2 additional aberrations) for the presence of GATA2 mutations. Of a total cohort of 102 patients with monosomy 7, GATA2 mutations were noted in 35 patients (34%). GATA2-mutated patients were significantly older at diagnosis of MDS compared to GATA2-wild type patients (median age at diagnosis 12.5 vs. 4.2 years, p<0.01). In fact, the youngest child with MDS and GATA2 mutation was 4.4 years of age. In contrast, the 5-yrs-overall survival did not differ between patients with monosomy 7 with or without GATA2 (64% vs. 67%).

In summary, GATA2 deficiency accounts for 7% of all primary childhood MDS and a third of all primary MDS cases with monosomy 7. To our knowledge this is the largest cohort of patients with MDS that follows this novel transcriptopathy. Further investigations will be critical to better define the penetrance, clinical characteristics and prognosis of this disease.

O 09

Juvenile myelomonocytic leukemia: Five genes – how many subtypes?

Charlotte M. Niemeyer[1], Brigitte Strahm[1], Michael Dworzak[2], Barbara De Moerloose[3], Henrik Hasle[4], Jan Stary[5], Markus Schmugge[6], Marek Ussowics[7], Marry van den Heuvel-Eibrink[8], Marco Zecca[9], Jonas Abrahamson[10], Marc Bierings[11], Victoria Bordon[3], Susanne Matthes-Martin[2], Petr Sedlacek[5], Franco Locatelli[12]

[1] *Pediatric Hematology and Oncology, University Childrens Hospital Freiburg, Germany*
[2] *St. Anna Children's Hospital , Vienna, Austria*
[3] *University Hospital, Ghent, Belgium*
[4] *University Hospital Skejby, Aarhus, Denmark*
[5] *University Hospital Motol, Prague, Czech Republic*
[6] *University Children's Hospital, Zurich, Switzerland*
[7] *University Hospital, Wroclaw, Poland*
[8] *Sophia Children's Hospital, Rotterdam, The Netherlands*
[9] *University Children's Hospital, Pavia, Italy*
[10] *Queen Silvias Children's Hospital, Gothenburg, Sweden*
[11] *University Hospital for Children, Utrecht, The Netherlands*
[12] *Ospedale Bambino Gesù, Rome, Italy*

During the last few years it became apparent that JMML is a very heterogeneous disease. In most cases it is encoded by germline or somatic mutations in 5 genes: NF1, CBL, PTPN11, NRAS and KRAS, and thus has been associated with the rasopathies Noonan(-like) syndrome and neurofibromatosis type 1 (NF1). Among 488 children with JMML studied by EWOG-MDS 51 patients were known to have Noonan (-like) syndrome with germline mutations in PTPN11, NRAS, KRAS, CBL. A normal karyotype was observed in all but three of these children, and overall survival (OS) at 5 years was 0.72 (0.58-0.86) irrespective whether HSCT was performed or not. In contrast, a clinical diagnosis of neurofibromatosis (NF1) had been made in 48 children, and JMML in NF1 proofed fatal unless hematopoietic stem cell transplantation (HSCT) was performed. Mutational analysis performed in 246 of the remaining 389 patients identified somatic mutations in PTPN11, KRAS and NRAS in 116, 46 and 47 children, respectively. In 37 of the 203 children with complete typing none of these abnormalities was present (all negative group). Normal chromosomal studies were observed in 74%, 66% and 60% of the NF1, PTPN11 mutated and the all negative group, respectively. Interestingly, monosomy 7, and other aberrations were noted in 52% and 10% of KRAS mutated patients, respectively, but only in 7% and 5% of the NRAS mutated group (P<0.05). Five of the patients with NRAS mutation and normal karyotype are long term survivors without HSCT (5.5 to 27 years after diagnosis) compared to none of the children from the NF1, PTPN11 mutated, KRAS mutated or all negative group. Event-free survival (EFS) following HSCT differed significantly among the mutational groups with 0.38 (0.29-0.49), 0.43 (0.25-0.61), 0.45 (0.24-0.66), 0.69 (0.54-0.84) and 0.72 (0.56-0.88) for children with PTPN11 mutation, NF1, NRAS mutation, KRAS mutation and the all negative group (P<0.01). Multivariate analysis identified mutational type and age at diagnosis as independent prognostic factors for EFS following HSCT. Most importantly, we showed that in JMML subtypes with a low relapse incidence following HSCT (KRAS mutated and all negative group) transplant related mortality exceeds the relapse incidence, while in other subtypes (PTPN11 mutations, NF1) leukemic relapse is the most common failure. Current transplant strategies for children with JMML need to be revised to accommodate these differences among mutational subgroups.

O 10

Reduced intensity conditioning for children with refractory cytopenia of childhood: results of the EWOG-MDS study SCT RC RIC 06.

Brigitte Strahm[1], Peter Bader[2], Albert Catala[3], Michael Dworzak[4], Marry Vandenheuvel[5], Barbara De Moerloose[6], Markus Schmugge[7], Owen Smith[8], Petr Sedlacek[9], Franco Locatelli[10], Charlotte Niemeyer[11]

[1] *Medical Center - University of Freiburg Center for Pediatrics Department of Pedatric Hematology and Oncology, Freiburg, Germany*
[2] *Department of Pediatric Hematology and Oncology, University Children's Hospital Frankfurt, Germany*
[3] *Hospital Sant Joan de Deu, Barcelona, Spain*
[4] *Department of Pediatric Hematology and Oncology, St. Anna Children's Hospital, Vienna, Austria*
[5] *Erasmus Medical Center, Rotterdam, and Dutch Childhood Oncology Group, the Hague, The Netherlands*
[6] *Department of Pediatric Hematology-Oncology, Ghent University Hospital, Belgium*
[7] *Department of Hematology and Oncology, University Children's Hospital, Zurich, Switzerland*
[8] *Paediatric Oncology and Haematology Dept. Of Oncology/ Haematology, Our Lady´s Hospital for Sick Children, Ireland*
[9] *Department of Pediatric Hematology and Oncology, University Hospital Motol, Prague, Czech Republic*
[10] *Department of Pediatric Hematology and Oncology, IRCCS Ospedale Bambino Gesù, Rome, University of Pavia, Italy*
[11] *Pediatric Hematology and Oncology, Center of Pediatrics and Adolescent Medicine, University of Freiburg, Germany*

Objective: Refractory cytopenia of childhood (RCC) is the most common subtype of myelodysplastic syndrome (MDS) in this age group. Patients with RCC without chromosomal aberrations were eligible for hematopoietic stem cell transplantation (HSCT) with a reduced intensity conditioning regimen (RIC) consisting of fludarabine and thiotepa. Here we report the outcome of children with RCC included in the prospective EWOG-MDS study SCT RC RIC 06.

Patients and Transplant Procedure: Eighty three patients (42 males/41 females) were diagnosed with RCC at a median age of 10.3 (0.8-17.9) years. Patients were transfusion-dependent for platelets (68) and/or red blood cells (57) or had neutropenia (70). None of the patients had an abnormal karyotype. Twenty three patients received immunosuppressive therapy prior to HSCT. The median time to HSCT was 169 days

(49 days-5.2 years). Patients were grafted from a matched sibling donor (MSD) (28), an alternative family donor (1) or a matched unrelated donor (MUD) (54). Stem cell source was bone marrow (76) or peripheral blood (7). All patients were prepared with thiotepa (15 mg/kg) and fludarabine (160 mg/m²). Prophylaxis for graft-versus-host-disease (GVHD) was CSA +/- MTX/MMF +/- anti-thymocyte globuline (ATG) for MSD, and CSA, MTX/MMF and ATG for patients transplanted from an MUD.

Results: After a median follow-up of 2.1 (0.4-6.2) years 78 patients are alive, resulting in a probability of overall survival of 0.94 (0.88-1.00). Graft failure or delayed haematopoietic recovery was the main cause of treatment failure. Five patients (6%) experienced primary graft failure (GF). Median time to neutrophil and platelet engraftment was of 26 (10-43) and 29 (0-307) days, respectively. Three patients developed secondary GF 76, 155 and 541 days after HSCT. Eleven patients (13%) received a second allograft (7) or a stem cell boost (4) for primary or secondary GF (8) or delayed platelet engraftment (3). Four patients died of transplantation associated causes following the second allograft or stem cell boost. Chimerism analysis performed in peripheral blood revealed ≥ 95% donor haematopoiesis at all times in 65/78 (83%) patients including one patient with secondary GF and three patients with delayed platelet engraftment. In the remaining 13 patients mixed chimerism (MC) was associated with secondary GF (2) or insufficient hematopoietic recovery (3) in five whereas seven patients with MC did not experience problems. One patient with MC died due to EBV associated lymphoproliferative disease 45 days after HSCT. The cumulative incidence of acute GVHD grade II-IV and grade III-IV was 23% and 12%, respectively. Twenty one of 76 (27%) patients at risk developed chronic GVHD which was mild (n=12), moderate (n=5) or severe (n=4). Outcome was comparable for patients grafted from MSD or MUD, and male sex was the only variable associated with a significantly worse outcome.

Conclusion: In summary, the conditioning regimen with thiotepa and fludarabine offered an excellent survival for patients RCC despite a considerable risk of graft failure and delayed platelet engraftment. In some patients secondary graft failure or insufficient haematopoietic recovery was observed despite complete donor chimerism in peripheral blood.

O 11

Copy number variations and IKZF1 mutations In pediatric CML

Manuela Krumbholz[1], Josephine Tauer[2], Geertruy te Kronnie[3], Arif Ekici[4], Meinolf Suttorp[2], Markus Metzler[5]

[1] *Children's Hospital of the University of Erlangen, Germany*
[2] *University Hospital Dresden, Department of Pediatrics, Germany*
[3] *University of Padua, Lab. Hemato-Oncology, Italy*
[4] *University Erlangen-Nuremberg, Institute of Human Genetics, Germany*
[5] *Children's Hospital of the University of Erlangen, Germasny*

Background: Chronic myeloid leukemia (CML) is a rare disease in children. Clinical and molecular differences between pediatric and adult patients indicate that CML in children and adolescents is not simply a reflection of CML in the usual age group >50 years. Detailed molecular analysesof the genomic *BCR-ABL1* breakpoints in a cohort of 60 pediatric individuals revealed a different breakpoint distribution compared to adult CML. Especially the observed bimodal breakpoint distribution pattern in the *BCR* gene and a higher proportion of breakpoints within *Alu* repeat regions vary between pediatric CML and adult CML and resembles the pattern observed in adult *BCR-ABL1*-postitive acute lymphoblastic leukemia.

Methods: To identify secondary genetic variations, in addition to the *BCR-ABL1* fusion gene, high resolution whole-genome microarray analyses using Affymetrix Cytogenetics Whole-Genome 2.7M or CytoScan® HD arrays were performed in a selected sub-cohort of pediatric CML patients from the German CML-paed II trial. Twenty individuals diagnosed in chronic phase (CP) and two individuals diagnosed in blast crisis (BC) were screened. Genome wide copy numbers variations (CNVs) were analyzed in pairs; each patient's remission sample (complete cytogenetic response) was used as a reference DNA for the patient's diagnosis sample to exclude CNVs that are not somatically acquired mutations.

To further evaluate the incidence of *IKZF1* mutations and their impact on the disease progression in pediatric CML patients, deep sequencing of *IKZF1* was performed in 52 individuals in CP and 3 individuals in BC.

Results: In contrast to adult CML patients in whom about 25% of individuals in CP exhibit detectable CNVs, genomic aberrations were observed in 60% of pediatric CML patients in CP (1.9 CNVs per case). All identified CNVs were private and no recurrent genetic aberration was associated with early disease manifestation in children. No differences between patients with or without detectable CNVs could be observed with regard to age at diagnosis or therapy response.Two patients in BC showed an increased number of CNVs (6.5 CNVs per case) which is in accordance with data from adult patients, indicating that additional secondary genetic events are associated with disease progression. One of the patients in lymphoid CML-BC harbored a deletion involving the *IKZF1* gene, an aberration frequently observed in advanced disease stages in adult CML and ALL. Deep sequencing identified no additional mutation within *IKZF1* in both CML-BC patients. Interestingly, sequencing analyses of 52 pediatric patients in CP revealed a single nucleotide insertion resulting in a premature stop codon, E35 and Y348 respectively, in two individuals. One patient developed imatinib resistance 1.5 years after treatment onset. The second patient underwent stem cell transplantation 1 year after initial diagnosis.

Conclusion: In contrast to adult CML our investigations revealed an unexpected high proportion of pediatric patients with detectable CNVs (60%) in CP. This may be associated to a higher genomic instability in leukemic cells in children and adolescents. Aberrations at the *IKZF1* gene are preferentially observed in advanced disease stages or imatinib resistant patients which is in line with findings in adult CML.