fresh Vs thawed BM mononuclear (MNC) cells from 3 JMML patients and 9 controls. In addition, we extended the immunophenotypic characterization of GM-CSF responding cells by applying 7-colors flow cytometry (LiveDead dye/CD33/CD34/CD14/CD45/ STAT5/CD38) in two post-BMT JMML samples. *Results and Discussion.* The aberrant STAT5 response was confirmed in all thawed JMML samples, although to a lesser extent as compared to the fresh samples. We also evaluated the STAT5 expression by scaling the maximum response to 100% as proposed by Kotecha *et al.* This approach improved the resolution between JMML and normal samples. The application of 7-color flow cytometry allowed the simultaneous identification of two GM-CSF responding populations: the $CD34^-/CD33^+/CD14^+/CD38^{low}$ cells described by Kotecha et al , and the $CD34^+/CD33^+/CD14^-/CD45^{low}$ cells reported by our group. We speculate that the latter could represent the more "stem" population and the former being the GM-committed precursor cells. The extension of this approach to a larger series of JMML samples will help to investigate further biological insights of JMML and to evaluate to what extent this new assay may be considered as a tool for the diagnostic work-up of JMML patients.

POSTERS

PO-01

CHRONIC URTICARIA AS FIRST SIGN OF MYELODYSPLASTIC SYNDROMES

C Micalizzi, J Svahn, F Scuderi, T Lanza, M Faraci, L Banov, M Calvillo, O Burlando, F Fioredda, D Caprino, C Dufour

Gaslini Research Institute, Genova, Italy

We report two patients with MDS presenting with recurrent urticarioid episodes. *Patient 1*. 8-year-old boy with chronic urticaria for 8 months. He had mild macrocytic anemia (Hb 9.5 g/dL, MCV 95fl), moderate neutropenia (ANC 1×10^9/L), monocytosis (1.8×10^9/L), normal platelet count (222×10^9/L) with giant elements. Eosinophils and IgE levels were normal, while hypergammaglobulinemia (IgG 2714 mg/dL, IgA 633 mg/dL) was present. HbF was increased (4.4%) and ferritin was 212 ng/dL. A double red cell population with A and O blood groups (acquired group was O) was detected. Bone marrow biopsy showed hypercellularity with increased granulopoiesis (54%), 20% monocytes, ALIP, increased reticulin, 10% myeloid blasts and trilineage dysplasia. Karyotype revealed chromosome 7 monosomy. Final diagnosis was RAEB. JMML was excluded since progenitor growth in colony assay was in clusters but without spontaneous proliferation and because RAS and PTPN11 oncogenes were not mutated. The boy is in complete continuous remission 6 months after HLA matched sibling transplant. *Patient 2.* 14-year-old boy, healthy until 2 months before first observation, when recurrent cutaneous urticarioid lesions on abdomen and thorax plus edema of tibio-tarsic joints were observed. These symptoms were treated with intermittent low dose steroids. Initially, blood count showed mild leukopenia (4×10^9/L) and 37% ANC. Two months later WBC was 8.2×10^9/L with 20% ANC, 28% lymphocytes, 47% blast cells, hemoglobin was 10.7 g/dL with normal MCV (85fl), platelet count was 117×10^9/L. Bone marrow biopsy showed overt AML(M4Eo) with trilineage dysplasia; a complex karyotype was present: 51,XY,+8,+13, +20,+21,inv(16). Diagnosis was secondary leukemia arising from MDS, and urticaria was the first sign. In conclusion, children with chronic urticaria must be screened for hematological malignancies.

PO-02

PROHEPCIDIN IN MYELODYSPLASIA

NM El Hossainy,[1] MM Mattar[2]

[1]Asisstant lecturer Faculty of medicine, Cairo University; [2]Professor of Hematology, Faculty of medicine, Cairo University, Egypt

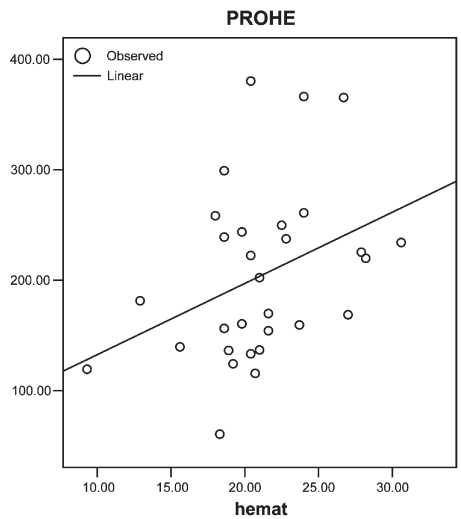
Objectives. 1. Determine main drive factor control hepcidin release whether the erythroid drive which suppresses it or the iron overload which enhance its release. 2. To compare our results with those of other anemias of chronic illnesses in order to find out if we can use the measurement of this prohormone for classification of anemias. Patients and methods: 30 adult myelodysplastic patients and 20 healthy adults. Serum prohepcidin was measured by an immunosorbent Statistics were done by SPSS. *Results.* A statistically significant difference in means of prohepcidin between the two groups. There was weak positive correlation only between prohepcidin and HCT.

Table 1. Correlation analysis between prohepcidin and other hematological parameters in MDS patients.

Variables	Prohepacidin		
	r	*p*	*Significance*
STFR	-0.018	0.962	NS
HCT	0.364	0.048	S(+)*
Ferretin	-0.043	0.821	NS
Blood units	0.021	0.911	NS

**Correlation is significant at the 0.05 level (2-tailed).*

Discussion. In the case group the mean prohepcidin was 203.99 ug/L with no statistical significant difference regarding sex or age subgroups in patients. But comparisons between cases and control group revealed a statistically significant difference between them. When comparing our results to other types of anemia, enhanced levels of prohepcidin (148.1 ug/L) in patients with chronic renal impairment. In contrast, concentrations of pro-hepcidin were significantly decreased in patients with hereditary hemochromatosis (70.2 ug/L) and the patients with rheumatoid arthritis (115.0 ug/L).There was weak positive correlation only between prohepcidin and hematocrite . Shih et al found that prohepcidin levels correlated positively with hematocrite that was the only significant predictor of plasma prohepcidin level. we can conclude that MDS is a disease with iron overload and variable degrees of erythroid acivity. Prohepcidin level in general increases in this anemia unlike other anemias with iron overload. To use prohepcidin or hepcidin in differentiating different anemias need further studies.



PO-03

THIOPURINE S-METHYL TRANSFERASE POLYMORPHIC STATUS, 6-MERCAPTOPURINE, AND TNF-ALPHA ANTAGONISM IN THE DEVELOPMENT OF MYELODYSPLASTIC SYNDROME / MONOSOMY 7 IN A CHILD WITH CROHN'S DISEASE

A Piccin,[1] U Rovigatti,[2] C Mc Mahon,[1] J Kelly,[3] B Bourke,[4] A O'Marcaigh,[1] OP Smith[1]

[1]Departments of Hematology, [3]Cytogenetics, [4]Gastroenterology, Our Lady's Children's Hospital and Trinity College University, Dublin, Ireland; [2]Retrovirus Centre, University of Pisa Medical School, Pisa, Italy

An association between inflammatory diseases, purine analogues and anti TNF-α and malignancy is not new. While 6-mercaptopurine (6-MP) and infliximab has been associated with non-Hodgkins lymphoma, the development of other clonal haematopoietic disorders(leukaemia or myelodysplasia) is less clear. Thiopurine S-methyl transferase (TMPT) is a drug-metabolizing enzyme. The TPMP gene is polymorphic, and 3 variant alleles account for the majority of persons with intermediate or low TPMT activity. Clinical studies shown higher risk of second cancers in children with acute lymphoblastic leukaemia treated with oral 6-MP. Infliximab®, a chimeric mouse/human anti-TNF-α antibody was the first agent generated to selectively target TNF-α and has shown good efficacy in inflammatory conditions, but induces a lack of tumour suppression. We report a case of a child with Crohn's disease (CD), who developed MDS/Monosomy7 and Diabetes Insipidus (DI) while treated with 6-MP and Infliximab®.

An 8 year old boy with CD responded to corticosteroids, because relapses, 6-MP (40 mg/m^2) and Infliximab® (20 mg/Kg) were added. Three yearS after commencing 6-MP and Infliximab®, developed pancytopenia and DI. Marrow aspirate showed trilineage dysplasia with monosomy 7. TPMT analysis showed intermediate enzymatic activity. He underwent successful MUD-HSCT. Three years after HSCT he is well, with 100% donor chimerism. Could anti-TNF-α therapy alone have initiated dysplasia by dysregulating stem cell differentiation cytokines (IL-1, IL-6, IL-8 and GM-CSF)? If so, did the use of a thiopurine plus anti- TNF-α targeting lower the threshold to MDS evolution especially in the setting low TPMT activity. The association of DI, monosomy 7 and myelodysplasia is intriguing. In a previous report 2 adults showed similar findings with low TGFβ-1. TGF-β1 and TNF-α are key

regulators of haematopoiesis via acceleration of c-kit degradation. Given the similar phenotype of DI, monosomy 7 and myelodysplasia, c-kit regulation may be modulated by TGF-β1/TNF-α Novel immunosuppressive therapies, such as anti TNF-α and 6MP, for inflammatory conditions may be efficacious, however haematological/ cytogenetic monitoring should be considered.

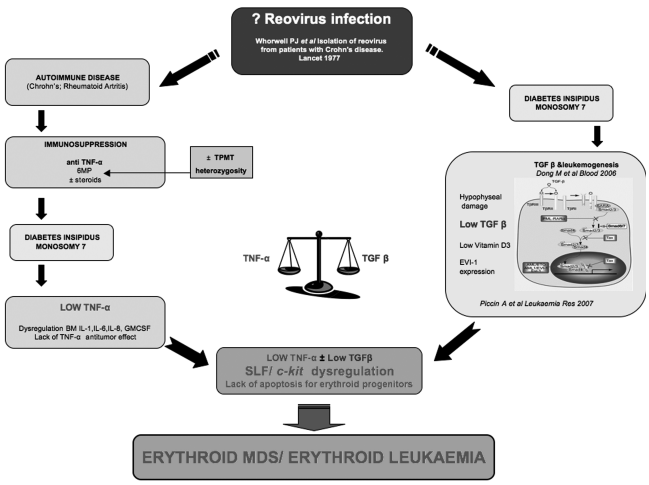


Figure 1.

PO-04

MYELODYSPLASTIC SYNDROME AS AN EARLY MANIFESTATION OF FANCONI ANEMIA

N Valizadeh

Urmia University of Medical Sciences, Urmia, Iraq

Background. Fanconi anemia is an autosomal recessive genetic disorder. It may be presented by anemia, thrombocytopenia, aplastic anemia. Skeletal anomalies (Radious and thumb malformation), short stature and cardiac, gatrointestinal and renal anomalyies are other manifestations. Patients with Fanconi anemia may developed AML,bone marrow failure,and myelodysplastic syndrome in the course of their disease.we want to report a 16 y/o female with fanconi anemia presented with MDS. *Case Report.* A 16 y/o female admitted with easy fatigability, dyspnea on exertion and dizziness. Family history was positive for two sisters with fanconi anemia. Pysical exam revealed pallor and short statuere. Laberatory findins: WBC=1700, Hb=2.2, MCV=112,PLT=70,000. LDH=600 Total & Direct Bill and Serum B12 & Folate levels were normal. Renal and liver function tests and abdominal sonography and echocardiography all reported normal. Bone marrow aspiration and biopsy showed cellularity =65-70%, Myelodysplastic changes in erythroid and myeloid series and increased in Number of dysplastic megakaryocytes and many micromegakaryocytes. Diagnosis of MDS secondary to Fanconi anemia was made according to family history and clinical and laboratory findigs and she refered to performing cytogenetic study and chromosomal breakage test and evaluation for allogenic Bone marrow transplantation. *Result.* MDS was as early manifestation of Fanconi anemia in this patient. *Conclusion*. We suggest investigation for Fanconi anemia in younger patients(age<20y) with myelodysplastic syndromes. *Key words.* Fanconi anemia, Myelodysplastic syndrome, bone marrow transplantation

PO-05

RAS AND FLT3-ITD GENE MUTATIONS IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES IN INDIA: AIIMS EXPERIENCE

R Chaubey, S Sazawal, M Mahapatra, R Saxena

Department of Hematology, All India Institute of Medical Sciences,New Delhi, India

Background. Chromosomal abnormalities and molecular detection has potential importance for diagnosis and prognosis of MDS, although the mechanisms underlying the development of MDS and their progressive evolution to AML are still largely unknown. Since, no studies have been reported from India on the prevalence of N-RAS, K- RAS point mutation in codon 12 and FLT3-ITD mutations in patients with MDS, we undertook this study. *Objective.* To find out the frequency of RAS and FLT3-ITD mutations in Indian MDS patients. *Method.* DNA and RNA were extracted from bone marrow/peripheral blood. Using RT-PCR the patients were screened for length mutations in FLT3 gene. PCR-RFLP and nested PCR-RFLP were used for the detection of point mutation in codon 12 of N-RAS and K-RAS. *Results.* A total of 53 patients (median age 39 yrs, range 9-78yrs; M: F 2:1; median TLC-3.9×10^9/L, range 0.8-116×10^9/L Median platelet count- 87×10^9/L, range 1-349×10^9/L, Median hemoglobin -6.8 g/dL, range 2.7-16.1 g/dL, were studied. One out of 53 patients (2%) was found positive for N-RAS and four patients were positive for K-RAS (8%) mutation. FLT3-ITD mutation was studied in 47 patients; all the patients were found negative. The mean observation of all the patients was 30 months and the median overall survival was 28 months. Nine patients died during follow up. The presence of N-RAS codon 12 mutation was associated with the poor survival. FLT3-ITD mutation was not observed in any of our cases, which is in contrast to 3% reported from the West. *Conclusion.* Thus, it appears that the RAS and FLT3 mutations are uncommon in MDS patients in India.

PO-06

THE FEATURES OF 65 CHINESE PEDIATRIC PATIENTS WITH *DE NOVO* MYELODYSPLASTIC SYNDROMES

S Hu,[1] W Gu,[2] H He,[1] Y Chai[1]

[1]*Hematology&Oncology Department, Children Hospital of Soochow University, Suzhou city;* [2]*The Third Affiliated Hospital, Soochow University, Changzhou city, China. Shaoyan Hu and Weiying Gu contribute to the manuscript equally and should be considered as the first co-author*

Objective. The relationship between MDS and AML is complex and incompletely understood. We try to discuss this question by retrospectively analyzing clinical data in Chinese children with *de novo* MDS. Method: Clinical data of 65 pediatric MDS patients from the recent 13 years were recorded. The diagnosis and typing of MDS were established according to the recent pediatric modification of the WHO classification. Patients of RC were treated with ATRA or/ and cyclosporine, RAEB and RAEB-t with intensive chemotherapy. *Results.* There were 46 boys and 19 girls with a median age of 6 years. 17 patients presented with RC(26.15%), 27 with RAEB(41.54%), 21 with RAEB-t(32.31%). Pallor, febrility, petechia and hepatosplenomegly involved accounted for 54.05%, 37.84%, 13.51% and 40.54% respectively. Sixty (39/65) percent of the patients had clonal chromosome abnormalities and forty (26/65) percent as normal karyotypes. Rates of abnormality varied among subtypes: 31.2% in RC, 63% in RAEB and 76.2% in RAEB-t. Numeral chromosome abnormality, including -5/5q, +7/-7, +8, +21 were more common. 29 kinds of fusion genes products were negative, which were more than 40% positive in AMLs. No RC patient obtained CR. Only 5 patients achieved CR with a CR rate of 10.42% (5/48). 12 patients transformed AML in a time-dependent way. The median DFS time of the MDS patients with abnormal karyotype was lower than that of the patients with normal karyotype (6 *vs.* 30 months, p=0.000). *Discussion*. Children with MDS shared the similar clinical manifestations with AML. Conventional cytogenetics still remains the basic technique in identifying chromosomal abnormalities associated with pediatric MDS. Alleviative treatments without HSCT were not curable ways. All forms of MDS with different likelihoods of transition to AML perhaps have different mechanisms to operate in different patients. New more sensitive techniques to detect genetic alterations will provide additional insights on the question.

PO-07

PREDICTING RESPONSE TO IMMUNOSUPPRESSIVE THERAPY IN CHILDHOOD APLASTIC ANEMIA

N Yoshida,[1] H Yagasaki,[2] Y Kosaka,[3] R Kobayashi,[4] H Yabe,[5] T Kaneko,[6] M Tsuchida,[7] A Ohara,[8] T Nakahata,[9] S Kojima[2]

[1]Department of Hematology and Oncology, Children's Medical Center, Japanese Red Cross Nagoya First Hospital, Nagoya; [2]Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya; [3]Department of Hematology and Oncology, Kobe Children's Hospital, Kobe; [4]Department of Pediatrics, Sapporo Hokuyu Hospital, Sapporo; [5]Department of Cell Transplantation, Tokai University School of Medicine, Isehara; [6]Department of Pediatrics, Kiyose Children's Hospital; [7]Department of Pediatrics, Ibaraki Children's Hospital; [8]Department of Pediatrics, Toho University School of Medicine, Tokyo; [9]Department of Pediatrics, Kyoto University Graduate School of Medicine, Kyoto, Japan

Background. Immunosuppressive therapy (IST) with antithymocyte globulin (ATG) and cyclosporine (CyA) provides response rates of 50-70% for childhood aplastic anemia (AA), but predictive markers of response to IST have not been well defined. We investigated the clinical relevance of HLA, a minor population of PNH-type cells, and a specific auto-antibody associated with AA in pediatric patients and reported that there was no correlation between these markers and IST response (Yoshida N, *et al.* Br J Haematol, 2008). *Objective.* To investigate whether clinical and laboratory findings before treatment could predict the IST response. *Methods.* We prospectively assessed the correlations between IST response and pretreatment findings including age, sex, interval between diagnosis and treatment, etiology, severity, white blood cell (WBC) count, neutrophil count, hemoglobin level, reticulocyte count, and platelet count in a cohort of a multicenter AA-97 study in Japan. *Results.* Between 1997 and 2006, 312 newly diagnosed AA children were enrolled and treated with a combination of ATG and CyA. The median age at diagnosis was 8 years (range, 1-17 years). Of the 312 patients, 156 had very severe disease, 107 had severe disease, and 49 had moderate disease. The median interval between diagnosis and treatment was 15 days (range, 1-180 days). The overall response rate was 56%. In multivariate analyses, lower WBC count (p=0.008), shorter interval between diagnosis and therapy (p=0.02), and male sex (p=0.02) were predictive markers of better response. Notably, response rate was inversely related to the interval between diagnosis and treatment. *Conclusion*s. Pretreatment clinical and laboratory findings influence the response to IST. Response is well correlated with WBC count rather than neutrophil count or severity of disease. IST should be started as soon as possible after diagnosis of AA, given that the response rate worsens as the interval between diagnosis and treatment increases.

PO-08

VERY SEVERE ACQUIRED APLASTIC ANEMIA: HISTORICAL OUTCOME OF PATIENTS TREATED BY ALLOGENEIC BONE MARROW TRANSPLANTATION FROM MATCHED SIBLING DONORS. A STUDY BY THE SPANISH GROUP FOR BLOOD AND MARROW TRANSPLANTATION IN CHILDREN (GETMON)

A Muñoz-Villa, M Diaz de Heredia, MA Diaz, I Badell, A Martinez, M Gonzalez-Valentin, MA Dasi, P Gomez, E Bureo, T Olive, JM Perez-Hurtado, MG Vicent, MS Maldonado on behalf of GETMON

Hospital Ramon y Cajal-Universidad de Alcala, Hospital La Paz, Hospital Niño Jesus, Madrid; Hospital San Pau, Hospital Valle Hebron, Barcelona; Hospital La Fe, Valencia; Hospital Reina Sofia, Cordoba; Hospital Valdecilla, Santander; Hospital V. Rocio, Sevilla, Spain

Objectives. Allogeneic haematopoietic stem-cell transplantation (AHSCT) from a matched sibling donor is the treatment of choice for very severe acquired aplastic anaemia (VSAA) in children. Experience with this approach from Spanish Working Party for Blood and Marrow Transplantation in Children in two sequential time periods (1982-1990 and 1991-2004) is reported. *Patients and methods.* Sixty two consecutive patients with a median age of 10 years were transplanted; 18 in the 1982-1990 period and 44 in the 1991-2004 period. Conditioning regimen consisted mainly of irradiation and cyclophosphamide in the first time period (72% of patients) and cyclophosphamide ± anti-thymocyte globulin (62%) in the second. Graft versus host disease prophylaxis consisted of cyclosporine in most patients (57/62). *Results.* Fifty one patients are alive and disease-free at a median follow-up of 127 months. Five years probability of event-free survival (EFS) is 82%. The EFS increased from 61 % to 91 % along the two time periods. Eleven patients died from graft failure or rejection (3), acute or chronic graft versus host disease and infection (4) or multi-organ failure (4). Univariate analysis identified two significant prognostic factors: interval diagnostic/transplant and time period of transplant (for both p=0.03). *Discussion.* This experience evidence the improvement in the results with AHSCT from a matched sibling donor in VSAA during the last two decades, with a current EFS of 90% of patients.

PO-09

ABSENCE OF HEMOGLOBIN F EXPRESSION ON BONE MARROW ERYTHROBLASTS IDENTIFIES A DISTINCT SUBSET OF ACQUIRED APLASTIC ANEMIA PATIENTS WITH GOOD RESPONSE TO IMMUNOSUPPRESSIVE THERAPY

M Ito, R Ichihashi, M Fujino, S Kojima[1]

Department of Pathology, Japanese Red Cross, Nagoya 1st. Hospital; [1]Department of Pediatrics, Nagoya University Graduate School of Medicine, Japan

Background. Acquired aplastic anemia (AA) is clinically well defined, but regarded as a heterogeneous disease. Especially, the distinction between AA and hypoplastic myelodysplastic syndrome (Hypoplastic MDS) is often problematic. We have previously reported that the fetal hemoglobin (HbF) expression on erythroblasts is frequently observed in MDS cases. HbF expression on erythroblast is not genomic event but epigenetic mechanism that might be speculated correlation with DNA hypomethylation. In order to clarify a diagnostic utility of HbF expression on erythroblast in hypoplastic bone marrow, we retrospectively analyzed bone marrow specimens by using immunohistochemistry. *Materials and methods*. We have reviewed the clinicopathological data of our bone marrow pathology file from 1995 to 2007. We have selected 46 patients clinically diagnosed as AA who were treated with combined immunosuppressive therapy (IST) using anti-thymocyte globulin (ATG) and cyclosporine A (CSA). Bone marrow trephine biopsy or clot material was used for HbF and p53 immunostaining. *Results*. HbF was detected in 37 patients (80%) and p53 in 13 patients (28%). Combined with chromosomal analysis, morphologic reevaluation suggested that our series were clinicopathologically divided into 4 groups; HbF-negative AA (9), HbF-positive AA (19), MDS (16), and AA/MDS overlap syndrome (2). HbF-negative AA group had a significantly lower neutrophile count (0.40 *vs.* 0.54 109/L, p=0.047), higher hemoglobin level (7.4 *vs.* 6.2 g/dL, p=0.019), and higher response rate (100% *vs.* 53% p=0.012) than HbF-positive AA group. Of 18 patients who did not respond to IST, HbF-positive erythroid colonies were detected in all patients. We observed 2 HbF-positive AA patients later developed chromosomal abnormalities but not in HbF-negative patients. *Conclusion.* HbF-negative AA might to be a pure immune-mediated aplastic anemia, which was well respond to IST. This may have different pathophysiology from HbF-positive AA or MDS. HbF on erythroblast might be useful predicting marker for IST response.

PO-10

INCIDENCE OF MALIGNANCY IN A FAMILY OF A PATIENT WITH FANCONI ANAEMIA

M Ferrara, L Capozzi, R Russo

Department of pediatrics II University of Naples, Italy

Objective. The aim of the study was to investigate the incidence of malignancy in a family of a patient with FA. Methods. The incidence of malignancies has been retrospectively investigated in 72 relatives of a patient with FA coming from a town next Benevento in Campania, Italy whose parents were consanguineous. In 28 living relatives DEB test was negative. Data were compared with 67 controls of a family coming from the same area. Statistical analysis was performed by student T test. Significant differences were set at p"0.05. *Results.* We recorded 2 cases of aplastic anemia (3.3%), 4 of leukemia (6.6%), 1 of MDS (1.6%), 1 of pancreatic cancer (1.6%), 1 of retinoblastoma (1.6%), 1 of lung cancer (1.6%), 4 of breast cancer (6.6%), 3 of liver cancer (5%). In controls there were 1 case of LLA (1.4%), 1 of liver cancer (1.4%), 1 of lung cancer (1.4%), 2 of breast cancer (2.9%). Significant differences were observed (p=0.016 C.I. 0.33-2.67). *Discussion.* Until recently more than 1200 cases of FA have been reported, but the true frequency is higher. The incidence of leukemias and tumors in FA is evaluated greater than 15% but the true incidence may be higher for unknown causes. This finding is

interesting because the studies *in vitro* regarding defects in DNA repair and cellular change suggest that FA might be a premalignant condition. It has been suggested that even a single gene for FA was thought to confer a risk of malignancy. So in possible heterozygotes for FA an increased incidence of malignancy has been reported. This data is interesting because the frequency of heterozygotes has been estimated by IFAR of 0.3-1% while in Italy they are about 1.1-1.4%. Our findings show a significant higher frequency of malignancy in possible heterozigotes suggesting that the question of cancer risk in these subjects should be re-examined in a larger numbers of cases. Haematologists and oncologists should examine in the future the problem of aplastic anemia and cancer risk in FA heterozygotes.

PO-11

PANCYTOPENIA- FIRST EXPRESSION OF SEVERAL BLOOD DISEASES [A 10 YEARS STUDY]

A Perolla, T Caja, P Xhumari, P Pulluqi, A Pushi

Department of Hematology QSUT Nene Tereza, Tiranë, Albania

Pancytopenia is a medical condition in which there is a reduction in the number of red and white blood cells, as well as platelets. Is diagnosed in the presence of: anemia HCT value <0.35 in women, <0.40 in men), leucopenia(WBC 3.5×10^9/L) thrombocytopenia (PLT <150×10^9/L). *Purpose.* To evaluate the data about pancytopenia, as the annual incidence, age-related incidence, sex – related incidence, the annual and geographical distribution, and also the etiology of pancytopenia. *Metode*. All admitted patients with pancytopenia were studied and a total of 87 adult patients were selected for the study by non-probability convenient sampling. This study was carried in the Department of Hematology, QSUT during a period of 10 years: from September 1998 to September 2008. Our study was limited by its restriction to hospitalized patients. Statistical methods include descriptive statistics (mean, median, etc). Cross tabulation was carried out to find out the correlation among different variables. *Results.* Median annual incidence of Pancytopenias in our study is 8,7 new cases /year, and median incidence 3,82 cases/100 000 habitants. The annual incidence of pancytopenias is increasing during the last years in our country [0,91/100 000 habitants]. According to the data we have found a slight predominance in females [51%]. We have found a higher incidence of Pancytopenias in Tirana, due to the movement of the population in to the capital city. *Conclusions.* The results show that Aplastic Anemia [22 pts], Myelodysplastic syndromes [18 pts] and Acute leukaemias [16 pts] constitute the predominant etiology of the pancytopenias in our hospitalized patients. The incidence is higher in the age-group population of over 55 years. We recomand bonne marrow examinations as indispensable to make a differential diagnosis in pancytopenias.

PO-12

CHILDHOOD PAROXYSMAL NOCTURNAL HEMOGLOBINURIA/APLASTIC ANEMIA (PNH/AA) SYNDROME - ANALYSIS FOR PNH CLONAL EVOLUTION AFTER IMMUNOSUPPRESSIVE THERAPY

M Sukova,[1] E Mejstrikova,[2] S Pekova,[3] J Cermak,[4] D Pospisilova,[5] J Stary[1]

[1]Department of Pediatric Hematology/Oncology, University Hospital Motol, Prague; [2]Childhood Leukemia Investigation Prague, Charles University, Prague; [3]Laboratory of DNA Diagnostics, Charles University, Prague; [4]Institute of Hematology, Prague; [5]Palacky University Hospital, Olomouc, Czech Republic

Paroxysmal nocturnal hemoglobinuria (PNH) is rare clonal hematopoetic disorder, characterized by clinical symptoms of intravascular hemolysis, thrombosis and bone marrow failure, resulting from deficiency of glycosylphosphatidylinositol anchored proteins (GPI-AP) on hematopoetic cells. Association with aplastic anemia (AA) is explained by selective proliferative advantage for PNH clone provided by aplastic marrow. PNH/AA syndrome was reported in few retrospective series of adult AA with incidence 10-25% and typical non-hemolytic, non-thrombotic course. Childhood aplastic anemia treated by allogenic SCT or antithymocyte globulin (IST) has favorable prognosis, long-term outcome is limited by the risk of relapse and clonal evolution. To assess incidence and clinical course of PNH/AA syndrome in children analysis for PNH clone evolution was performed in a series of Czech pediatric AA patients. Standard immunophenotyping of granulocytes/erythrocytes for GPI anchored molecules (CD55,CD59,CD66c,CD14,CD16,CD24) was used for PNH clone screening. Between 1996-2006 61 consecutive patients were diagnosed with AA in Czech republic. All 27 (44%) tested for PNH at diagnosis had no detectable PNH clone. 46 patients were assigned to IST therapy. With median follow-up 81 months, survival rate in IST group was 90,7%, EFS 74,6%, relapse rate 24% and clonal-MDS evolution 0%. Out of 27 evaluable patients (7 SCT for non-response, 4 deaths, 8 LFU) 6 (22%) had significant PNH clone (10-65% of gran/ery). All but one had clinical symptoms of PNH: Budd-Chiari syndrome (1), hemolysis (2), recurrent pancytopenia (2). In all examined (5) molecular analysis revealed somatic mutation of PIG-A gene. In our series, PNH as additive event criterion has significantly negative impact on EFS (41,6%). Size of the clone seems to predict seriousness of clinical course, including thrombotic events. Regarding long interval to PNH presentation (2-11 years after IST) we suggest long-term follow-up with prospective yearly testing for PNH in all IST patients. SCT should be considered in children with bone marrow failure and significant PNH clone.

Supported by grant VZ-FNM MZ 64203.

PO-13

SALVAGE ALLOGENEIC STEM CELL TRANSPLANTATION IN A CHILD WITH SEVERE APLASTIC ANEMIA IN THE COURSE OF LIFE-THREATENING POTENTIAL INVASIVE FUNGAL LUNG INFECTION WITH PERI- AND MYOCARDIAL INFILTRATION

I Meyts, M Renard, J Maertens,[1] A Uyttebroeck

Department of Pediatrics, Pediatric Haemato-Oncology; [1]Department of Haematology, University Hospitals Leuven, Belgium

The patient was diagnosed with severe aplastic anemia at the age of 13y8m. Past medical history was uneventful. Her younger brother had died 3 years before due to acute myeloid leukemia with relapse in full acute respiratory distress syndrome. Two other sibs age 14y and 5y were healthy. HSCT was planned with a HLA-identical sibling, with conditioning according to the EWOG-MDS SCT RC RIC-06 protocol (fludarabine, thiotepa, ATG). 3 days prior to start of the conditioning, she was admitted with fever, cough, dyspnea. White blood cells were 700/mcl, 100% lymphocytes. CRP was 230 mg/L. Chest X-ray showed left upper lobe pneumonia. Disease progressed with CRP rising under amikacin-ceftazidim-vancomycin. Bronchoalveolar lavage culture grew Enterobacter cloacae and antibiotics were switched to meropenem. The patient continued to deteriorate with ground glass opacities spreading over both lungs and antifungal treatment with caspofungin (d7) and later amfotericine was initiated(d16). Cardiac ultrasound showed perimyocarditis with myocardial dysfunction (d18). Because of suspicion of invasive aspergillosis, not responsive to caspofungin and amfotericin, voriconazole was started (d20). Of note, galactomannan remained (-) throughout the disease course. CRP rose over 600 mg/L, fever persisted and the patient further deteriorated. The conditioning protocol was changed to fludarabine-cyclophosphamide-ATG (start d20) and cyclosporine for Graft-versus-Host prophylaxis due to renal toxicity. Repeat cardiac ultrasound (d22) showed dense material in the pericardial effusion. She was transplanted (3.58×10^6/kg CD34$^+$ cells/kg) at d27 after admission. At D4 after HSCT focal epileptic seizures occurred. MRI of the brain showed subcortical and cortical contrast-enhanced lesions suggestive of invasive Aspergillosis. Following transplantation, CRP levels started to decline. G-CSF was initiated D+4 after HSCT and there was neutrophil engraftment at D+13. At D+30 donorchimerism was 90-95%. Voriconazole was stopped atD+90, after resolution of brain and lung radiological signs. At the latest follow-up at 16 monts post-HSCT, the patient is doing excellent. *Conclusion*. We describe a patient with potential invasive fungal (Aspergillus) infection with pneumonia, peri- and myocardial infiltration and probable central nervous system involvement. Voriconazole treatment and HSCT performed during life-threatening infection, proved beneficial with survival up to 16 months after HSCT.

PO-14

INCIDENCE OF APLASTIC ANAEMIA IN KHUZESTAN PROVINCE, IRAN: A RETROSPECTIVE SINGLE-CENTRE STUDY

F Rahim,[1,2] M Panahi,[3] I Irashid[4]

[1]Research Center of Physiology, Ahwaz Jondishapur University of Medical Sciences, Ahwaz; [2]Apadana Clinical Research Center, Apadana Hospital, Ahwaz; [3]Department of Histology, Ahwaz Jondishapur University of Medical Sciences, Ahwaz; [4]Pathology Labs, Shafa Hospital, Ahwaz Jondishapur, Iran

Introduction. Aplastic anaemia (AA) is a rare but serious disorder with high mortality and morbidity rates. The incidence of AA worldwide is

2-5 patients/million/year. There is paucity of studies on this disorder from Iran. The aim of the study is to find out the incidence of AA in Khuzestan province, Iran. *Patients, Materials, and Methods.* The study was conducted at the Research Center of Thalassemia and Hemoglobinopathies (PCTH), Khuzestan province, Iran, from 21 March 2002 through 21 March 2005.

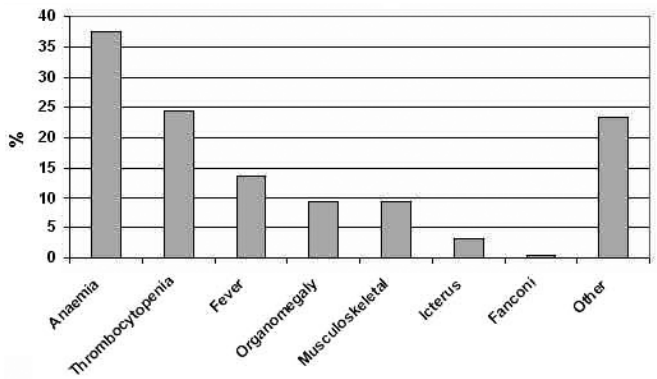


Figure 1. The prevalence of sign and symptoms of aplastic anaemia in the study groups.

This centre covers the 4.3 million population of Khuzestan province (~20% of Iran's population). All the haematological findings and bone marrow biopsy specimens were studied at Shafa Hospital, Jondishapur University of Medical Sciences, which is the onlyoncology centre in Khuzestan province. Patients were diagnosed as having AA if they satisfy two or more of the following criteria: (1) leukocytes <3500/mm^3, (2) platelets <50,000/mm^3, and (3) haemoglobin <10.0 g/dL or haematocrit <30%, in addition to bone marrow features compatible with AA. *Results.* A total of 1753 patients were examined during the study period. Of them, 257 (14.6%, 95% CI: 13.1-16.4%) satisfied AA criteria, giving an incidence of 20 (95% CI: 13-29) cases/million individuals/year in Khuzestan province, Iran. The age distribution of AA showed a bi-modal pattern; males and females aged 15-30 years, the majority of patients falling under this category, were affected equally. There was a gradual decline in the incidence over the studied years. *Conclusion*. The incidence established in this study is less than incidences from other parts of the world. This may reflect the role of environmental factors in aetiology of bone marrow suppression. *Keywords.* Anaemia, aplastic anaemia, incidence, Khuzestan, Iran.

Table 1. Incidence of aplastic anaemia in selected countries.

Country	*Incidence/million/year*
UK (10)	2.3
France (11)	1.4
Japan (12)	31-48
Thailand (13)	5.7
China (14, 15)	19-21
Turkey (16)	1.14
USA (17)	2.5
Brazil (17)	2.4
Mexico City (18)	3.9

PO-15

HORSE ANTITHYMOCYTE GLOBULIN IN ACQUIRED APLASTIC ANAEMIA; IS LESS MORE?

VME Neefjes,[1] RYJ Tamminga[2]

[1]Department of Paediatrics, Royal Aberdeen Children's Hospital, UK; [2]Beatrix Children's Hospital, University Medical Centre Groningen, Division of Paediatric Oncology/Haematology, the Netherlands

Acquired aplastic anaemia (AA) is an uncommon and life-threatening childhood disorder. Although the aetiology is unknown, clinical observations suggest an immune-mediated disease mechanism. Treatment with (usually) horse antithymocyte globulin (ATG) in combination with ciclosporin has excellent results. However, the optimal ATG dose for treatment of AA in children has not been determined. *Objective.* To determine the optimal dosage of ATG in the treatment of paediatric AA. *Methods.* We performed a systematic literature search. Additionally, two case reports of children unintentionally treated with low dose ATG are described. *Results.* We found that dosage with ATG in AA varies widely (total dose 60-420 mg/kg) whilst no obvious therapeutic advantage can be ascribed to any treatment schedule. *In vitro* data suggest that considerably lower dosage of ATG might be as effective. Both case reports describe children who achieved remission of AA after treatment with a total dose of 15 mg/kg ATG. *Discussion. In vitro* data as well as the two case reports suggest that it may be possible to treat AA in childhood with a considerably lower dose of ATG than currently used. The optimal dose of ATG in treatment of AA should be determined in a clinical trial.

PO-16

APLASTIC ANEMIA SECONDARY TO DENGUE VIRUS INFECTION: A CASE REPORT

R Pallota,[1] L Gomes[1]

[1]Escola Bahiana de Medicina e Saude Publica, Salvador, Bahia, Brazil

Introduction. Aplastic anemia is a blood dyscrasia mediated by aberrant immune response inducing bone marrow failure, idiopathic in most cases. The development of Aplastic Anemia after dengue infection is uncommon, however, a case has been registered in Salvador, Bahia, Brazil. *Case report.* A 36 year old female, with history of Hashimoto´s Thyroiditis, was infected by dengue virus, complicated by anemia and cutaneous cellulites. Blood exam showed anemia and thrombocytopenia. Serology for IgM Dengue was positive and serology to FAN, HBV, HCV, FR and HIV were negative. Seven days after hospitalization, the decreasement in blood cells levels required transfusion of 3 units of red cells and 4 units of platelets by aphaeresis. Bone marrow biopsy detected cellularity lower than 5%. As the diagnosis of aplastic anemia was confirmed, the immunosuppressant was initiated, with Cyclosporine (5 mg/kg/day) and Corticoid (1 mg/kg/day). Four weeks after the treatment begging, she presented recuperation of the white cells count and neutrofilia, but bone marrow celularity did not recover. ATG was added to the immunosuppressant therapy with excellent response after 8 weeks of treatment. *Conclusion*. In the present case, the patient continued presenting pancytopenia even after the dengue infection resolution, which supports the suspicion of aplastic anemia induced by its infection. As she had antecedents of auto-immune diseases, it is possible that the dengue infection worked as a co-factor for exacerbation of the immune response, already unregulated.

PO-17

USE OF PEGYLATED GRANULOCYTE-COLONY STIMULATING FACTOR IN CHILDHOOD SEVERE APLASTIC ANEMIA

S Vallero, P Quarello, A Lorenzati, D Renga, E Rivetti, R Laudati, L Farinasso, F Timeus, U Ramenghi, P Saracco

Pediatric Onco-hematology , University of Turin, Torino, Italy

Objective. To describe the case of a patient with severe aplastic anemia (SAA) treated with pegylated granulocyte-colony stimulating factor (PG-CSF). To our knowledge, this is the first report of the use of PG-CSF in childhood SAA. *Methods.* The patient, a 13-year-old girl, presented with trilinear cytopenia. Blood count, bone marrow aspirate and biopsy showed SAA. No PNH clone was evidenced. Cytogenetics was normal. No HLA-matched siblings were found, so the patient was treated with anti-thymocyte globulin (ATG) and cyclosporine A (CSA). Due to persistent neutropenia and oral mucositis, G-CSF was started on day +80 from diagnosis. However, since the patient refused daily G-CSF injections, PG-CSF was administered subcutaneously every 2-3 weeks at a dose of 100 mcg/kg on days 85, 100 and 115.

Results. After PG-CSF administration, no adverse reaction was evidenced in our patient. PMN count was 170/mcl before the start of PG-CSF. PMN zenith was documented 14 days after the first dose (1,190/mcl), 7 days after the second dose (9,530/mcl), and 4 days after the third dose (17,480/mcl). PMN level remained above 1,000/mcl in all subsequent controls.

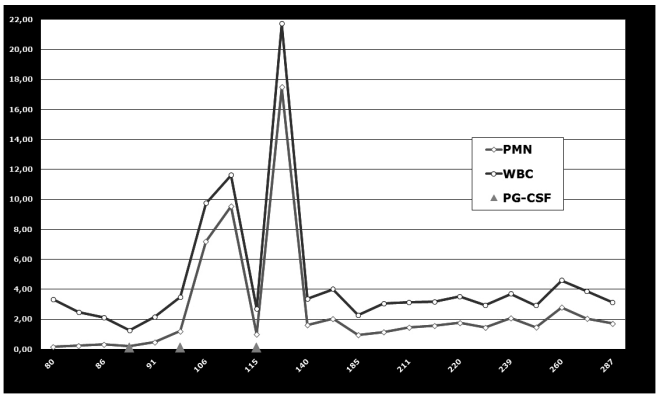


Figure 1.

The patient is currently transfusion-independent, but is still partial responder;surveillance for clonal disease is negative. A second ATG course is to be planned. Search for HLA-matched unrelated donor has been opened. *Discussion.* PG-CSF has been used in adult and pediatric cancer patients after high-dose chemotherapy and as a mobilizing agent; its use is also reported anecdotally in severe congenital neutropenia. Compared to G-CSF, PG-CSF seems to be equally safe and tolerable. Risk of myelodysplastic evolution is probably comparable. We propose to extend the use of PG-CSF also in children with SAA, the major advantages being a better compliance and higher quality of life. However, caution in both G-CSF and PG-CSF routine use is mandatory: strict cytogenetic surveillance and clinico-hematological monitoring is recommended.

PO-18

ETIOPATHOGENESIS OF CHILDHOOD APLASTIC ANEMIA - A SINGLE CENTER STUDY OF 94 CASES

M Bhattacharyya, S Tyagi, M Mahapatra, VP Chaudhury, R Saxena

Hematology Department, All India Institute of Medical Science, New Delhi, India

Objectives. Aplastic anemia in children is a serious disease . Inspite of recent improvement in treatment modalities fatality is still quite high. Epidemiological investigations in aplastic anemia, especially in children are scanty. Association with other illness, biologic status, radiation , chemical exposure, drugs and viral infections are well established. Determination of potentially preventable etiologies therefore assume particular importance in childhood aplastic anemia. *Materials and methods.* Detail history and a thorough physical examination. Complete hemogram, reticulocyte count, bone marrow biopsy. Screening for paroxysmal nocturnal hemoglobinuria, HbsAg, Anti HCV, Anti HEV. Skeletal survey and ultrasonography of abdomen. Cytogenetic study including stress cytogenetics Xylose absorbtion test and stool fat excretion (for suspected Shawchman Diamond Syndrome). *Results.* Ninety four children with aplastic anemia studied. Forty one (43.6%) were inherited while 53 (56.38%) were acquired. Eleven (11.7%) patients had history of exposure to toxic chemicals. Drugs were incriminated in 4 (4.2%). Post-hepatitis aplastic anemia noted in seven. Screening for paroxysmal nocturnal hemoglobinuria (PNH) were positive in 8 (8.5%). Majority of the inherited variety-thirty seven amongst 41cases of inherited aplastic anemia were conclusively diagnosed as Fanconis anemi. Only 2 (4.8%) were Dyskeratosis Congenita and 1 (2.4%) each Shawchman Diamond Syndrome and Down Syndrome. *Discussion*. An underlying etiopathological factor can be detected in a large no of childhood aplastic anemia cases if carefully searched for. Treatment & prognosis largely depend on these factors.

PO-19

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA CLONES IN CHILDREN WITH ACQUIRED APLASTIC ANEMIA: A SINGLE CENTRE STUDY

F Timeus, N Crescenzio, P Quarello, A Doria, L Foglia, S Pagliano, A Lorenzati, S Vallero, L Farinasso, P Saracco

Pediatric Onco-hematology, University of Turin, Italy

Introduction. The presence of paroxysmal nocturnal hemoglobinuria (PNH) clones is a frequent finding in acquired aplastic anemia (AA). Some studies in adults with AA found a correlation between the presence of PNH clones at diagnosis and a favourable response to immunosuppressive therapy (IST). However, the biological significance of PNH clones in AA is still controversial and in pediatric patients needs to be established. Aim of our study was to assess the incidence of PNH cells in children with AA at diagnosis and during follow up after IST. *Materials and methods*. A cohort of 19 caucasian children diagnosed with AA, between 1990 and 2008, and classified according to Camitta criteria: 15 (79%) severe or very severe (SAA) and 4 (21%) non severe (nSAA), were studied for the presence of PNH cells. The median age at diagnosis was 10,5 years, 12 (63%) were males and 7 (37%) females. The use of flow cytometry to test PNH started in 1998, with 12 patients having flow cytometry follow-up of PNH clones since diagnosis. PNH cells were detected by the lack of expression of CD 59 on granulocytes. The presence of a population CD 59– > 0,15% was defined as abnormal. All the patients but three (who initially received related bone marrow transplantation, BMT) were treated with IST according to EBMT protocol (anti-tymocyte globulin, cyclosporine, CSA, ± G-CSF). *Results*. among the 12 patients followed since diagnosis a PNH clone was present in 7 (58%) (range 0.2-1%). Of these PNH positive patients, 5 were treated with IST. Four showed a partial/complete response after 1 or 2 IST courses and remained PNH positive; one non responder developed a RAEB, received aploidentical BMT and is still alive. Among the PNH negative patients at diagnosis, 3 received IST, showed a partial response and so far remained PNH negative. Among the 7 patients diagnosed before 1998, all treated with IST, follow up for PNH clones started within 1 year from diagnosis in 3 patients and off therapy in the remaining four. In 2 complete responders, followed still during CSA treatment and persistently PNH negative, the appearance of a PNH clone occurred at time of relapse. *Conclusions.* The present single centre study confirms the high incidence of minor PNH clones in children with AA. A clear correlation between this marker and the response to IST is controversial, according to a recent observation in a larger cohort of Japanese children. In PNH negative patients with long term follow up, the appearance of a PNH clone has been predictive of relapse.

PO-20

CYCLOSPORIN A RESPONSE AND DEPENDENCE IN CHILDREN WITH ACQUIRED APLASTIC ANAEMIA: AN UPDATE OF A MULTICENTRE RETROSPECTIVE STUDY WITH LONG-TERM OBSERVATION FOLLOW-UP

P Saracco, P Quarello, AP Iori, M Zecca, D Longoni, J Svahn, S Varotto, GC Del Vecchio, C Dufour, U Ramenghi, A Bacigalupo, A Locasciulli; Bone Marrow Failure Study Group of the AIEOP (Italian Association of Paediatric Haematology Oncology)

Paediatric Onco-Haematology, University of Turin, Italy

Introduction and obbjectives. Immunosuppressive therapy (IST) with antithymocyte globulin and cyclosporin A (CyA) is the standard treatment for children with acquired aplastic anaemia (AAA) lacking a matched donor. In our previous study of 42 children with AAA diagnosed from January 1991 to December 1999 and treated with IST we observed an overall survival of 83% at 10 years and a cumulative incidence of relapse of 16% at 10 years that resulted significantly associated with rapid CyA discontinuation. The CyA-dependence without a predictive marker was observed in 18% of responders and the cumulative incidence of MDS/AML was 8% at 10 years, with a significant correlation with both G-CSF cumulative dose and second IST. We concluded that IST with a slow CyA tapering course is an effective treatment with a low-relapse rate in these cases (Saracco *et al.,* BJH 2007). In this report we provide an update of our previous study with a 24 months longer follow up. *Methods and results.* We updated follow up of alive patients at 31st December 2008. A comprehensive surveillance protocol for minor PNH clones and MDS/AML evolution has been designed and applied for these patients in the last 12 months. Overall survival at the last follow-up was 85%. The 35 survivors have been followed for a median of 142 months (range: 49-209): 21 are in remission and off CyA, 6 are still CyA-dependent, 1 has mild haemolysis PNH and is off all treatment and 6 are alive after HSCT. One child had been lost to follow up. A slow CyA tapering schedule was performed in 84% of patients. Cumulative incidence of MDS/AML was 8% at 12 years; no additional evolution to MDS/AML was observed in our cohort. Analysis of minor PNH clones is under investigation. *Discussion.* This updated long-term fol-

low-up of children with AAA confirms that IST with a slow CyA tapering is an effective treatment with a low-relapse rate in these cases. Considering the risk of clonal evolution we strongly recommend a focused surveillance strategy.

PO-21

IMMUNOSUPPRESSION THERAPY IN CHILDREN WITH SEVERE APLASTIC ANAEMIA

M Mohamed,[1] E Juraida,[1] A Rahman,[2] BS Menon,[1] C Ho,[1] R Khuzaiah,[1] Z Muda,[1] I Shahnaz Othman,[1] L Yin Sear,[1] A Thevarajah,[1] AM Rivai,[1] HM Ibrahim[2]

[1]The Haemato-Oncology Unit, Paediatric Department, Kuala Lumpur Hospital, Malaysia; [2]Paediatric Department, University Putra, Malaysia

Background. Severe aplastic anaemia (SAA) is a life threatening disease characterised by pancytopenia and hypoplastic bone marrow. Without treatment, children with SAA are at risk of severe bleeding and infection that can lead to death. The treatment of SAA is based on allogeneic bone marrow transplantation BMT) and immunosuppressive therapy. *Method.* This is a retrospective single-centre study on the incidence of SAA and outcome of those treated with combined immunosuppressive therapy (CIST) in two different 4- year time periods: 1997-2000 and 2004-2007. *Results*. There were a total of 15 children diagnosed with SAA in 1997-2000, of which 8 are males and 7 are females. Their age ranges from 7 months to 11 years. Eight patients underwent BMT, 4 had CIST and 3 died before definitive treatment were carried out. From 2004-2007, there were 14 children diagnosed to have SAA of which 10 are males and 4 are females and their age ranges from 11 months to 12 years. Four patients underwent BMT, 7 had CIST, 2 died before treatment and one was lost to subsequent follow-up. A total of 11 children had CIST using similar drugs and protocol in both the time periods. Of these 11 children, 8 children had their blood counts normalised after treatment while one child had a partial response (PR). One child failed to respond to CIST and had a one antigen mismatched sibling BMT following that and recovered. One girl required repeated cyclosporin (CSA) and prednisolone at intervals of 6 to 18 months after being taken off CSA of the CIST regime. Of the 10 patients who responded to CIST, we find that the granulopoeitic series takes a shorter time to recover (absolute neutrophil count > 1000/microml at a median 2 months) while the platelets counts only normalised after immunosupression was taken off in 6 children. Three children's platelet count recovered within 61 to 153 days after starting CIST while one child's platelet count remains within 25-30 000/microml after 18 month on CSA (PR). *Discussion*. The number of children who was treated for SAA in our centre during both the time periods does not differ much. Children with SAA who does not have a donor for BMT should be treated with CIST as an alternative treatment as it shows good response rate. Treatment should be commenced as soon as possible before patient develop complications of marrow failure.

PO-22

IS THE RISK OF DEVELOPING AML OR MDS INCREASED IN X-LINKED NEUTROPENIA?

K Beel,[1] MM Cotter,[2] P Vandenberghe[1]

[1]Center for Human Genetics, University Hospital Leuven, Leuven, Belgium; [2]Department of Haematology/Oncology, Our Lady's Children's Hospital, Crumlin, Dublin 12, Ireland

Introduction. In 2001, we described X-linked neutropenia (XLN) as a novel subtype of severe chronic neutropenia (SCN) in a three- generation family with five affected men and with an 843T>C WAS mutation, resulting in an L270P gain-of-function mutation in the GTPase binding domain (GBD) of the Wiskott-Aldrich Syndrome protein (WASP) (Devriendt, et al 2001). Since our original report, two more cases have been described, one with an S272P and one with an I294T mutation, the latter presenting with myelodysplasia, in a series of 14 SCN males without an ELA-2 mutation (Ancliff et al. 2006). We recently discovered two more XLN families and one more XLN case. The first family is a large XLN kindred with 10 affected males an 8 female carriers with an I294T WASP mutation (Beel *et al.* 2008). Similar to the L270P mutation, the I294T also causes constitutive activation of WASP. The second newly diagnosed case of XLN had the L270P mutation, and, based on SNP-Array comparison, is probably related to the L270P XLN family that we described originally. The last newly diagnosed XLN case was discovered when screening for WAS mutations in a cohort of 16 Irish XLN patients (9 male, 7 female). He had also an L270P mutation. *Objective.* The risk of transformation to MDS/AML in XLN remains unclear. One of two cases reported by Ancliff *et al.* reportedly presented as MDS (Ancliff *et al.*, Blood 2006). In addition, two out of five affected adult males of the original L270P family developed MDS or AML, with monosomy 7, after a prolonged disease course and under G-CSF. Acquired truncating CSF3R mutations were found in leukaemic samples, suggesting an involvement of G-CSF in malignant transformation. In contrast, no cases with MDS or AML were discovered in the second L270P family or in the I294T family. In the latter, G-CSF use was not frequently administered and most cases are younger than in the first family. The diagnosis of XLN is sometimes made only at a later age. In addition, the maturation arrest at the promyelocyte/metamyelocyte stage can masquerade as myelodysplasia. We therefore investigated the frequency of activating WAS mutations in cases primarily presenting as AML or MDS. *Methods.* We screened exons 7-10, encoding the GBD of WASp (residues 230-381) in 231 patients, 134 male cases (36 cases <18 y) of MDS (22%) or AML (78%), including 61 cases (26%) with monosomy 7. *Results.* No relevant intronic WAS GTP-ase binding domain (GBD) mutations were observed in a series of 231 paediatric and adult MDS or AML. The large I294T family allowed us to describe the phenotype in detail: the degree of neutropenia is variable and does not seem to correlate with the severity of infections; low-normal IgA levels and low NK cells are additional features of XLN. On the other hand, inverted $CD4^+/CD8^+$ ratios were not a feature in this family. Unlike in our L270P XLN family, X-inactivation pattern in female carriers of the I294T family was inconsistent, suggesting that selection against I294T WASP might not be strong enough to cause consistent skewing (Beel *et al*. 2008). *Discussion.* In conclusion, two cases of MDS or AML have been observed in the L270P XLN family, but no myeloid malignancies were documented in a large kindred with I294T XLN. Therefore, XLN does not inherently seem to predispose to myeloid malignancies. The cases in the L270P XLN might be related to more liberal use of G-CSF in this pedigree. This is further supported by the presence of CSF3R mutations in the leukemic phase. Finally, we have not identified cases that initially presented as MDS or AML. Based on this, and on the mild infectious phenotype of XLN, we recommend that G-CSF be used sparingly in XLN and restricted to infectious episodes.

Table 1.

	Median age (range)	*MDS*	*AML*
Male <18	12 (1-17)	8	28
Male >18	66 (19-89)	22	76
Female <18	12 (0.2-18)	6	15
Female >18	64 (19-82)	14	62

PO-23

IMPROVING CONGENITAL NEUTROPENIA DIAGNOSIS: MUTATIONAL ANALYSIS OF ELA2 AND HAX1 GENES. A SINGLE CENTRE EXPERIENCE

A Catala, S Rives, T Toll, M Camós, R Berrueco, N Conde, A Quintero, JI Arostegui,[1] J Estella

Haematology department Hosptial Sant Joan de Deu; [1]Immunology department Hospital Clinic, Barcelona, Spain

Severe chronic neutropenias are represented by a heterogeneous group of disorders with very different clinical outcomes. Recently, genetic mutations causing many of these diseases have been identified. Mutations in the *ELA2* gene represent the most common cause of congenital neutropenia. They are responsible for familial and sporadic cases of cyclic (CN) and severe congenital neutropenia (SCN) whereas *HAX1* mutations cause autosomal recessive congenital severe neutropenia (Kostmann disease). *Objective.* To evaluate mutations in the *ELA2* and *HAX1* genes to highlight their use as a new tool in the diagnosis of severe neutropenia. *Methods*. Since February 2007 we have evaluated the mutational status of both genes in every child with severe chronic neutropenia, either by specific amplification of known mutations, or by gene sequencing. *Results.* 19 patients were evaluated. The mutated cases are shown in the Table 1.

Table 1.

n	Age diagnosis	Diagnosis	Gene/ mutation	Familiar study	Severe infections	G-CSF	Outcome	Follow up
1	2y 4m	CN	ELA2 / IVS4+1	mother carrier	no	yes	>1500ANC	8y
2	10m	SCN	ELA2 / C194X	not performed	no	yes	1000 ANC osteopenia / liver pseudotumor	10y
3	5m	AI	HAX1 / GGAGAA*+	father carrier	no	no	recovered	2y
4	7m	SCN	ELA2 / A32V	negative	no	yes	>1000ANC osteopenia	14y
5	2m	SCN	ELA2 / S38W*	negative	yes	yes	1000ANC multiple infections	1y
6	3y	CN	ELA2/ V36D*	negative	no	yes	>1500ANC	1,5y

*not reported; +possible polymorphism. AI: autoimmune neutropenia

Discussion. All our patients affected of NC or NCS had ELA2 gene mutated. The study of the mutational status is been of great help as a new diagnostic tool at our hospital. It should be implemented in the routine study of severe chronic neutropenia. Although further work is needed, recent studies were able to relate certain ELA2 mutations with more severe phenotype and increased risk of developing leukemia/myelodisplasia. Such patients could benefit from a more exhaustive follow up or even early bone marrow transplantation.

PO-24

NON-SYNDROMIC CONSTITUTIONAL RUNX1 DELETION PRESENTING AS THROMBOCYTOPENIA WITH MYELODYSPLASIA

MB Bierings,[2] SN van der Crabben,[1] E van Binsbergen,[1] MGEM Ausems,[1] A Buijs[1]

[1]*Dept. Medical Genetics,* [2]*Dept. Pediatric Hematology/Oncology, UMC Utrecht, The Netherlands*

Objective. Congenital 21q22 RUNX1 gene mutations are the cause of autosomal dominant familial platelet disorders/AML. Recently, four patients were reported with syndromic thrombocytopenia due to constitutional microdeletions in 21q22 (including RUNX1). These patients showed variable degrees of growth restriction, dysmorphic features and mental retardation (MR). *Methods and Results*. We report on a 5-year-old Moroccan boy, born to consanguineous parents, with a history of frequent haematoma and increased bleeding tendency, caused by thrombocytopenia. Growth and psychomotor development was normal as well as dysmorphologic evaluation. BM aspirates revealed an active, atypical megakaryopoiesis with dysplastic features. G-banding of BM showed a 46,XY karyotype. FISH analysis revealed loss of 1 copy in RUNX1 and loss of 21qter, suggesting an interstitial and a terminal deletion on chromosome 21q. FISH analysis on T-lymphocytes, cells from the urinary tract and derived by mucosal swaps demonstrated the constitutional nature. High-resolution oligo array-CGH (105K Agilent platform) confirmed a 1.6 Mb interstitional deletion, including RUNX1, and a 2.2 Mb terminal deletion of chr.21. Cytogenetic and FISH analysis of the mother was normal. *Discussion*. We present a case with non-syndromic thrombocytopenia with *de novo* 21q deletions. Our results confirm the role of haploinsufficiency of RUNX1 in thrombocytopenia. This case emphasizes the necessity to molecularly investigate 21q22 status in patients with both 'isolated' and syndromic thrombocytopenia .We will discuss our findings in relation to candidate genes on 21q22 involved in MR.

PO-25

CHILDHOOD APLASTIC ANEMIA – SEARCH FOR INHERITED STATUS

M Bhattacharyya, S Tyagi, M Mahapatra, VP Chaudhury, R Saxena

Department of Hematology All India Institute of Medical Sciences New Delhi, India

Objectives. Aplastic anemia in children is often a part of inherited bone marrow failure syndromes which includes – Fanconi ,s anemia, Dyskeratosis Congenita and Shawchman Diamond Syndrome. Clinical profile, complications, therapy and prognosis of these group of patients differ from acquired aplastic anemia. With this background, childhood aplastic anemia patients attending hematology OPD were investigated. *Materials and methods.* A detail history and a thorough physical examination. Complete hemogram, reticulocyte count, bonemarrow biopsy. Screening for PNH HBsAg, Anti HCV, Anti HEV. Skeletal survey and ultrasonography of abdomen. Cytogenetic study including stress cytogenetics Xylose absorbtion test and stool fat excretion (for suspected Shawchman Diamond Syndrome). *Results.* Ninety four children with aplastic anemia studied. Underlying inherited marrow failure syndrome detected in 41 (43.6%).Thirty seven (90%) of theses were Fanconi ,s anemia, 2 (4.8%) were Dyskeratosis Congenita and 1 (2.4%) each Shawchman Diamond Syndrome and Down Syndrome. Four (10.8%) patients of Fanconis anemia had other associated risk factors. Out of 37 patients of Fanconis Anemia, Physical anomalies were detected in 15 amongst 37 cases of Fanconis anemia. *Discussion.* 43.6% of childhood aplastic anemia were due to inherited bone marrow failure syndromes and majority were Fanconis Anemia. This was higher compared to previous reports which vary from 4-30%. Physical anomalies detected in 15 out of 37 patients (40.5%). Twenty two patients (53.6%) diagnosed as Fanconis Anemia did not have any physical anomaly. Neal S Young et al reported no physical anomalies in 14% patients. Higher incidence in the present study most likely due to availability of stress cytogenetics. Search for chromosmal breaks should be done in all children with aplastic anemia.

PO-26

A FURTHER PEDIGREE WITH GERMLINE RUNX1 MUTATION AND PROPENSITY TO MYELOID MALIGNANCIES

T Ripperger,[1] D Steinemann,[1] G Göhring,[1] C Niemeyer,[2] B Strahm,[2] B Schlegelberger[1]

[1]*Institute of Cell and Molecular Pathology, Hannover Medical School, Germany;* [2]*Divison of Pediatric Hematology and Oncology, Department of Pediatric and Adolescent Medicine, University of Freiburg, Germany*

Objective. Heterozygous germline mutations in RUNX1 are causative genetic alterations in familial platelet disorder predisposing to the development of MDS and/ or AML. We report here on another pedigree of familial MDS/ AML with a heterozygous germline mutation in RUNX1 in affected family members. *Subjects.* The two investigated first degree relatives developed myeloid malignancies with different clinical severity at different ages. The index patient presented with MDS-related AML (MDR-AML) at age 13. Cytogenetic analysis displayed an interstitial deletion in 5q and an additional structural aberration in 2q. While her twin brother, one younger and one older brother, and her mother are clinically healthy, MDR-AML was diagnosed in her 47-year-old father six months earlier. No history of thrombocytopenia or platelet defects was reported in the family. *Results.* In both patients, a heterozygous nonsense mutation was detected in exon 3 of RUNX1 (NM 001001890.2) by direct sequencing. The mutation (c.520C>T, p.Arg174X) causes a premature truncation at the end of the runt homology domain (RHD) leading to a predicted dominant negative effect. *Discussion.* Up to now, less than 30 pedigrees with familial platelet disorder/ myeloid malignancy (FPD/MM) are known. While most RUNX1 mutations in FPD/MM cluster in RHD and are unique to individual pedigrees, a broad range of intrafamilial and interfamilial clinical variability characterizes FPD/MM. This phenotypic variability can, at least partially, be explained by the hypothesis of multistep leukaemogenesis in FPD/MM. The accumulation of cooperating gene alterations as potential second hits may lead to distinct clinical courses in individuals carrying the same germline mutation in RUNX1. Underlining this hypothesis, we report here on a novel pedigree with a heterozygous germline mutation in RUNX1 which was already described as somatic mutation in a case with sporadic, atypical CML with trisomy 21 and in another pedigree with FPD/MM.

PO-27

A JAPANESE PEDIGREE WITH RUNX1 MUTATION RESULTING IN FAMILIAL PLATELET DISORDER WITH PROPENSITY TO ACUTE MYELOGENOUS LEUKEMIA

D Hasegawa, C Ogawa, S Hirabayashi, M Park, Y Hayashi, A Manabe, R Hosoya

Department of Pediatrics, St. Luke's International Hospital, Tokyo; Department of Hematology-Oncology, Gunma Children's Medical Center, Shibukawa, Japan

Familial Platelet disorder with propensity to acute myelogenous leukemia (FPD/AML) is a rare autosomal dominant disease characterized by thrombocytopenia, abnormality of platelet function, and a propensity to develop myelodysplastic syndrome (MDS) and acute myelogenous leukemia (AML). The disorder was shown to be caused by germline mutations in the RUNX1, which is a key regulator of definitive hematopoiesis and of myeloid differentiation. Here, we report a new Japanese pedigree with FPD/AML harboring RUNX1 mutation. The proband was diagnosed with refractory cytopenia at the age of 10 after 8-year follow-up with mild to moderate thrombocytopenia. Cytogenetic analysis revealed normal karyotype and fluorescent in situ hybridization showed neither monosomy 7 nor trisomy 8. Her elder sister also was followed for 10 years with mild thrombocytopenia, but the morphological findings of peripheral blood or the bone marrow were not dysplastic. While her father died of MDS at the age of 43, her paternal aunt developed MDS at the age of 49 and remains in complete remission for 10 years after successful allogeneic cord blood transplantation. Her paternal grandfather and uncle also had a history of thrombocytopenia. Direct sequencing analysis of RUNX1 in the proband and her sister revealed a one-base deletion in exon 7 resulting in a frameshift mutation and an early truncated protein. This mutation was not detected in their mother. Inherited RUNX1 mutations clustered to the N-terminal region in exons 3 to 5, which affect runt homology domain. Mutations in the C-terminal region, detected in the presented pedigree, have been reported less frequently so far, and are thought to affect transactivation domain. Although FPD/AML is thought to be rare, the screening for RUNX1 mutation may be indicated in children suspected of having familial MDS/AML.

PO-28

SUCCESSFUL UNRELATED BONE MARROW TRANSPLANTATION FOR TWO PATIENTS WITH AMEGAKARYOCYTIC THROMBOCYTOPENIA WITH RADIO-ULNAR SYNOSTOSIS IN JAPAN

Y Ohtsuka, M Sugita, Y Nakajima, J Ono, N Okamoto, Y Yokokawa, K Yoneyama, T Kaneko, T Tanizawa

Department of Pediatrics, Hyogo Collage of Medicine, Hyogo, Japan and Department of Hematology-Oncology, Kiyose Children's Hospital, Tokyo, Japan

Objectives. Amegakaryocytic thrombocytopenia with radio-ulnar synostosis (CTRUS) is a rare inherited bone marrow failure syndrome that has the potential to progress to pancytopenia that behaves like TAR.The diagnosis of CTRUS is made later when pronation-supination of the forearm is discovered to be restricted. Hox 11a was reported to be abnormal in the initial cases but at least 1 other case did not have this abnormality. Hematopoietic stem-cell transplantation (HSCT) is presently the only curative treatment approach. We used a reduced intensity transplantation regimen in CTRUS patient with aplastic anemia.We reported two cases of CTRUS who underwent successful unrelated bone marrow transplantation. *Methods.* Conditioning regimen consisted of Flu, ATG, CY, and TLI (300cGy). For GVHD prophylaxis, all received FK506 and short course MTX. The number of transplanted nucleated cells were 6.35, 6.8 108/kg. All patients underwent an alternative donor SCT. The each age at transplant for patients were 8, 18 months. *Results.* The patients had rapid and durable engraftment at day +13, +16 with minimal complications.No regimen related toxicity were observed, and no viral re-activation (CMV, EBV, VZV) were seen. Each patients developed acute GVHD involving skin only (grade II).One of two patients developed clonic GVHD (skin only limited type). All patients are alive and transfusion independent with each follow-up time of 34, 29 months. *Conclusions.* Reduced intensity conditioning might be a feasible approach to stem-cell transplantation in patients with CTRUS who do not have a related donor.

PO-29

A CASE OF CONGENITAL DYSERYTHROPOIETIC ANEMIA TYPE II

M Ferrara, L Capozzi, R Russo

Department of Pediatrics, Second University of Naples, Italy

Objective. The diagnosis of Congenital Dyserythropoietic Anemia type II (CDA II) can be performed in early childhood, but it is often difficult due to poor knowledge of the disease and the various differential diagnoses that have to be excluded. This study underlines differential diagnostic difficulties particularly with HS. *Methods.* A 6 year-old child was admitted to our DH with HS diagnosis. At presentation he had pallor, splenomegaly, jaundice gallstones, skull changes. Hb, MCV, ferritin level, bilirubin value, aptoglobin level, bone marrow morphology, analysis of UGT 1A gene, Pink test, EPO, sFTR and RPI value, HAM test and PAGE of erythrocyte membrane protein were performed. *Results*. Patient had Hb 10.4 g/dL MCV 83 fl, ferritin level 42.9 ng/mL, unconjugated bilirubin 1.67 mg/dL, undetectable aptoglobin level. Molecular analysis of UGT 1A gene showed homozygous genotype (TA)7/(TA)7. Pink test was positive in the propositus (76%), but negative in the parents. Bone marrow aspirate showed erythroid hyperplasia with M/E ratio (1/1.6) and multinuclearity of intermediate and late erythroblasts. Increased value of EPO (380 IU/L) and sTFR concentration (6.5 mg/L), low RPI value (0.8%) were found. HAM test was positive. SDS PAGE of erythrocyte membrane protein showed alteration of banda 3 protein. Diagnosis of CDA II was performed. Patient was splenectomised because of worsening of anemia (Hb 6.5 g/dL) and splenomegaly. *Discussion.* CDA II is a rare autosomal recessive disorder characterized by mostly moderate anemia and ineffective erythropoiesis. CDA II is also known as HEMPAS as this disease is characterized by hereditary erythroblastic multinuclearity with acidified serum test. In our study we analyzed the parameters expressing ineffective erythropoiesis but the follow-up of clinical course of disease is necessary to evaluate not only the severity of anemia, but also the highest risk of iron overload, with liver cirrhosis, diabetes and cardiac failure in later life.

PO-30

LIVER FIBROSIS AND TELOMERE MAINTENANCE: A FAMILY WITH DYSKERATOSIS CONGENITA

I Furlan, U Graubner, A Karow, CP Kratz, CM Niemeyer

Division of Pediatric Hematology and Oncology, Department of Pediatric and Adolescent Medicine, University of Freiburg; Department of Hematology and Oncology, University Children's Hospital, Munich, Germany

Here we present a family with two siblings followed for idiopathic liver fibrosis by gastroenterologists for years. Alpha-1-antitrypsin deficiency and M. Wilson had been ruled out. No metabolic disorders were found. Both siblings developed increasing pancytopenia with high MCV in early childhood. Fanconi anemia was excluded. For further evaluation of unclear cytopenia the EWOG-MDS study center was involved in the diagnostic process. As cytopenia and liver fibrosis are features of dyskeratosis congenita (DC), TERC gene was analysed. TERC mutation analysis revealed a mutation c.300G>C in the conservative region (CR) 4-5 in both siblings. This specific mutation has never been described before in DC patients, but there are other mutations in CR 4-5 which cause DC. As TERC associated DC is dominantly inherited, we analysed the family. The same mutation was found in another sister and in the mother. The sister has only mild leukopenia, while the mother has abnormalities of hair and teeth, but normal blood counts. The father and another sister are unaffected. Abnormal telomere maintenance resulting in accelerated telomere shortening is one of the causes of acquired and inherited bone marrow failure. Several genetic associations are known. The genotype/ phenotype correlation in DC patients is complex and other factors/ modifiers additionally contribute to organ damage. The wide range of clinical manifestations of DC results from the excessive telomere erosion causing not only genomic instability (propensity to malignant disease as AML, MDS), but also loss of stem cells (bone marrow failure) and impaired regeneration (liver and pulmonary fibrosis, skin abnormalities). For TERC mutations disease anticipation as in our family has been shown. We conclude that liver fibrosis and pancytopenia are a rare characteristic of inherited bone marrow failure syndromes as DC.

PO-31

COMPARATIVE ANALYSIS OF SHWACHMAN-DIAMOND SYNDROME TO OTHER INHERITED MARROW FAILURE SYNDROMES

SK Hashmi,[1] C Allen,[1] R Klaassen,[2] CV Fernandez,[3] R Yanofsky,[4] J Wu,[5] J Champagne,[6] M Silva,[7] JH Lipton,[8] J Brossard,[9] Y Samson,[10] S Abish,[11] M Steele,[12] K Ali,[13] N Dower,[14] U Athale,[15] L Jardine,[16] JP Hand,[17] J Beyene,[18] Y Dror[1]

[1]Marrow Failure and Myelodysplasia Program, Division of Haematology/Oncology and Cell Biology Program, Research Institute, The Hospital for Sick Children and the University of Toronto, Toronto, Ontario; [2]Children's Hospital of Eastern Ontario, Ottawa, Ontario; [3]Isaak Walton Killam Hospital for Children, Halifax, Nova Scotia; [4]CancerCare Manitoba, Winnipeg, Manitoba; [5]British Columbia Children's Hospital, Vancouver, British Columbia; [6]Hôpital Ste.Justine, Montréal, Québec; [7]Queen's University, Kingston, Ontario; [8]Princess Margaret Hospital, Toronto, Ontario; [9]Centre U Sante de l'Estrie-Fleur, Sherbrooke, Quebec; [10]Centre Hospital University Quebec-Pav CHUL, Sainte-Foy, Quebec; [11]Montreal Children's Hospital, Montreal, Québec; [12]Alberta Children's Hospital, Calgary, Alberta; [13]University of Saskatchewan, Saskatoon, Saskatchewan; [14]University of Alberta/Health Sciences Centre, Edmonton, Alberta; [15]McMaster Children's Hospital/McMaster University Health Sciences Centre, Hamilton, Ontario; [16]Children's Hospital of Western Ontario, London, Ontario; [17]Janeway Child Health Centre, St. John's, Newfoundland; 18Population Health Sciences, Research Institute, The Hospital For Sick Children, Toronto, Ontario, Canada

Objectives. Shwachman-Diamond syndrome (SDS) is an inherited bone marrow failure syndrome (IMFS) with a high risk of leukemia. The full clinical phenotype is still evolving. To better define the SDS clinical spectrum and genetic background, we analyzed 34 cases of SDS enrolled on the Canadian Inherited Marrow Failure Registry (CIMFR) and compared them to patients with the other four most common IMFSs on the CIMFR: Diamond Blackfan Anemia (DBA, 39 patients), Fanconi Anemia (FA, 35 patients), Kostmann Neutropenia (KN, 10 patients) and Dyskeratosis Congenita (DC, 9 patients). *Methods*. The CIMFR is a prospective multicenter study to register all patients with inherited marrow failure syndromes (IMFSs) in Canada. Herein, we extracted data on SDS patients enrolled on the CIMFR, and compared it to other IMFSs. *Results.* SDS is currently the third most prevalent disease on the CIMFR, with a male to female ratio of 15:16. Compound heterozygosity in the SBDS gene was found in 77% of the patients tested. At diagnosis, 90% of the patients had neutropenia, 32% thrombocytopenia and 61% anemia. Initial bone marrow showed hypocellularity in 62% of patients and granulocytic hypoplasia in 42%. Eighty-six percent of patients had pancreatic dysfunction and 67% required enzyme replacement therapy. Sixty two percent had short stature and 19% had metaphyseal dysostosis. One patient developed insulin-dependent diabetes mellitus. At a median follow-up of three years on the registry, 6% of SDS patients developed severe aplastic anemia, 19% clonal marrow cytogenetic abnormalities, 19% MDS and 6% leukemia. Thirty-three percent of the SDS patients needed treatment for cytopenias, compared to approximately 91% for the non-SDS IMFS patients. *Conclusion*. SDS is a common IMFS and is associated with frequent progression to MDS, however SDS requires less treatment for cytopenias than the other 4 IMFSs above.

PO-32

MALIGNANT MYELOID TRANSFORMATION IN INHERITED BONE MARROW FAILURE SYNDROMES

C Segbefia, R Klaassen, CV Fernandez, R Yanofsky, E Sherek, J Champagne, M Silva, JH Lipton, J Brossard, Y Samson, S Abish, M Steele, K Ali, N Dower, U Athale, L Jardine, JP Hand, C Allen, J Beyene, Y Dror

From the Marrow Failure and Myelodysplasia Program, Division of Haematology/Oncology and Cell Biology Program, Research Institute, The Hospital for Sick Children and the University of Toronto, Toronto, Ontario; Children's Hospital of Eastern Ontario, Ottawa, Ontario; Isaak Walton Killam Hospital for Children, Halifax, Nova Scotia; CancerCare Manitoba, Winnipeg, Manitoba; 5British Columbia Children's Hospital, Vancouver, British Columbia; Hôpital Ste.Justine, Montréal, Québec; Queen's University, Kingston, Ontario; Princess Margaret Hospital, Toronto, Ontario; Centre U Sante de l'Estrie-Fleur, Sherbrooke, Quebec; Centre Hospital University Quebec-Pav CHUL, Sainte-Foy, Quebec; Montreal Children's Hospital, Montreal, Québec; Alberta Children's Hospital, Calgary, Alberta; University of Saskatchewan, Saskatoon, Saskatchewan; University of Alberta/Health Sciences Centre, Edmonton, Alberta; McMaster Children's Hospital/McMaster University Health Sciences Centre, Hamilton, Ontario; Children's Hospital of Western Ontario, London, Ontario; Janeway Child Health Centre, St. John's, Newfoundland; Population Health Sciences, Research Institute, The Hospital For Sick Children, Toronto, Ontario, Canada

Background and Objectives. Inherited bone marrow failure syndromes (IBMFSs) are a group of rare, heterogenous genetic disorders with a significant risk of malignancy, particularly malignant myeloid transformation (MMT) into myelodysplastic syndrome (MDS) and acute leukemia. The incidence, pathogenesis and outcome of the various stages along the process of MMT in IBMFSs are unclear. Also, although clonal marrow cytogenetic abnormalities (CMCA) such as monosomy 7 are considered an important diagnostic and prognostic factor in IBMF-related MDS/AML, the true significance of isolated finding of CMCA in an otherwise well patient is unclear. In this study we analyzed the prevalence of CMCA, MDS and AML in patients enrolled on the Canadian Inherited Marrow Failure Registry (CIMFR), and the clinical characteristics of these patients. *Method.* The CIMFR is a prospective multi centre study established in January 2001 to enroll all patients with IBMFS. Clinical, hematologic and cytogenetic data was extracted from the CIMFR database and analyzed. MMT was defined as having either CMCA or prominent bilineage morphologic dysplasia or increased percentage of marrow blasts (≥5%) or a combination of the above. *Results.* As of October 31, 2008, 237 patients were enrolled on the CIMFR. Thirty three patients (14%) were identified with MMT. Of the 33 patients, 21 patients were male and 12 were female. Nine out of 33 patients (27%) had Fanconi anemia, 7 (21%) had Shwachman-Diamond syndrome, 12 (36%) had unclassified syndromes, 1 dyskeratosis congenita 1 had Kostmann neutropenia, 1 amegakaryocytic thrombocytopenia and 2 had constitutional trisomy 8. In thirty one cases of the 33 (94%) the initial MMT phase was CMCA/MDS; of those patients, 65% had RC, 16% RCEB, 13% RCD and 6% RCRS. CMCA was observed in 29/33 (88%). At follow up, four of the 31 CMCA/MDS patients (13%) develop AML; 3 had monosomy 7. One of the 33 patients developed pre-B ALL without a preceding identified MDS phase. An additional patient with constitutional trisomy 8 was diagnosed with MDS (RC phase) due to hypercellular bone marrow, severe cytopenia and no evidence of peripheral blood cell destruction. CMCA was common among the patients; the probability of having a CMCA by the age of 23 years was 36%. At follow-up of the CMCAs 36% underwent hematopoietic stem cell transplantation due to severe cytopenia or excess blasts. Overall survival of all patients with CMCAa at 32 months of follow up was 71%. *Conclusion.* Despite the short-term follow-up of the patients on the CIMFR, a relatively high prevalence of CMCA/MDS was found. CMCAs are associated with high incidence of severe marrow dysfunction and a risk of progression into advanced MDS/AML. The annual follow-ups performed on the patients on the CIMFR will help to determine the natural history and significance of CMCA in patients with IBMFS.

PO-33

NRAS, KRAS, PTPN11 AND NF1 MUTATIONS LEAD TO *IN VITRO* AND CLINICAL DIFFERENCES IN JUVENILE MYELOMONOCYTIC LEUKEMIA

B Perez,[1,2] J Lachenaud,[1,2] S Pereira,[1] ML Menot,[2] C Liger,[1] F Zassadowski,[2] C Alberti,[3] C Chomienne,[2] B Cassinat,[2] H Cavé[1,2]

[1]Department of Genetics and [3]Service of Public Health, Hôpital Robert Debré; [2]INSERM U718, IUH-Hôpital Saint Louis, Paris, France

Objectives. JMML is a rare pediatric malignant myeloproliferative/myelodysplastic disorder, characterized by monocyte proliferation. An underlying genetic syndrome (Noonan syndrome or Neurofibromatosis) is often associated. RAS signalling pathway is pathologically activated in JMML and leads to hypersensitivity to GM-CSF and spontaneous *in vitro* growth of myeloid progenitors. We genetically characterized a cohort of patients with JMML and questioned the possible correlation between genetic status, *in vitro* growth characteristics, clinical presentation and outcome.

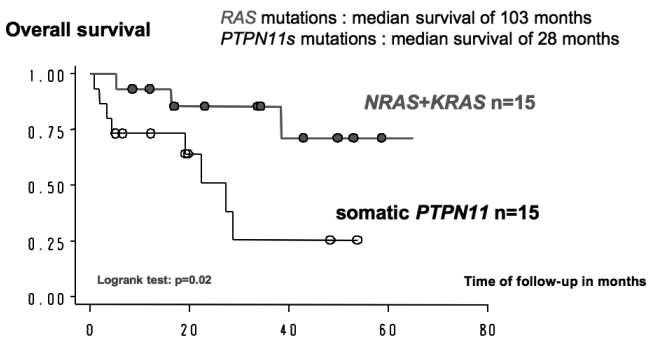


Figure 1. Survival of patients according to the genetic status N/KRAS versus somatic PTPN11. Patients with germline PTPN11 and NF1 were not included to assess survival because the disease progression could be due to the genetic syndrome.

Methods. PTPN11, NRAS and KRAS were sequenced in 51 patients with JMML. The presence of germline mutations was checked. Mutations of NF1 were investigated in patients with clinical neurofibromatosis. *In vitro* growth of myeloid progenitors was performed with and without growth factors. Clinical and biological features were collected. *Results*. PTPN11, NRAS, KRAS and NF1 were mutated in 43%, 18%, 14% and 6% of cases respectively. Only 10/51 (20%) patients had no mutations. 9 patients (18%) had a germline PTPN11 or NF1 mutation. Spontaneous growth of myeloid progenitors was observed in 70% of patients; 26% had only an increased growth of myeloid progenitors with growth factors. Growth characteristics were significantly different according to the genetic status (p=0.01). Clinical presentation did not differ according to genetic subgroups. However, significantly lower platelet counts were noted in patients with somatic PTPN11 mutations, and higher monocytosis in patients with inherited diseases (NS or NF1), who were also the youngest. More importantly, patients with somatic PTPN11 mutations had a lower 2-year overall survival (25% *vs.* 75%; p=0.02) than those harbouring RAS mutations. *Discussion*. Although mutations found in JMML all lead to RAS dysregulations, they are associated with *in vitro* and clinical differences, suggesting that they do not have an identical impact on intracellular signalling pathways.

PO-34

DEL(5Q) IN A PATIENT WITH JMML, NEUROFIBROMATOSIS 1 AND PARVOVIRUS B19 INFECTION

S Vallero, S Aschero, L Garbarini, R Mazzone, N Crescenzio, A Iavarone, E Ricotti, E Vassallo, F Nesi, L Cordero di Montezemolo, L Farinasso

Pediatric Oncohematology, Children Hospital Regina Margherita, University of Turin, Italy

Objective. We report the peculiar karyotype of a 4-year-old boy with neurofibromatosis 1 (NF1) and a picture of JMML with active Parvovirus B19 infection at diagnosis. We discuss the finding of a karyotype seldom reported in JMML and the meaning of Parvovirus infection in presence of progressive disease with a clonal chromosomal aberration. *Methods.* The patient, previously followed-up for NF1, was sent to our Centre for fever, petechiae, hepatosplenomegaly and lymphadenopathy. Thrombocytopenia (39×10^9/L), leucocytosis (18.91×10^9/L), monocytosis (2.31×10^9/L) and mild anemia (90 g/L) was evidenced. HbF was elevated (14.9%). Search for BCR-ABL rearrangement was negative. Spontaneous growth in colony assay was evidenced. PB and BM smear showed monocytosis and myelodysplastic features. PB and BM blasts were 10%. Viral infections were screened by serology and PCR. Cytogenetic analysis was performed on both PB and BM. *Results.* G-banding showed del(5q) in 4/10 metaphases on PB and BM. LSI FISH with a 5q31.2 probe confirmed the presence of the deletion in 35% of nuclei. Serology for Parvovirus showed IgM positivity at diagnosis, PCR for Parvovirus was positive in all subsequent controls, despite multiple infusions of immunoglobulin (0.4 g/kg). The patient received low-dose cytarabine and 6-mercaptopurine with partial benefit and subsequently underwent allogeneic stem cell transplantation from unrelated donor. *Discussion*. Del(5q) is very unusual in JMML. In our patient, such aberration could have triggered a clonal proliferation in the setting of a predisposing condition (NF1). Our patient fulfilled primary and secondary diagnostic criteria for JMML. Del(5q), along with the worsening haematological picture, helped us decide to treat our patient as a true JMML despite the persistent Parvovirus infection. The presence of a viral infection does not exclude the diagnosis of JMML. The hypothetical causative role of viruses in the pathogenesis of JMML remains unclear.

PO-35

HLA-IDENTICAL UMBILICAL CORD BLOOD TRANSPLANTATION FROM A SIBLING DONOR IN JUVENILE MYELOMONOCYTIC LEUKEMIA

ACH de Vries,[1] RGM Bredius,[2] AC Lankester,[2] M Bierings,[3] M Trebo,[4] P Sedlacek,[5] CM Niemeyer,[6] M Zecca,[7] F Locatelli,[7] MM van den Heuvel-Eibrink[1]

[1]Pediatric Oncology/Hematology, Erasmus Medical Center, Sophia Children's Hospital, Rotterdam, The Netherlands; [2]Department of Pediatrics, Leiden University Medical Center, Leiden, The Netherlands; [3]Department of Pediatric Hematology/Oncology, UMCU/Wilhelmina Children's Hospital, Utrecht, The Netherlands; [4]Department of Pediatrics, St Anna Children's Hospital, Vienna, Austria; [5]Department of Pediatric Hematology/Oncology, Charles University, University Hospital Motol, Prague, Czech Republic; [6]Department of Pediatrics, University of Freiburg, Freiburg, Germany; [7]IRCCS Policlinico San Matteo, Pediatric Hematology/Oncology, Pavia, Italy

Background. Currently, for juvenile myelomonocytic leukemia (JMML) stem cell transplantation is the only curative treatment option. Although successful unrelated donor umbilical cord blood transplantations (UCBTs) have been reported, series of HLA-identical sibling donor UCBTs in JMML are not available. *Patients and methods.* From the European Working Group on Childhood MDS (EWOG MDS) registry 5 JMML patients who underwent a fully HLA matched sibling UCBT were identified. *Results.* The median age at diagnosis was 18 months (range 15-30 months). None of the patients had clinical signs of NF1. In one case a *PTPN11* mutation was found. In two other cases no mutation in *RAS* or *PTPN11* was found. In one case no mutation analysis was performed, in four cases the conditioning regimen consisted of busulfan, cyclophosphamide and melphalan and in one case of cyclophosphamide, etoposide and total body irradiation. All patients engrafted slowly (ANC > 500 uL: median 33 days, range 10-35 days, platelets > 20.000/uL: median 53 days, range 38-77 days). In 3 patients acute graft versus host disease was noticed (grade 1 and 2), no chronic graft versus host disease was reported. Two patients relapsed after the initial transplantation and underwent a second transplantation with marrow of the initial donor. One of them is in second complete remission and the other died after a second relapse. One patient developed increased donor chimerism from day 42 without any clinical sign of relapse. She was treated with DLI, 6-mercaptopurine and 13-cis retinoic acid respectively. She is still in complete remission 5 years after transplantation. *Conclusion.* This EWOG series illustrates that, although this needs to be confirmed in larger series, transplantation with relatively immunologically naive cord blood stem cells from a HLA-matched sibling is feasible in selected cases of JMML.

PO-36

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH FLUDARABINE-CONTAINING REGIMEN IN CHILDREN WITH JUVENILE MYELOMONOCYTIC LEUKEMIA

M Yabe, A Manabe, Y Ohtsuka, K Watanabe, M Imamura, M Kaneda, R Miyachi, J Inagaki, Y Ebihara, N Yoshida, K Sakashita, K Horibe, M Tsurusawa

The JMML committee of the Japanese Pediatric Leukemia/Lymphoma Study Group, Tokyo

Objective. Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative treatment for juvenile myelomonocytic leukemia (JMML), however, relapse has been represented as the main cause of treatment failure after HSCT. We report the outcome of 25 patients (pts) with JMML, who were given unmanipulated hematopoietic stem cell after a uniform conditioning comprising busulfan (Bu), fludarabine (Flu), and melphalan (LPAM), without irradiation. *Methods.* Between 7/01 and 11/08, 25 pts with JMML received HSCT. There were 18 males and 7 females, aged 0.5 to 6.8 years. Five pts received HLA-matched related HSCT, and 20 pts from alternative donors, 12 of whom from HLA-mis-

matched (MM) donors. Conditioning was Bu (16 pts: 560-645 mg/m^2 given orally, 9 pts: 16-24 mg/kg given intravenously), Flu (120 mg/m^2) and LPAM (180-210 mg/m^2). Most pts given allograft from alternative donors received tacrolimus and short-term methotrexate to prevent graft-versus-host disease (GVHD). *Results*. Five pts failed to engraft, all of whom had received allograft from HLA-MM donors. Another pt, receiving transplant from HLA-MM cord blood, presented a secondary marrow failure 53 days after HSCT because of hemophagocytic syndrome. The rate of acute-GVHD (II <) and chronic-GVHD was 8/20 (40%), 8/17 (47%), respectively. Four pts died of transplantation-related causes and four pts, including two pts who had graft failure, had hematological relapse. Six pts received second HSCT, and three are alive. Overall, 18 children remain alive in first complete remission after HSCT (range 1-88 mo), and 14 pts are alive event-free. *Discussion*. Our study indicates that HSCT, after Bu/Flu/LPAM regimen, may cure more than 50% of pts with JMML using alternative donors. This conditioning was well tolerated and appeared to be effective for JMML, but graft failure was seen in five who had received HLA-MM HSCT. To overcome graft failure, particularly in MM HSCT, it may be necessary to use other immunosuppressive agents.

PO-37

EXPRESSION OF P53 IN MYELODYSPLASTIC SYNDROME, JMML AND NON-NEOPLASTIC BONE MARROW FAILURES IN CHILDREN

C Dahl,[1] GB Kerndrup,[2] J Stary,[3] H Hasle[1]

[1]Department of Pediatrics, Aarhus University Hospital Skejby, Denmark; [2]Department of Clinical Genetics, Vejle Hospital, Denmark; [3]Department of Pediatric Hematology Oncology, Charles University, Prague, Czech Republic

Objectives. Altered expression of the tumor suppressor gene p53 has been shown to be a valuable tool for studying bone marrow failure in adults and is a prognostic factor in myelodysplastic syndrome (MDS) where increased p53 expression is associated with disease progression. The expression of p53 was significantly higher in patients with refractory anemia as compared to aplastic anemia. The expression of p53 was concluded to be helpful differentiating hypocellular refractory anemia from acquired aplastic anemia. In children with leukemia the expression of p53 is elevated in the majority of patients with unfavorable prognostic features suggesting a role of the protein in development of high-risk leukemias. Very few data on the expression of p53 in children with MDS are available. *Methods.* This study analyzed immunohistochemically the expression of p53 at the protein level in bone marrow trephines from 138 children with MDS (n=53), acute myeloid leukemia (AML) (n=10), juvenile myeloid leukemia (JMML) (N=20) and non-neoplastic conditions (suspicion of MDS, aplastic anemia, Diamond Blackfan anemia, idiopathic thrombocytopenic purpura , Fanconi anemia and others) (n=55). *Results.* By immunohistochemistry 33 of the 53 patients with MDS expressed p53. MDS was divided into refractory cytopenia (RC) and advanced MDS (RAEB and RAEB-t). In the group of children with RC 15% had a level of p53 protein expression more than 5 % whereas in the group of children with advanced MDS, 56 % of the children had a p53 level more than 5%. In patients with AML 9 of the 10 patients were p53 positive with expression levels between 8-60%, only one bone marrow biopsy was found with just 1% p53 positive cells. Expression of p53 was studied in 20 JMML patients, 13 of the bone marrow biopsies were positive for p53 at levels between 1 and 23%. In the group of patients with non-neoplastic disorders 55 biopsies were examined and 7 of them stained positive for p53, only 3% from this group of patients had p53 expression of more than 5 %. Table 1 shows the percentage of p53 positive patients in the different groups of diseases and children with non malignant diseases. *Discussion.* Our data suggests that p53 can be used as a diagnostic marker in childhood MDS. We plan to study p53 expression as a prognostic marker in childhood MDS.

Table 1.

p53 (%)	*RC (n=26)*	*RAEB and RAEB-t (n=27)*	*AML (n=10)*	*JMML (n=20)*	*Non-malignant conditions (n=55)*
0	50%	26%	10 %	35%	87%
1-5	35%	19%	10 %	25%	10%
>5	15%	56%	80 %	40%	3%

PO-38

A CASE OF MYELODYSPLASTIC SYNDROME WITH HYPOCELLULER FIBROSIS

A Akyay,[1] L Olcay,[1] M Ertem,[2] I Kuzu,[2] N Bozdoğan,[1] E Ünal Ince,[2] T Ileri,[2] B Tavil,[1] A Sayili,[2] Z Uysal[2]

[1]S.B. Ankara Oncology Research and Training Hospital, Pediatric Hematology and Pathology Clinics, Ankara; [2]Ankara University Medical Faculty, Pediatric Hematology and Pathology, Ankara, Turkey

A nine-year-old girl was admitted to our hospital, because of pallor which lasted for nearly six years. It was learned that she did not benefit from previous iron therapies. For five years, she developed occasional nasal and gingival bleeding and/or ecchymoses. There was first degree parental consanguinity. Her physical examination was normal except paleness. Her hemoglobin was 4.9 g/dL; hematocrit 15.8%; platelet count 37×10^9/L; white blood cell count: 3.2×10^9/L, MCV 81 fl; reticulocyte index 0.79%. The peripheral blood smear revealed 62% lymphocytes, 27% neutrophils, 7% stabs, 2% monocytes, 1% metamyelocytes, poikilocytosis, anisocytosis of erythrocytes and segmentation abnormalities of granulocytes. Bone marrow aspiration smear was hypocellular and revealed 3% blasts, 21% lymphocytes, 10% neutrophils, 44% normoblasts, 3% stabs, 8% myelocytes, 11% monocytes. The cellularity of bone marrow biopsy was below 10%; myeloid and erythroid series were condensed in paratrabecular region. There were small groups of dysplastic and micro megakaryocytes and no ring sideroblasts. Reticulin and trichrom stains revealed reticulin fibrosis with concomitant collagenization (grade 4 fibrosis). Bone marrow cytogenetic examination revealed 46 XX and monosomy 8. Blood chemistry, ferritin, vitamin B12, folic acid, hemoglobin electrophoresis, abdominal ultrasonography, CD55, CD59, immunoglobulin G, A, M levels were normal; viral serologic markers, diepoxybutane test, anti nuclear antibody, anti deoxyribonucleic acid, anti cardiolipin antibodies were negative. She was diagnosed as MDS with hypocellular fibrosis. Allogeneic bone marrow transplantation was performed from her 6/6 HLA matched sibling. Hypoplastic MDS with fibrosis, whether primary or secondary, is very rare in childhood. Pathological examination of bone marrow biopsy is essential to distinguish between hypocellular MDS, aplastic anemia and MDS with hypocellular fibrosis.

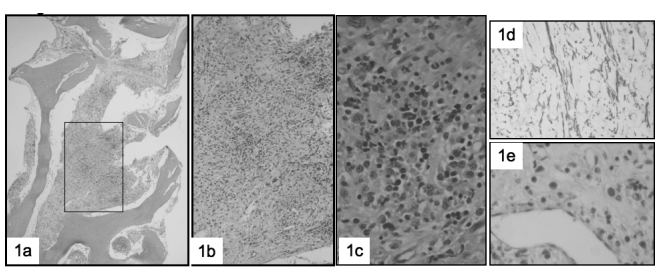


Figure 1. Morphology of the bone marrow biopsy. a) Fibrotic bone marow without any fat cells, but decrease in cellularity (HE) b) High power view showing one of the scattered cellular areas with fibrosis (HE). c) The erytroid precursors and increased immature granulocytic lineage with maturation failure and scattered megacaryocytes were composing the cellularity. The background was highly fibrotic (HE). d) Reticulin staining revealed the meshwork of increased thick reticulin fibers (Gomori reticulin). e) Trichrome staining revealed the collagen production consistent with grade IV reticulin fibrosis.

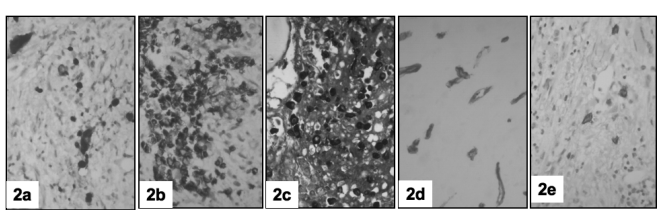


Figure 2. Immunohistochemistry revealed the cellular composition of the bone marrow. a) With CD61 several micromegakaryocytes were demonstrated. b) Glycophorin A showed the increase in immature erytroid precursors. c) Myeloperoxidase was positive in the background but the dark brown staining in the cytoplasms of the myeloid precursors revealed the decrease in granulocytic series comparing to erytroid precursors. d) CD34 was positive only on endothelial cells and e) CD117 was positive only on reactive

mast cells. The last two stem cell markers revealed that there weren't any blastic transformation. (Peroxidase, DAB).

PO-39

IMMUNOPHENOTYPIC PROFILE IN PEDIATRIC PATIENTS WITH MYELODYSPLASTIC SYNDROME IN RIO DE JANEIRO, BRAZIL

MH Ornellas,[1,2] MM Campos,[2] C Diniz Athaide,[2] B Evangelho Gomes,[2] K Wagner-Souza,[2] T de Souza Fernandez,[1,2] HR Diamond[2]

[1]Department of Pathology and Hematology, Medical School Rio de Janeiro State University; [2]Bone Marrow Unit , National Cancer Institute, Rio de Janeiro, Brazil

Introduction. Recently flow cytometry became an useful tool for diagnosis of MDS. In the great majority efforts to analyze MDS by flow cytometry have been reported in adults and information on the immunophenotypic characteristics in pediatric patients continues scanty. This is due to the rarity of these disorders in children that represents less than 5% of all malignancies. The goal of this study was to explore the immunophenotypic differences between pediatric patients with MDS and normal individuals, including changes in distribution of cell lineages as well as phenotypic aberrations and blockades in cell maturation pathways. *Methods*. We evaluated 28 bone marrow samples from 25 patients (14 males and 11 females) until 21 years old by multiparameter flow cytometry. Some of them could be studied in more than one time and the evolution was observed. All of them were submitted to myelogram, bone biopsy, cytogenetic, cytochemistry. As control group, 8 healthy pediatric donors for allogenic bone marrow transplantation were analysed. For immunophenotypic study monoclonal antibodies with FICT: CD3, CD4, CD16, CD19, CD22 ,CD38, CD7, CD14, CD15, CD36, CD61; PE (CD8, CD10, CD13, CD33, CD34, CD56, CD117, CD11b, glycophorin A, CD71, HLA- DR and CD45 PERCP were employed. For data analysis the PAINT-GATE software (BDB) were used. *Results.* All specimens were adequate for flow cytometric analysis and 2 or more abnormalities were found in all of them. In MDS the proportion of bone marrow myeloid $CD34^+$ cells was higher than in normal patients but the lymphoid progenitors ($CD34^+CD19$) were less represented. Upon analyzing the granulo-monocytic differentiation pathway, MDS patients showed an increased proportion of monocytic cells with a decreased percentage of cells of neutrophil lineage, leading to a lower neutrophil/monocytic cell ratio. Maturational arrests in the monocytic and in the neutrophil differentiation pathway were observed. *Conclusions.* Our results show that, in addition to an abnormal distribution of the bone marrow cell compartment, MDS patients frequently show aberrant phenotypes and maturational arrests. Some of these features may help in cases in which the diagnosis of MDS is questionable.

PO-40

THE PATHOLOGICAL FINDINGS AND SURVIVAL IN PEDIATRIC BONE MARROW FAILURE SYNDROMES. A SINGLE CENTRE REVIEW

RJ Leguit, MCA Bruin, MB Bierings, JG van den Tweel

University Medical Centre Utrecht, Depts of Pathology and Paediatrics, The Netherlands

To study the distinguishing morphological features of pediatric bone marrow failure syndromes and their survival, we studied all bone marrow biopsies (BMBs) of pediatric patients in the UMC Utrecht from 1990 until 2008, which were performed because of cytopenia. Out of a total of 160 biopsies, the study group comprises 24 children, with the following clinical diagnoses: - Fanconi anemia (5), Blackfan-Diamond Anemia (6); - Congenital (a)megakaryocytic thrombocytopenias (7); - Kostman syndrome / severe congenital neutropenias (4); Shwachman-Diamond syndrome (2). Four patients with Fanconi anemia received allogenic stem cell transplantation (aSCT), of whom one died of sepsis after12 months. Mean follow-up and survival of the 3 surviving patients is 103 months. The fifth patient was sent to another hospital for treatment and was lost for follow-up. All 6 patients with Blackfan-Diamond anemia were treated with Prednison and / or blood transfusions. All patients were alive at follow-up (mean follow-up 134 months). The 2 patients with Shwachman syndrome only received antibiotic prophylaxis and were alive and well at age 1 and 18 years, respectively. The latter developed a monosomy 7 in his bone marrow at age 19. Of the seven patients with congenital thrombocytopenia, three were diagnosed with amegakaryocytic thrombocytopenia, who all three received aSCT. One of them died within 3 weeks after her second aSCT because of an invasive Aspergillus infection due to prolonged neutropenia as a result of non-engraftment. Of the other four patients, one was transplanted when he developed AA. The surviving patients (N=6)) had a mean follow-up of 154 months. Of the five patients with severe congenital neutropenia one patient received aSCT; in addition to her cyclic neutropenia, she was also known with a congenital hypoplastic anemia. All patients are alive at follow-up (102 months). Regarding the morphology, in the cases of Blackfan-Diamond anemia, BMBs were normo-, to hypocellular with an aplastic or hypoplastic (dys)erythropoiesis. However, some dysgranulopoiesis and dysmegakaryopoiesis was seen as well, so that a MDS can be considered as well on the morphology alone. The congenital neutropenias consistently show insufficient maturation of the granulocytic series from the (pro)myelocyte level in the BMB. The other cell lines were normal. The two cases of Shwachman-Diamond syndrome had hypocellular BMBs, one of them showed some associated dysmegakaryopoesis. In cases of congenital thrombocytopenia, dyshematopoiesis was often seen in erythropoieis and granulopoiesis. In patients with Fanconi anemia, BMBs were hypocellular and often showed some dysplastic features reminiscent of myelodysplastic syndromes. The differential diagnosis with MDS can be very difficult and additional workup is sometimes necessary to come to an adequate diagnosis. In conclusion, survival in pediatric bone marrow failure syndromes seems generally good and the morphology of the bone marrow is often characteristic. However, despite the characteristic morphologic features, dyshematopoietic features are sometimes also observed making the interpretation of bone marrow biopsies in these patients still challenging.

PO-41

JAK2 V617F MUTATION IN ACUTE MYELOID LEUKEMIA SECONDARY TO PH NEGATIVE CHRONIC MYELOPROLIFERATIVE DISORDERS

CR Rinaldi,[1] P Rinaldi,[1] V Martinelli,[1] R Ciancia,[1] L Gugliotta,[2] AM Vannucchi,[3] T Barbui,[4] B Rotoli,[1] F Pane[1]

[1]AF Ematologia, Università Federico II, DBBM e CEINGE Centro di Biotecnologie Avanzate; [2]Ematologia, Arcispedale S. Maria Nuova, Reggio Emilia; [3]Dipartimento di Area Critica, Università degli Studi, Firenze, [4]Ospedali Riuniti, Bergamo, Italy

Objective. Transformation to acute myeloid leukemia (AML) is a known complication of myeloproliferative disorders (MPDs). Recently, Theocharides *et al*. have published an interesting report about the incidence of JAK2 negative blasts from patients affected by secondary AML derived from JAK2 mutated MPDs. *Methods.* We collected, by cell sorting, blast cells and mature cells from total bone marrow of 13 patients newly diagnosed of secondary AML [7 derived from primary myelofibrosis (PMF); 3 from polycythemia vera (PV) and 3 from essential thrombocythemia (ET)]. All the samples were genotyped for JAK2-V617F mutation by ASO PCR. At MPD diagnosis, JAK2-V617F was detectable in 7 of 13 patients (3 of 7 PMF; 3 of 3 PV and 1 of 3 ET). All patients had received cytoreductive treatment with HuOH. *Results.* In our cohort of patients we found that JAK2-V617F mutation was still present at the blast transformation in both compartments (blasts and mature cells) in 6 of 7 JAK2 mutated MPDs. Only 1 of 7 patients developed JAK2-V617F negative AML starting from a mutated MPD. Interestingly, the negativity for the mutation was confirmed in blast cells but also in the rest of mature-myeloproliferative bone marrow tissue. Surprisingly we also described a case of JAK2-V617F mutated AML from a wild type MPD but even in this case the positivity occurred in mature and blast compartments. The remaining 5 wild type JAK2 MPDs maintained the same JAK2 status during blast crisis. Two JAK2 positive AML from JAK2 positive MPD (1 ET and 1 PV) achieved CR after induction treatment while the others did not respond to the therapy. *Discussion.* According to our preliminary results, in contrast to the previous study, we conclude that JAK2-V617F positive MPD yields JAK2-V617F negative AML at the same frequency of a JAK2-V617F negative MPD transforming in JAK2 mutated AML. Furthermore we wanted to underline how any modifications in the JAK2 integrity or the persistence of the previous status involved the entire bone marrow during leukemic transformation suggesting that the leukemic hit could take place in a common ancestor precursor able to modify entirely the genomic signature of the disease.

PO-42

CONGENITAL MYELOPROLIFERATIVE DISEASE WITH GATA-1 MUTATION IN A PHENOTYPICALLY NORMAL INFANT

S Hirabayashi, K Arima, T Kamiya, D Hasegawa, C Ogawa, A Manabe, R Hosoya, E Ito[1]

St. Luke's International Hospital, Tokyo; [1]Hirosaki University Graduate Schiool of Medicine, Hirosaki, Japan

Introduction. Transient abnormal myelopoiesis (TAM) is a leukemoid reaction occurring in ten to twenty percent of Down syndrome (DS) newborn infants. Acquired somatic mutations in exon 2 of the hematopoietic transcription factor GATA-1 have been found in individuals with TAM with DS. On the other hand, TAM of phenotypically normal neonates is often associated with mosaic DS with acquired GATA-1 mutations. Recently, the other type of TAM was reported: non-DS with GATA-1 mutations. We report an additional case with TAM in a phenotypically normal infant with GATA-1 mutation and an unique chromosomal abnormality were positive. *Case report*. A 1-day-old boy presented with purpura over the face, trunk and limbs. He had no dysmorphic features apparently, and had undergone a normal vaginal delivery at 40 weeks of gestation following an uncomplicated pregnancy. He was referred to our hospital at 12 days of age. On admission, he had splenomegaly (4 cm). WBC count was 11600/uL, with 37% of blast cells, Hb was 12.4g/dL, and Plt was 54000/uL. The karyotype of the peripheral blood was 48, XY, +der(1), +21 (20/20). A bone marrow aspirate consisted of 13% blast cells, 68% of which expressed CD7, 70% CD33, and 40% CD34 antigens. Mutation in the GATA-1 gene was present in the peripheral blood. Interphase FISH analysis of buccal mucosa cells showed 2 normal 21st chromosomes in 100 out of 100 cells, confirming that trisomy 21 were restricted to hemopoietic lineage. In the absence of exacerbation or profound marrow failure he has been observed without treatment. After free of transfusion 6 month after birth, he progressed to AML-M7. He was treated with chemotherapy, but he suffered from hemophagocytosis. After 86 days neutropenia, BM recovered spontaneously. We performed 3 additional courses of intensive chemotherapy. The patient is in remission for 10 months now. *Discussion.* The questions are three folds: Mosaic DS was really excluded in this case? Did this patient have TAM or congenital MPD? Was it possible that acquisition of extra chromosome 21 and GATA-1 mutation occurred at the same time in the prenatal period?

PO-43

FREQUENCY OF JAK2 V617F IN INDIAN PATIENTS WITH CHRONIC MYELOPROLIFERATIVE DISORDERS: AIIMS EXPERIENCE

S Sazawal, J Bajaj, B Sharma, R Bhargava, R Chaubey, M Mahapatra, R Saxena

All India Institute of Medical Sciences, New Delhi, India

B*ackground*. Chronic myeloproliferative disorders (CMPD's) are clonal hematopoietic stem cell disorders and include polycythemia vera (PV), essential thrombocythemia (ET), idiopathic myelofibrosis (IMF). Sometimes it is not possible to differentiate some cases from reactive disorders however, recently described point mutation JAK2V671F a potential candidate aids in this differentiation. *Objective*. Since, its frequency varies in different populations we aimed to looked for the presence of JAK2V617F mutation in Indian patients with CMPD's. *Methods.* A total of 150 patients; PV (n=80), ET (n=19), IMF (n=51), 35 non MPD patients and 25 healthy controls were studied. Suspected MPD cases were subjected to haemogram, red cell mass, erythropoietin . All cases and controls were screened for JAK2 V617F mutation detection by DNA tetra-primer amplification refractory mutation system. *Results.* The JAK2 V617F mutation was positive 82% of PV, 70% of ET and 52 % of IMF. The mean age of JAK2 positive patients was 53years (range 28-73years) and JAK2 negative was 44years (range15-83years) (p=0.01). The overall presence of JAK2 mutation was associated with a higher hemoglobin level (p=0.041), a higher white blood cell count (p=0.007), higher age (p=0.01). Homozygosity was observed in 75% of PV, 100% of ET and 56% of IMF.The mutation was not detected in any of the non MPD patients or the healthy controls. *Conclusion.* The JAK2 V617F mutation can be frequently detected in Indian patients with MPD disorders hence should be incorporated into the initial evaluation of patients suspected of MPD. Thus, mutation screening for V617F serves as a tool to diagnose MPD's and may determine a subgroup which would require special therapy.

PO-44

ESSENTIAL THROMBOCYTHAEMIA IN CHILDREN: CLINICAL AND LABORATORY CHARACTERISTICS

D Pospisilova,[1] M Horvathova,[2] J Veselovska,[2] R Solna,[2] J Kucerova,[2] J Stary,[3] K Petrtylova,[3] S Pekova,[4] V Divoky[2]

[1]Department of Paediatrics, University Hospital, Olomouc, [2]Department of Biology, Faculty of Medicine, Palacky University, Olomouc, [3]Department of Paediatric Haematology-Oncology, University Hospital Motol, Prague, [4]Department of Clinical Biochemistry, Haematology and Immunology, Na Homolce Hospital, Prague, Czech Republic

Introduction. Essential thrombocythaemia (ET), a Ph-negative chronic myeloproliferative disorder, is usually a disease of middle age and is extremely rare in paediatric population. *Patients, methods and results.* We have analysed 9 girls and 6 boys diagnosed with ET in the Czech population between 1987-2007. Platelet count at the time of diagnosis was 681-2428×10^9/L (median 1720×10^9/L); haemoglobin (Hb) and leukocytes were within normal range. Splenomegaly was found in 8 children. The median follow-up was 19 months. The diagnosis was made during routine blood analysis in majority of cases. No thrombosis or major bleeding was observed at the time of diagnosis; symptoms of microvascular obstruction were present in 5 patients. Erythropoietin hypersensitivity of haematopoietic progenitors *in vitro* was found in 11/13 patients, with EEC formation in 9/11. JAK2 V617F mutation in peripheral blood leukocytes and separated platelets was detected in one female patient with monoclonal hematopoiesis and borderline level of Hb. Analysis of EECs revealed rare JAK2 V617F heterozygous or homozygous colonies in 3/5 examined patients. Our data suggests that childhood ET patients could bear minor JAK2 V617F-positive subclones. These patients did not show any phenotypic differences from the cohort. *Discussion.* The clinical picture in our paediatric patients was milder than in adults, JAK2 V617F mutation is rare. However, the paediatric patients should be monitored for possible disease progression to clonal haematopoiesis, acquisition of JAK2 V617F mutation, or evolvement into clinical picture of PV. It seems that low JAK2 V617F mutant allele burden can occur even in early childhood and it may remain stable for several years. During this asymptomatic period, the disease can be diagnosed accidentally. Only large multicentral studies and long-term monitoring of ET paediatric patients can bring novel insights into this field. Supported by grant NR/9471, Ministry of Health, Czech Republic.

PO-45

CYTOPENIA PROGRESSING SLOWLY TOWARDS FULL-BLOWN MDS-RC, THREE CASE REPORTS

A Janda,[1] J Cermak,[2] K Petrtylova,[3] Z Cerna,[4] Z Zemanova,[5] M Markova,[2] E Mejstrikova,[1] P Smisek,[1] J Stary [1]

[1]Department of Pediatric Hematology and Oncology, University Hospital Motol, and 2nd Medical School, Charles University, Prague; [2]Institute of Hematology and Blood Transfusion, Prague; [3]Department of Transfusion Medicine, University Hospital Motol, Prague; [4]Department of Pediatrics, Faculty of Medicine in Pilsen, Charles University, Prague; [5]Centre of Oncocytogenetics, Institute of Clinical Biochemistry and Laboratory Diagnostics, 1st Medical School, Charles University, and General Teaching Hospital, Prague Czech Republic

We discuss 3 cases with insufficient BM function present since childhood where the dg. of MDS-RC could be made not until adulthood. In patient 1 bicytopenia (leukopenia, trombocytopenia) was detected in infancy, later megaloblastic anemia and mild hypogammaglobulinemia developed. The first biopsy of BM (3 y-o) was normal; 6 y later the BM was hypercellular with predominance of megakaryocytes; hyperplastic megakaryocytes and B cell proliferation, still without clear dysplasia were seen when 15 y-o; 6 y later dysplastic hemopoiesis in all lineages and reticular fibrotisation were detected and dg. of MDS-RC was made. Apart from repeated respiratory infections, he suffered from severe phlegmona with life-threatening sepsis shortly before HSCT (22 y-o). Patient 2 was treated since her infancy for numerous infections of respiratory and urinary tract, asthma, chronic abdominal pain and diarrhea. She was found leukopenic with markedly low number of B cells in the periphery, with normal immunoglobulin levels and megaloblastic anaemia when 12 y-o. BM biopsy done 10 years later revealed hypocellular BM with mild dysplastic features of megakaryocytes and mild dyserythropoiesis; a year later (22 y-o) peripheral cytopenia progressed,

dysplastic changes were present in all lineages, dg.of MDS-RC was stated and HSCT performed. Pancytopenia in patient 3 has been detected since her 17 y when she suffered from tiredness, vertigo and bleeding diathesis. Hypoplastic BM was confirmed and diagnosis of acquired aplastic anaemia was made 3 years later; 5 years later it was reclassified to MDS-RC. She refused BMT and IST has been continued until now (31 y-o). In all the patients the known congenital BM failure disorders were excluded, they had normal karyotypes and no relevant cytogenetic abnormalities. G-CSF was applied only transiently. Having experience with these patients, we highlight the need for close and long-term monitoring of patients with cytopenia as the full picture of MDS-RC may develop later in life. Supported with grant VZ FNM 64203.

PO-46

MYELODYSPLASTIC 5Q(DEL) CLONES ARE ABLE TO REACH TERMINAL DIFFERENTIATION INTO ENUCLEATED RED BLOOD CELLS

L Garderet,[1,2,3] L Kobari,[1,2] C Mazurier,[1,4] C De Witte[1,2]
M-C Giarratana,[1,2] C Pérot,[5] H Lapillonne,[1,2,6] L Douay[1,2,4,6]

[1]INSERM, UMR S 938, Proliferation and differentiation of stem cells, Paris; [2]UPMC Univ Paris 06, UMR S 938, Proliferation and differentiation of stem cells, Paris; [3]AP-HP, Hôpital Saint Antoine, Service d'Hématologie, Paris; [4]Etablissement Français du Sang Ile de France, Ivry-sur-Seine; [5]AP-HP, Hôpital Saint Antoine, Service de Cytogénétique, Paris; [6]AP-HP, Hôpital Armand Trousseau, Service d'Hématologie Biologique, France

We propose a model of *in vitro* generation of mature red blood cells (RBC) from human hematopoietic stem cells (HSC) applied to the 5q(del) syndrome as pertinent for this pathology .The proliferation rate of CD34$^+$ cells from 5q(del) patients was 33 fold lower than that of controls throughout culture (p<0.0001). The erythroid commitment was similarly massive in both groups. Erythroid cells from patients continued their proliferation/differentiation and massively achieved complete maturation into enucleated RBC, as did the controls.(p=ns). Overall, taking into account the rates of expansion and levels of enucleation, we calculated that one CD34$^+$ cell from patients generated 32 times less RBC than one CD34$^+$ cell from normal bone marrow (p<0.0001). This decrease in RBC generation in 5q(del) patients could be partly related to the altered clonogenic capacity of the bone marrow samples: mean number of BFU-E in patients was 27±9 colonies/104 cells as compared to 748±105 colonies/104 cells in normal marrow (p<0.0001).We then addressed the question of whether these RBC generated *in vitro* originated from non deleted patient clones (NP) and/or from 5q(del) patient clones (PC) compared to normal clones (NC). A limiting dilution assay (LDA) was performed starting from CD34$^+$ cells. PC were able to generate enucleated RBC to a similar extent as NC (p=ns).

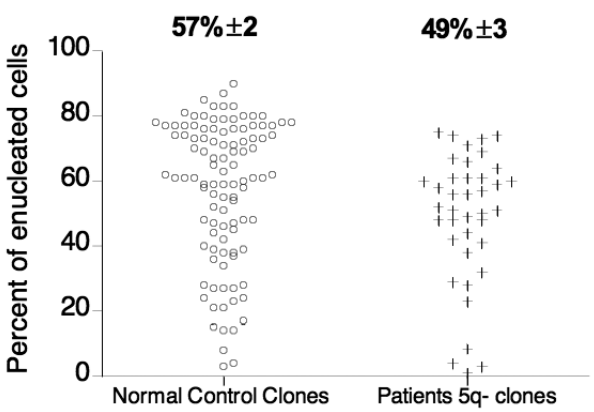


Figure 1. Wells were analyzed for the percentage of enucleated RBC on day 18, each dot representing the result for one well. Data are from three patients and three normal controls. 5q(del) clones were able to generate enucleated RBC to a similar extent as normal controls: mean level of enucleation: 49±3% *vs.* 57±2% respectively (p=ns).

These results imply that one CD34$^+$ PC cell generated 88 times less RBC than one CD34$^+$ NC cell. We further studied RPS14 gene expression at the single cell level in LDA. While the gene expression was at least half in the pathological clones, enucleation rate was similar to the normal clones. Taking the 5q(del) syndrome as a model, we show that contrary to what has been thought until now, the anemia is not linked to an incapacity of the progenitor cells to differentiate into enucleated RBC, but arises solely from the defective proliferation of the HSC committed to the erythroid line.

PO-47

A COMBINED IMMUNOSTIMULATORY AND IMMUNOINHIBITORY SIRNA WITH ANTI-LEUKEMIC PROPERTIES IN A RAT MODEL OF ACUTE MYELOID LEUKEMIA

PO Iversen,[1,2] E Semaeva,[3] DR Sorensen,[4] H Wiig,[3] M Sioud[5]

[1]Department of Nutrition, IMB, Faculty of Medicine, University of Oslo; [2]Department of Hematology, Ullevål University Hospital, Oslo; [3]Department of Biomedicine, University of Bergen, Bergen; [4]Center for Comparative Medicine, Rikshospitalet University Hospital, Oslo; [5]Department of Immunology, Molecular Medicine Group, The Norwegian Radium Hospital, Oslo, Norway

Objective. The recognition that the graft-versus-leukemia (GVL) effect is important for eradication of the leukemic cell clone, points to immunomodulation as possible treatment for acute myeloid leukemia (AML). In addition to GVL-induction, vaccines have been developed, but often with dismal results. This may be due to limitations of breaking tolerance to leukemic cells and/or insufficient activation of T cell cytotoxicity. To break self-tolerance against leukemic cells, we now tested dendritic cells (DCs) loaded with leukemic antigens and a bifunctional siRNA targeting IL -10 and simultaneously activating toll-like receptors (TLRs), on leukemogenesis. *Methods.* Monocytes were isolated from rat blood and cultured with GM-CSF and IL-4 to induce immature DCs. Leukemic proteins were isolated from BNML rats, a model of human AML. BNML rats were treated with DCs harbouring leukemic antigens and a combined immunostimulatory (TLR-ligand) and anti-IL-10 siRNA, as an adjuvant. Bone marrow leukemic cellularity was determined with antibody-staining and flowcytometry, whereas extramedullar dissemination was measured with spleen weight and matrix-metalloproteinase activity. *Results. In vitro* the siRNA inhibited IL-10 production in DCs and stimulated production of TNFalpha, implying activation of TLRs 7/8. Vaccination of BNML rats with the loaded DCs induced specific T cell cytotoxicity. Significantly, leukemic rats treated with the siRNA lived longer, had less leukemic cell mass in their bone marrows and less extramedullar dissemination of the leukemic disease post mortem compared with rats given inactive siRNA. *Discussion*. Vaccination with leukemic antigens and an immunostimulatory siRNA targeting IL-10, can break tolerance to leukemic cells. Specifically, this strategy enhanced survival and reduced both leukemic cell mass and metastasis. Collectively, our data demonstrate the possible usefulness of this bifunctional siRNA as an immunomodulatory drug with anti-leukemic properties.

PO-48

PROLIFERATIVE EFFECTS OF GPD AND GPT ON HUMAN BONE MARROW HAEMOPOIETIC PROGENITOR CELLS

Y-P Niu, X-D Qian, J-Q Qian

[1]School of Pharmaceutic Science, Zhejiang Univerisity of Technology, Hangzhou, P.R.China; [2]Zhejiang Province Hospital of TCM, Hangzhou P.R., China

Objective. To observe the effect of ginsenosides panaxadiol (GPD) and ginsenosides panaxtrol Saponin (GPT) on proliferation of human bone marrow hemopoietic progenitor cells (HPC). *Methods.* GPD and GPT were separated and purified from ginsenosides, and the effects on HPC were studied using *in vitro* hemopoietic progenitor cell colony forming technique, by observing the proliferation of human burst forming unit-erythroid progenitor (BFU-E), colony-forming unit-erythroid (CFU-E), colony-forming unit-granulocyte/macrophage (CFU-GM) and colony-forming unit-pluripotent hemopoietic progenitor (CFU-Mix) *in vitro* after GPD and GPT stimulation. *Results.* Different concentration of GPD (2.5-200 g/mL) could stimulate the proliferation of HPC obviously, showing increase of CFU-E, BFU-E, CFU-GM and CFU-Mix by 54.9±6.3%, 48.8±5.1%, 27.6±4.2% and 48.9±3.9% respectively, which was higher than that of the control group. While stimulated by GPT of the same concentration, the CFU-E and BFU-E was lower that that of control significantly (p<0.05); when the terminal concentration of GPT was 200 g/mL, CFU-E and BFU-E was zero respectively. In the CFU-GM culture, GPT in concentration of 12.5 g/mL could cause the proliferation increased by 29.7±2.2% (p<0.05), but in concentration of 100 g/mL and

200 g/mL, it showed inhibitory effect on CFU-GM, the inhibition rate being 48.6±3.9% and 100% respectively. *Discussion.* Ginseng is a traditional Chinese medicine that has been used in treating anemia for thousands of years. The main component is ginsenosides, which is composed of GPD and GPT. Previous studies have reported that Ginsenosides could promote hematopoiesis by stimulating proliferation of BFU-E and CFU-E. However, It had different effects on CFU-GM. The data indicates that GPD is the effective component of ginsenosides in stimulating proliferation of human bone marrow HPC. GPT is an component with inhibitory action on proliferation of BFU-E and CFU-E and its effect on CFU-GM was depending on its concentration.

PO-49

EX VIVO EXPANSION AND HEMATOPOIETIC RECONSTITUTION ABILITY OF ISOLATED CD34+CD59+ CELLS FROM PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

B Han, J Xiao, Y Wu, Y Zhong, W Sun

Hematology, Peking Union Medical Collegue Hospital, Beijing, China

Paroxysmal nocturnal hemoglobinuria (PNH) is a clonal hematopoietic stem cell disorder characterized by intravascular hemolysis, venous thrombosis, and bone marrow (BM) failure. Until now, allogeneic(allo) hematopoietic stem cell transplantation is still the only way to cure PNH. Eculizumab, although very promising, is not the eradication of the disease because of raising the possibility of severe intravascular hemolysis if therapy is interrupted. Here we isolated the residual bone marrow normal progenitor cells (marked by $CD34^+CD59^+$) from PNH patients, tried to find a way of effectively expanding the progenitors cells *ex vivo*. *Object.* To expand $CD34^+CD59^+$ cells isolated from patients with PNH and observed the long term hemaotopoietic reconstruction ability both *ex vivo* and *in vivo*. *Method.* $CD34^+CD59^+$ cells from 13 patients with PNH and $CD34^+$ cells from 11 normal controls were isolated from the bone marrow mononucleated cells first with immunomagnetic microbead and then by autoclone flow cytometry sorting. The sorted cells were then cultivated under different conditions for two weeks to find out the optimal expansion. The long term hematopoietic supporting ability was evaluated by long-term culture in semi-solid medium ex vitro and long-term engraftment in irradiated immunodeficient mice *in vivo*. *Results.* $CD34^+CD59^+$ cells from patients with PNH can be expanded effectively *in vitro*, and the biggest expansion of $CD34^+CD59^+$ cells was 23.49±3.52 fold on the 7^{th} day. The best combination of hematopietic factors for *in vitro* expansion was SCF+IL-3+IL-6+FL+Tpo+Epo, and the most suitable time for harvesting was on day 7. Although the $CD34^+CD59^+$ PNH cells have impaired *ex vivo* proliferation and survival compared with normal $CD34^+$ cells, they remain strong colony-forming capacity in long-term culture. The peripheral blood cell count in lethally irradiated mice transplanted with expanded $CD34^+CD59^+$ PNH cells recovered on day 90, which was compatible with those transplanted with normal $CD34^+$ cells ($p>0.05$). The survival rate and human CD45 expression in different organs was similar between mice transplanted with $CD34^+CD59^+$ PNH cells and those with normal $CD34^+$ cells ($p>0.05$). On secondary transplantation, the peripheral blood cell count returned to almost normal on day 30 in mice transplanted either with PNH cells or with normal control cells. Lower CD45 percentage was found in secondary transplantation but no difference between mice transplanted with different cells. *Conclusion.* Isolated $CD34^+CD59^+$ cells from patients with PNH can be effectively expanded *ex vivo* and can support hematopoiesis both *in vitro* and *in vivo* in the long term. These data provide a new potential way of managing PNH with auto-PBSCT.

PO-50

GONADAL DAMAGE IN FEMALE ADULT CHILDHOOD CANCER SURVIVORS AFTER TOTAL BODY IRRADIATION, ASSESSED BY ANTI-MÜLLERIAN HORMONE

S Lie Fong,[1] JSE Laven,[1] FGAJ Hakvoort-Cammel,[2] I Schipper,[1] JA Visser,[3] APN Themmen,[3] FH de Jong,[3] MM van den Heuvel-Eibrink[2]

[1]Division of Reproductive Medicine, Department of Obstetrics and Gynaecology, Rotterdam; [2]Department of Paediatric Oncology/Haematology, Rotterdam; [3]Department of Internal Medicine, Rotterdam, The Netherlands

Background. To describe possible treatment-induced gonadal damage in a cohort of adult female childhood cancer survivors using anti-Müllerian hormone (AMH), the most sensitive marker of ovarian reserve. *Methods.* A total cohort of 185 long-term female survivors was included. They had been treated for acute lymphoblastic leukaemia, non Hodgkin lymphoma, acute myeloid leukaemia, Hodgkin lymphoma, neuroblastoma, sarcoma, nephroblastoma, Langerhans cell histiocytosis or germ cell tumour. The median follow-up time was 18.1 years (range 4.1-43.2 yr). Nine survivors had received a stem cell-transplant and all had been treated with a cyclophosphamide and total body irradiation based regimen. *Results.* Median AMH concentration in the total cohort was not different from healthy controls (median 1.7 *vs*. 2.1 g/L; $p=0.57$). There were no differences in AMH levels in subgroups allocated according to disease. In non-transplanted survivors only Hodgkin lymphoma survivors treated with ≥3 procarbazine containing chemotherapy cycles (median 0.5 g/L; $p=0.004$) and patients who had received abdominal irradiation had lower AMH levels than controls (median 0.2 g/L; $p=0.03$). Significantly lower serum AMH levels were observed in survivors after stem cell-transplant as compared with controls (median <0.1 g/L; $p<0.001$). *Conclusion.* Serum AMH were not detectable in survivors treated with total body irradiation, suggesting that they have decreased fertility or premature ovarian failure. In these survivors options for fertility preservation should be considered prior to starting treatment since they may be at risk for poor chances of pregnancy after assisted reproductive treatment.

PO-51

HAEMATOPOIETIC STEM CELL TRANSPLANTATION FOR PEDIATRIC MDS SINGLE CENTER STUDY

D Wójcik[1,2] J Musia,[1] E Gorczynska,[1] M Ussowicz,[1] A Dyla,[1] J Owoc-Lempach,[1] M Sociak,[1] D Sega-Pondel,[1] W Pietras,[1] K Kalwak[1]

[1]Dept of Pediatric Hematology/Oncology and BMT,Wroclaw Medical University, Wroclaw, Poland; [2]Dept of Pediatric Onkology,Haukeland University Hospital, Bergen, Norway

This single centre report on HSCT in children with MDS is challenging due to the toxicity of the preparative regimens aiming to minimize relapse risk and graft rejection. Retrospective analysis of 55 procedures in 49 children performed in Wroc aw. MDS-RA n=6,MDS-RAEB n=14, MDS-RAEB-t n=12, JMML n=16, sAML n=1. The most frequent cytogenetic abnormality was trisomy8 and monosomy7. Median time from diagnosis to HSCT was 6.8 (1.6-103) months. According to donor availability there were MUD-HSCT (n=26), haploidentical T cell depleted (TCD) PB HSCT (n=11) and MSD-HSCT (n=12). The vast majority received combined preparative regimen consisting of busulfan, cyclophosphamide and melphalan. GvHD prophylaxis was dependant on HLA matching varying from ciclosporine-A alone (3 mg/kg b.w.) to extensive *in vitro* TCD. The stem cells' source was PB (n=32), BM (21), CB (n=1) and TCD PB+BM (n=1). Seven patients underwent 2nd transplantation because of relapse (n=1), primary graft failure (n=1) or graft rejection (n=5). In patients achieving engraftment median time to WBC>1 was 14 days, ANC>0,5 was 15 days, platelets >50 was 26 days. The probability of overall survival (pS) and the event-free survival (pEFS) for the whole cohort was 41%. The best results were obtained in children transplanted from MSD (pS and pEFS=0.56) and from MUD (0.46), whereas pts transplanted from haploidentical donors had pS and pEFS=0.18. In the MSD and MUD recipients no difference was observed between the source of stem cells:PBSC recipients had pEFS=0.51 and BM recipients pEFS=0.46. Twenty-five deaths were recorded in the whole group,18 pts (36.7%) died of TRM and 7 pts due to relapse (14.3%). TRM was high resulting from significant proportion of complicated procedures using alternative donors including haploidentical PB HSCT.The results of MUD-HSCT and MSD-HSCT are statistically sim-

ilar.Urgent need for MUD search is warranted when MSD donor is lacking. Supported by grants of The Ministry of Science and Higher Education No 2PO5E12328 and The Ministry of Science and Higher Education No N406 063 31/2352.

PO-52

A NOVEL FORM OF MYELODYSPLASTIC SYNDROME ASSOCIATED WITH MENDELIAN SUSCEPTIBILITY TO MYCOBACTERIAL DISEASE

I Resnick, P Stepensky, M Weintraub, D Engelhard, D Averbukh, J Feinberg, S Boisson-Dupuis, J Bustamante, J-L Casanova

Hadassah Hebrew University Medical Center, Jerusalem, Israel and INSERM U550, Paris, France

Introduction. An Mendelian susceptibility to mycobacterial disease (MSMD) is a rare condition with defective interaction between APC and lymphocyte which are mediated by IFNgamma-IL12/IL23 signaling circuit. At present discovered mutations at several genes explain a molecular basis of a majority of MSMD. *Case report.* Patient DR, 11, presented with prolonged low grade fever, lymphadenopathy, cough. Lymph node biopsy revealed necrotizing granulomatous inflammation. On Ziehl Neelsen stain a number of acid fast bacilli were seen. Found pathogen was defined as Mycobacterium fortuitum. Gastric aspirate revealed M. gastri. Started treatment with isoniazid, rifampicin, doxilin gave full effect. Meanwhile his blood work up demonsrate high MCV (110fL), hemoglobin electropheresis showed increased HbF. Bone marrow evaluation showed evidence of myelodysplastic syndrome with trisomy 8 with normal karyotype of fibroblasts culture. Repeated immunophenotype analysis showed decreased number of B lymphocytes (CD19 2%, CD20 3%, HLA-DR 3%, CD21 5%) while other cell subsets were normal. Immunoglobuline levers were normal as well (IgG 1020 mg/dL, IgM 217 mg/dL, IgA 109 mg/dL). Plasma interferon IFN-gamma level was 0 pg/mL (ELISA). Results of IFN-gamma / IL12 signaling circuit evaluation showed no response both for IL12 and IFN-gamma with and without BCG. Patient underwent BMT from MUD with conditioning consisted of fludarabin 180 mg/m , busulfan 16 mg/kg, thiotepa 10 mg/kg and ATG (Fresenius) 40 mg/kg; antimycobacterial coverage with amikacin and ciprofloxacin up to d+80. Six month later immunosuppression and all other accompaining treatment was stopped and at present patient demonstrate no evidence neither MDS nor mycobacteriosis. *Conclusion*. We believe that this case represent an unknown form of MSMD which manifested as weakly virulent mycobacterial infection combined with a clonal hematopoietic stem cell disease (MDS). Potential molecular mechanisms are under investigation. BMT demonstrate a curative approach for this novel disease.

Author Index