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001

LONG-TERM RESULTS OF THE "PROSPECTIVE STUDY OF THE DIAGNOSIS AND TREATMENT OF MYELODYSPLASTIC SYNDROMES IN CHILDHOOD (EWOG-MDS 98)"

A. Strauss,[1] A. Yoshimi,[1] I. Baumann,[2] M. Ussowicz,[3] B. de Moerloose,[4] B. Schlegelberger,[5] M. Dworzak,[6] M. Schmugge,[7] M. Zecca,[8] A. Fischer,[1] P. Nöllke,[1] M.M. van den Heuvel-Eibrink,[9] J. Starý,[10] H. Hasle,[11] F. Locatelli,[12] C. Niemeyer[1]

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Introduction. EWOG-MDS 98 is a prospective, multi-center non-randomized study of diagnosis and treatment of pediatric MDS between 07/1998 and 06/2007. Major milestones in the course of the study were the description of myeloid leukemia of Down Syndrom as a separate entity as well as the definition of refractory cytopenia of childhood (RCC). Specification of pediatric MDS and growing understanding of its pathogenesis lead to the development of risk based treatment strategies. This study analyses long-term outcome of pediatric MDS as well as clinical, laboratory and genetic features. *Materials and Methods.* Registered were a total of 731 patients (<19 yrs.) with: primary MDS (RCC N=235, RAEB N=116, RAEB-t N=30, MDR-AML N=6), secondary MDS (therapy-related N=73, BMF N=39, others N=16), myeloproliferative syndromes (JMML N=158, JMML with Noonan Syndrome N=17, others N=4) and others (myeloid leukemia of Down syndrome N=34, AML with t(8;21) N=2, SAA N=1). *Results.* Median age at diagnosis was 7.4 years (0-18.8) with a male-female ratio of 1.47. The 10-year overall survival (OS) of all patients was 0.65. Median follow-up was 65.6 (0-170.1) months. OS of primary MDS was 0.74 (RCC 0.87 *vs.* advanced MDS 0.54, p<0.01). Primary therapy did not have an effect on survival for RCC (HSCT 0.85 *vs.* immunosuppressive therapy 0.93 *vs.* watch and wait 0.86, p=n.s.), but age at diagnosis was a significant factor for OS in RCC (<2 yrs 0.73 *vs.* 2-11yrs 0.81 *vs.* >12 yrs 0.81, p <0.01). First-hand stem cell transplantation was performed in 117 cases of RCC, 78 cases of advanced MDS and 146 cases of JMML (including 3 pts. with JMML and Noonan syndrome). OS of primary advanced MDS was 0.54 (RAEB 0.59, RAEB-t 0.51), that of secondary MDS and JMML (excluding Noonan syndrome) was 0.43 and 0.59, respectively. *Conclusions.* EWOG-MDS 98 provides an overview of current knowledge about pediatric MDS and JMML based on data of 731 patients collected in a transnational cooperation. Patients were registered systematically according to the FAB and WHO criteria, ensuring reliable data serving as groundwork for further investigations. The long-term results for RCC are excellent independent of primary therapy, but optimization in therapy is desirable for children with advanced MDS, secondary MDS and JMML.

002

MOLECULAR AND CYTOGENETIC CHARACTERISATION OF RELAPSE POST MYELOABLATIVE HAEMATOPOIETIC STEM CELL TRANSPLANTATION IN CHILDHOOD MYELODYSPLASIA AND JUVENILE MYELOMONOCYTIC LEUKEMIA

K. Pawliczak, F. Pinto, J. Chalker, G. Wright, S. Chatters, H. Kempski, S. Adams, K. Rao, J. Bartram, D. Edwards, A. Rao

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Introduction. Children with myelodysplastic syndromes (MDS) and JMML can only be cured with a haematopoietic stem cell transplant (HSCT). Sadly they face a 20-30% risk of relapse following a myeloablative conditioned (MAC) HSCT. These children often undergo second transplants which surprisingly can be curative for some. Currently there is a lack of robust clinical and molecular risk stratification criteria to identify children destined for relapse. Additionally the kinetics of relapse can vary from months to years following a MAC HSCT. Little is known about the clonal evolution of relapsing disease in myelodysplasia and JMML. Unravelling the clonal evolution of relapse offers the future potential to explore molecular and cellular targeted therapy pre and post HSCT. *Materials and Methods.* We conducted a longitudinal sequential retrospective study on a cohort of 3 children with MDS and 2 children with JMML who relapsed following MAC HSCT. Total of 10 samples were analysed. Genomic DNA was obtained from bone marrow mononuclear cells (n=5), whole bone marrow (n=5). DNA libraries were prepared using a Myeloid Sequencing Panel of 54 genes. Sequence data alignment and variant calling were performed using MiSeq Reporter Somatic Variant Caller Filtering and annotation of sequencing data was performed using the VariantStudio software with the threshold for mutation calling set at 5%. Human genome build 19 (hg19) was used as the reference. All variants identified by NGS were confirmed by Sanger sequencing. Cytogenetic analysis and chimerism studies were performed. *Results.* Table 1. Summary. Two children with JMML who relapsed post MAC HSCT had PTPN11 mutations. Despite immunomodulation, one child developed frank disease within 4 months with evidence of clonal evolution and the other child relapsed at 7 months. Two children with Refractory Cytopenia had complex cytogenetics, monosomy 7 and relapsed 2 years and 5 years respectively following MAC transplant with evidence of clonal evolution. A child with RAEB relapsed 10 months post MAC transplant with several additional clones. *Conclusions.* The clonal evolution of relapse in MDS and JMML following MAC HSCT suggests the persistence of pre-leukemic clone or the generation of new leukemic clones related to the original pre-leukemic clone but with further downstream mutations. Future studies focussing on mechanisms of relapse in MDS and JMML will broaden the focus for development of innovative therapies.

Table 1.

		Diagnosis		BMT	Relapse	
	Patient	Cytogenetics	Molecular Results (Variant Frequency)		Cytogenetics	Molecular Results (Variant Frequency)
MDS	Female 12y	45,XX,-7[5]/46,XX,-7,+21[1]/46,XX,-7,+mar[1]/ 45,XX,-7,-14,-14,-19,+mar1,+mar2,+mar3[1]/ 44,XX,-6,-7,+C,+16,-17,+18,inc[1]/ 55,XX,inc[1]/ 46,XX[2]/44,X,(random loss)[2] 81.25% Mono 7	GATA2 c.1081C>A, p.Arg361Ser (48%) GATA2 c.953C>T, p.Ala318Val (45%) SETBP1 c.2602G>T, p.Asp868Tyr (47%) ASXL1 c.1926 1927insG, p.Gly646Trpfs*12 (8%) CBL c.1259G>A, p.Arg420Gln (17%)	Bu/Cy/Mel/Camp 10/10 MUD Relapsed 2 years post transplant	45,XX,-7[1]/46,XY[121] 64.5% Mono 7	GATA2 c.1081C>A, p.Arg361Ser (25%) GATA2 c.953C>T, p.Ala318Val (25%) SETBP1 c.2602G>T, p.Asp868Tyr (24%) ASXL1 c.1926 1927insG, p.Gly646Trpfs*12 (19%) KRAS c.34G>A, p.Gly12Ser (26%)
	Male 6y	46,XY[13]/45,XY(random loss)[6]/ 45,XY,-7[1] 1.2% Mono 7	NRAS c.37G>T, p.Gly13Cys (40%) WT1 c.1110dupT, p.Val371Cysfs*14 (7%) NRAS c.181C>A, p.Gln61Lys (3%) WT1 c.1108 1109insCGGC, p.Arg370Profs*16 (4%)	Bu/Cy/Mel 10/10 MDS Relapsed 10 months post transplant	46,XY[18]/46,XX[2] (donor cells)	WT1 c.1110dupT, p.Val371Cysfs*14 (43%) NRAS c.181C>A, p.Gln61Lys (43%) WT1 c.1108 1109insCGGC, p.Arg370Profs*16 (43%) GATA2 c.207 208insTAG, p.Val70* (38%) EZH2 c.1571A>T, p.Asn524Ile (25%) PTPN11 c.182A>T, p.Asp61Val (12%)
	Female 6y	45,XY,der(1)t(1;3)(p32;q12),der(5) t(5:15)(q14;q21),-7[10] 60% Mono 7	GATA2 c.1081C>A, p.Arg361Ser (48%)	Bu/Cy/Mel/Camp 9/10 MMUD Relapsed 5 years post transplant	45,XY,der(1)t(1;3)(p32;q12), add(3)(q2?),der(5) t(5:15)(q14;q21),-7, del(13)(q21)[10] 87% Mono 7	GATA2 c.1081C>A, p.Arg361Ser (49%) WT1 c.1400_1401ins CTTGAA, p.Lys467_Thr468insAsnLeu (49%) PTPN11 c.182A>T, p.Asp61Val (45%)
JMML	Male 21 months	46,XY[20] Mono 7 Neg BCR/ABL1 Neg	PTPN11 c.182A>T, p.Asp61Val (46%) SETBP1 c.2608G>A, p.Gly870Ser (48%)	Bu/Cy/Mel/Camp 10/10 MUD Relapsed 7 months post transplant	46,XY[20] Mono 7 Neg	PTPN11 c.182A>T, p.Asp61Val (51%) SETBP1 c.2608G>A, p.Gly870Ser (49%)
	Male 17 months	46,XY[20] Mono 7 Neg Mono 5 Neg, 13% loss of Y	PTPN11 c.226G>A, p.Glu76Lys (47%)	Bu/Cy/Mel/Camp 9/10 MMUD Relapsed 4 months post transplant	45,X,-Y[19]	PTPN11 c.226G>A, p.Glu76Lys (47%)

003

Abstract not published accordingly to the authors.

004

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005

COMPARISON OF CLINICAL FEATURES BETWEEN APLASTIC ANEMIA AND REFRACTORY CYTOPENIA OF CHILDHOOD

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Introduction. The 2008 WHO classification proposed a new entity of refractory cytopenia of childhood (RCC) in children with myelodysplastic syndrome (MDS). A part of RCC cases meet the criteria of refractory cytopenia with multilineage dysplasia (RCMD) defined for adults with MDS. It is still unclear whether this WHO morphological classification reflects clinical outcomes. *Patients and Methods.* To enable diagnosis based on the WHO classification, the Japanese Society of Pediatric Hematology and Oncology established a central review system of bone marrow (BM) morphology in childhood BM failure (BMF) in February 2009. Peripheral blood and BM smears were reviewed by two pediatric hematologists, and the specimens from BM trephine biopsies and clot sections were reviewed by a hematopathologist. *Results.* From February 2009 to August 2014, 1,200 cases were prospectively reviewed. Among them, 678 were classified with BMF; 154 were classified with aplastic anemia (AA), 412 with RCC including 137 with adult-type RCMD, 43 with hepatitis-related BMF, 4 with paroxysmal nocturnal hemoglobinuria (PNH) and 65 with inherited BMF. To determine the clinical differences among AA, RCC, and RCMD, we compared laboratory and clinical data for 566 patients classified with AA, RCC excluding RCMD, and RCMD. According to the disease severity criteria for AA, 78% of the patients with AA had very severe or severe disease, whereas only 38% of the patients with RCC excluding RCMD and 28% of the patients with RCMD had very severe or severe disease ($p<0.001$). Chromosomal abnormalities were detected in 2 patients (1%) with AA (trisomy 8, n=2), 10 patients (4%) with RCC excluding RCMD (monosomy 7, n=2; trisomy 8, n=6; other, n=2), and 18 patients (13%) with RCMD (monosomy 7, n=5; trisomy 8, n=5; other, n=8) ($p=0.001$). Out of the 566 patients, 78 (AA, n=35; RCC excluding RCMD, n=37; RCMD, n=6) were treated with immunosuppressive therapy (IST) with rabbit antithymocyte globulin (ATG) and cyclosporine. Six months after the IST, the response rate to the IST was not significantly different between AA (40%) and RCC (49%). Although the number of patients was limited, the response rate to the IST was good in patients with RCMD (100%). *Conclusions.* Acquired BMF could be classified as AA, RCC excluding RCMD and adult-type RCMD on the basis of the BM morphology in children. The response rate to the IST was not different between AA and RCC. The entity of RCMD should be applied to also childhood MDS because patients with adult-type RCMD exhibited a significantly higher frequency of chromosomal abnormalities at the time of diagnosis.

006

MYELODYSPLASTIC SYNDROME OF CHILDHOOD WITH FIBROSIS: DESCRIPTION OF A NEW VARIANT OF HYPERCELLULAR MDS AND ITS DIFFERENTIATION FROM REACTIVE BONE MARROW DISORDERS WITH FIBROSIS

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Introduction and Background. Bone marrow fibrosis without excess of blasts and without underlying myeloproliferative disease is an extremely rare histologic finding in bone marrow biopsies of children and can either accompany hypercellular myelodysplastic syndrome or occur as a reactive lesion. *Materials and Methods.* Reanalysing the slides from bone marrow biopsies of 21 cases initially evaluated by the German EWOG-MDS study centre a histopathologic score was developed to define Myelodysplastic syndrome of childhood with fibrosis (MDS-F) based on the presence of at least a moderate marrow fibrosis (MF-2/3 according to WHO classification) and on the abnormalities of the megakaryocytes. In a second step the inter rater reliability of diagnostic criteria to differentiate MDS-F from reactive bone marrow conditions was analysed. *Results.* Atypia of megakaryocytes (round nucleus, binucleated cell, separated nuclei and especially micromegakaryocytes) was marked in MDS-F but not in reactive (toxic or/and inflammatory) conditions. Whereas initially 13 cases were diagnosed MDS-F by the pathologists and clinicians only 6 cases were considered MDS-F after reevaluation based on dysplasia of megakaryocytes. The overall agreement among 5 reference pathologists of EWOG MDS study was 79%, the inter rater reliability (Fleiss kappa) was 0.58. Genetic abnormalities were only present in the MDS-F group and not in reactive conditions. Patients responding to steroid therapy were only found in the non MDS group, whereas all 6 patients with MDS-F were treated with hematopoietic stem cell transplantation with matched unrelated donor. 4 of these patients died with a median survival of 10 months, 2 patients are still alive. *Conclusions.* MDS-F without blast excess is a rare variant of myelodysplastic syndrome of childhood and seems to have a worse prognosis. The atypia of megakaryocytes is a moderately reliable criterion to differentiate MDS-F from reactive bone marrow fibrosis, but final interpretation needs to incorporate clinical and cytogenetic findings.

007

IMMUNOPHENOTYPIC PROFILE OF JMML AND MDS PATIENTS FROM THE BRAZILIAN COOPERATIVE GROUP OF PEDIATRIC MYELODYSPLASTIC SYNDROME

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Introduction. Myelodysplatic syndromes (MDS) and JMML are rare in children, but enter the differential diagnosis of cytopenias of unknown origin. Multiparametric Flow Cytometry has been an useful tool to clarify the diagnosis in this context. *Objectives.* To compare the pattern of phenotypic abnormalities between MDS and JMML from children diagnosed by the Brazilian Pediatric MDS Cooperative Group that included immunophenotyping in the diagnostic work-up of the patients referred from 2012 until first quarter of 2015. *Patients and Methods.* 25 patients were analyzed: 10 JMMLs and 15 MDS. According to the Pediatric WHO Classification (Hasle.2002), 4 patients were RCC, 6 RAEB (2 with Down Syndrome), 3 RAEB-t and 2 secondary MDS (sec-MDS). Bone marrow was collected in EDTA and analysed by an 8-colors panel for the majority of patients (Euroflow). *Results.* median age was for JMML: 30 months, RAEB/RAEB-t: 7 years, secondary MDS: 19 years, RCC: 4 years. Concerning the immunophenotypic features, a decreased SSC of granulocytes was seen in 56% of the cases. Shift to the left was seen in 70% of JMMLs, 50% of RAEB/RAEB-t, 3/4 cases of RCC but not in sec-MDS. Monocytic lineage was increased in 9/10 cases of JMML but also in 3/6 RAEB/RAEBt e 2/4 RCC. No aberrant co-expressions were found in the myelomonocytic lineage. Increase in myeloid CD34+ cells was seen in 72% and abnormal coexpression of CD7 was found in 50% of RAEB/RAEB-t, 70% of JMMLs and none of RCCs and sec-MDS. B-cell precursors were decreased (corrected for age) in 100% of RCCs, 75%

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of RAEB/RAEB-t but only in 20% of the JMMLs. *Conclusions.* the immunophenotypic features of childhood MDS are similar to those described in adult patients. Hematogones were markedly decreased, even in very young patients, except in cases of JMML. Myeloid CD34+ cells were frequently increased and abnormal co-expressions had a similar frequency as in adults. So, multiparameter flow cytometry is feasible in children and may be useful not only for diagnosis, but also to examine prognostic features and to evaluate treatment response to hypomethylating agents as we have seen in the cases.

008

CLINICAL CHARACTERISTICS OF THERAPY-RELATED MYELODYSPLASTIC SYNDROME IN CHILDHOOD: A PROSPECTIVE REGISTRATION THROUGH THE JAPANESE SOCIETY OF PEDIATRIC HEMATOLOGY/ONCOLOGY

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Background. Intensive cytotoxic therapy has improved the outcome of childhood cancer, however it increases the risk of various subsequent late effects including secondary malignant neoplasms. Especially, therapy-related myelodysplastic syndrome and acute myeloid leukemia (t-MDS/AML) shows dismal outcome. The biological and clinical characteristics of t-MDS/AML have been clarified in adults but have not been fully described in children. *Patients and Methods.* From July 1999 to August 2014, 1818 children who were suspected of having MDS or bone marrow failure were prospectively registered into the Japanese Society of Pediatric Hematology/Oncology (JSPHO) database. Of those, 44 children (27 males and 17 females) who were diagnosed with t-MDS/AML were analyzed. *Results.* The median age at diagnosis of primary malignancies and subsequent t-MDS/AML was 4.7 and 9.6 years of age, respectively. Latency period from primary malignancies to t-MDS/AML varied from 0.8 to 15.3 years (median, 3.5 years). The primary malignancies were leukemia/lymphoma in 17 cases (12 acute lymphoblastic leukemia, 2 acute myeloid leukemia, 2 non-Hodgkin lymphoma and 1 Hodgkin lymphoma), solid tumor in 21 cases (10 neuroblastoma, 4 rhabdomyosarcoma, 2 hepatoblastoma, 2 Langerhans cell histiocytosis, 2 extracranial germ cell tumor and 1 osteosarcoma) and brain tumors in 5 cases (2 astrocytoma, 1 medulloblastoma, 1 intracranial germ cell tumor and 1 rhabdoid tumor). One child experienced multiple preceding malignancies (medulloblastoma and osteosarcoma). Latency period for development of t-MDS/AML was not different between hematological malignancies and solid tumors, 3.3 and 3.5 years, respectively, however, 2 patients who had astrocytoma showed long latency period (12.4 and 13.5 years). Most children showed mild pancytopenia and blasts were rarely detected in the peripheral blood (PB). According to the percentage of blasts in the PB and bone marrow, patients were classified as refractory cytopenia in 16, refractory anemia with excess blasts in 18, and AML with myelodysplasia-related chenges in 7. Three patients were diagnosed with chronic myelomonocytic leukemia. Structurally complex karyotype and abnormalities of chromosome 7 was the most common cytogenetic abnormalities. *Conclusions.* Despite of an attempt to restrict the use of topo-II inhibitors and radiotherapy, t-MDS/AML is still obstacle to childhood cancer survivors. Unravelling the genetic background of this dismal complication is mandatory.

009

CRITERIA FOR EVALUATING RESPONSE AND OUTCOME IN CLINICAL TRIALS FOR CHILDREN WITH MYELODYSPLASTIC SYNDROME AND JUVENILE MYELOMONOCYTIC LEUKEMIA

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Introduction. Based on the advanced understanding of molecular pathogenesis of myelodysplastic syndrome (MDS) and juvenile myelomonocytic leukemia (JMML), the development of potential novel agents is recently expanding. While hematopoietic stem cell transplantation remains the only curative therapy, children with these disorders increasingly receive such drugs in phase I-II clinical trials as pre-transplant therapy or therapy for relapse after transplantation. Therefore, the standardized response criteria for non-transplant therapy in children with MDS and JMML became necessary. Response criteria for MDS in childhood: The revised International Working Group response criteria in myelodysplastic syndrome (IWG-MDS, Cheson 2006) have been used widely in clinical trials in adults with MDS. Because of the difference in normal blood counts, clinical presentation, pathogenesis and treatment strategies between children and adults, some modifications are necessary. We propose modified IWG-MDS response criteria for children keeping structures and concepts of revised IWG-MDS, but adjusting such as erythroid response criteria according to ages of patients. Response criteria for JMML: Response criteria for standardized evaluation of treatment efficacy in patients with JMML were lacking. Therefore, we recently proposed the response criteria for JMML (Niemeyer, Haematologica, 2015). For the evaluation of non-transplant therapy, we defined 6 clinical (white blood cell count, platelet count, hematopoietic precursors and blasts in peripheral blood, bone marrow blast percentage, spleen size and extramedullary disease) and 3 genetic variables (cytogenetic, molecular and chimerism response) which serve to describe the heterogeneous picture of response to therapy in each individual case. For each of these variables (v), complete response (vCR), partial response (vPR) and progressive disease (vPD) were defined. Based on the cumulative response of these 9 variables, the clinical and genetic remission statuses are described. *Conclusions.* The international experts of MDS and JMML discussed to reach agreement on the response criteria for JMML and MDS. It is hoped that these criteria will facilitate the comparison of results between clinical trials in MDS and JMML in children.

010

GENETIC BACKGROUND OF IDIOPATHIC BONE MARROW FAILURE SYNDROMES IN CHILDREN

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Introduction. Appropriate classification of bone marrow failure syndromes in children is challenging, particularly with respect to the histological distinction between aplastic anemia (AA), refractory cytopenia of

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childhood (RCC), and refractory cytopenia with multilineage dysplasia (RCMD). Inherited bone marrow failure syndromes (IBMFS) further defy accurate diagnosis. In adult patients with AA, somatic mutations were frequently detected in myeloid malignancy-related genes such as DNMT3A, BCOR, and ASXL1. We aimed to characterize the genetic background of childhood AA/RCC/RCMD. *Patients and Methods.* We studied 168 patients with childhood idiopathic AA/RCC/RCMD. Target sequencing (n=168) was performed for 88 IBMFS-associated genes and 96 myeloid malignancy-related genes. Furthermore, whole-exome sequencing (WES, n=25) was performed with matched tumor/normal samples. *Results.* Only one germline mutation that was diagnostic of IBMFS was detected in our cohort (0.6%). It was an RTEL1 mutation, which supported the diagnosis of dyskeratosis congenita. WES, performed in 25 patients, detected only three somatic mutations, all of which affected BCOR. In target sequencing, 20 somatic mutations were detected in 19 patients (11.3%). BCOR (n=9) and PIGA (n=4) were recurrently mutated. The mutational frequency of DNMT3A and ASXL1 was very low (0.6%) in our cohort and was clearly different from that of an adult cohort. The difference in the frequency of somatic mutations in AA, RCC, and RCMD was not statistically significant (p=0.49). However, with regard to the mutated genes, two patients with RCMD carried U2AF1 plus SETBP1 and TP53 mutations, respectively, which are well-known predictors of poor prognosis in adult MDS. *Discussion.* In our cohort of children who were clinically diagnosed with AA/RCC/RCMD, the frequency of cryptic IBMFS was considered low. Furthermore, the frequency of the detectable somatic mutations in childhood AA was low compared with that in adult AA. No novel mutational target was identified with WES. Idiopathic bone marrow failure syndromes in children were characterized by a paucity of gene mutations irrespective of the histopathological classification. Mutations in adult MDS-related genes suggest a different form of molecular pathogenesis from other patients with RCMD. In conclusion, our study clarified the yet unrevealed genetic background of idiopathic bone marrow failure syndromes in children.

011

TARGET CAPTURE NEXT GENERATION SEQUENCING AS A MOLECULAR SCREENING SYSTEM FOR PEDIATRIC BONE MARROW FAILURE SYNDROMES

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Background. Precise classification of bone marrow failure syndromes in children is challenging but essential for appropriate clinical decision making. Differential diagnosis for congenital bone marrow failure syndromes (CBMFSs) from acquired aplastic anemia is very important to avoid ineffective immunosuppressive therapy and/or lethal complications of allogeneic hematopoietic stem cell transplantation. Significant advances in genomics were achieved by next-generation sequencing. It has influenced every field of medicine; bone marrow failure syndromes are of no exception. A large panel of newly identified causative genes of CBMFSs have been identified in the recent years; therefore, it is virtually impossible to establish a routine genetic diagnostic testing using conventional Sanger sequencing. *Patients and Methods.* To clarify the diagnostic efficacy of target sequencing with a custom capture bait, we performed target in 110 patients with CBMFSs. Target capture was performed using a custom bait (SureSelect®, Agilent Technologies), covering genomic lesions for >200 genes that are known to be mutated in CBMFSs and associated hematological disorders such as myelodysplastic syndromes and primary immunodeficiency disorders, followed by massively parallel sequencing using HiSeq 2000 (Illumina). *Results.* Using our established pipeline for massive parallel sequencing analysis (genomon: http://genomon.hgc.jp/exome/), we detected 1-10 candidate variants per patient. Diagnoses were based on variants with pathogenicities confirmed by published studies. Genetic diagnoses were possible in 49 patients (45%). The best efficacy was achieved in patients with Fanconi anemia (15/18, 83%). Encouraging results were obtained in patients with Schwachman-Diamond syndrome (4/6, 67%), and dyskeratosis congenital (5/11, 45%). Four genetic diagnoses (4%) were inconsistent with their corresponding clinical diagnoses, possibly because of overlaps in disease phenotypes. *Conclusions.* Compared with conventional genetic testing, target sequencing with next generation sequencer was proved to be effective in diagnosing CBMFSs.

012

OUTCOMES OF STEM CELL TRANSPLANTATION WITH FLUDARABINE AND MELPHALAN CONDITIONING FOR CHILDREN WITH ACQUIRED BONE MARROW FAILURE: A NATIONWIDE RETROSPECTIVE STUDY

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Background. Recently, we have experienced a certain number of children with acquired bone marrow failure (aBMF) who presented with bone marrow aplasia with full donor chimerism after stem cell transplantation (SCT). We named the complication "donor-type aplasia", and showed the association of it with fludarabine (FLU)/cyclophosphamide (CY)-based regimen which is one of the standard conditionings for aBMF. To reduce the risk for donor-type aplasia, the conditioning regimen for children with aBMF needs to be reconsidered. The purpose of the present study is to evaluate the outcomes of SCT using FLU/melphalan (MEL)-based conditioning instead of the standard FLU/CY-based regimen in children with aBMF. *Patients and Methods.* We retrospectively reviewed the clinical data of 603 patients (<16 years) with aBMF (aplastic anemia and refractory cytopenia of childhood) who received the first SCT from 2000 to 2013 in the JSHCT Registry. Totally, 49 patients received the FLU/MEL-based regimen. Of the 49, 21 patients received SCT from a related donor, whereas 28 received it from an unrelated donor. The stem cell source was bone marrow (BM) in 36 patients, peripheral blood (PB) in 1, or cord blood (CB) in 12. The conditioning regimen was based on FLU (100-180 mg/m^2) and MEL (70-180 mg/m^2), and ATG was included in the regimen in 29 patients, while low dose irradiation was used in 38 patients. *Results.* The 5-year overall survival and event free survival (EFS) of patients who received the FLU/MEL-based regimen was 91%. Engraftment was achieved in 98% of patients and secondary graft failure including donor-type aplasia was not observed. Notably, all patients who received bone marrow transplantation (BMT) were alive without any complication. We then compared the outcomes in the setting of BMT with the FLU/MEL-based regimen (n=36) to those with the FLU/CY-based regimen (n=270). The EFS was inferior in patients treated with the FLU/CY-based regimen, although this difference was not statistically significant (86% *vs.* 100%; P=0.07). With the FLU/CY-based regimen, engraftment was achieved in 98% of patients, whereas secondary graft failure including donor-type aplasia was seen in 21 patients. *Conclusions.* The FLU/MEL-based regimen provided excellent outcomes especially in the setting of BMT. Given this, a prospective study on SCT with this conditioning for aBMF children with high risk of donor-type aplasia is now planned by the Japan Childhood Aplastic Anemia Study Group.

013

MOLECULAR CHARACTERIZATION AND PHENOTYPE DELINEATION OF DBA PATIENTS WITH LARGE MICRODELETIONS ENCOMPASSING RIBOSOMAL PROTEIN GENES

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Introduction. Diamond-Blackfan anemia (DBA) is a congenital disease of ribosomal biogenesis. Its main features are severe macrocytic anemia with reticulocytopenia frequently accompanied by diverse syndromal anomalies. Typical presentation time is at young age, mostly during 1st year of life. Mutations of genes coding for ribosomal proteins (RP) have been described previously in ~50% of all patients (RPS19, RPL5, RPL11, RPS26, RPL35a, RPS24, RPS17, RPS10, RPS7 and RPL26) and the transcription factor GATA1. Additionally, recent studies confirmed recurrent large microdeletions affecting the genes RPS17, RPS19, RPS26, RPL5, RPL15 and RPL35a in patients with DBA. *Patients and Methods.* We investigated a group of 90 patients, who have previously tested negative for mutations in 12 known DBA-associated genes. SNP array, High Resolution CGH array and Quantitative PCR methods were utilized to determine Copy Number Variations (CNVs) within RP genes. Subsequently, a polysome profiling technique was utilized to confirm impaired ribosomal biogenesis. Finally, we conducted a chart review of DBA patients to identify clinical and phenotypic attributes associated with microdeletions. *Results.* Microdeletions in RP genes were identified in total 20 DBA patients. The most commonly affected gene was RPS17 with deletions in six cases, followed by loss of RPL5 in 5 cases (including one familial case) and RPS19, RPL11 and RPL35a with 3 cases respectively. 3 of 20 DBA patients with RP gene CNVs presented with intragenic deletions in RPL35a, RPS19 and RPL35a. Polysome profiling was eligible to confirm haploinsufficient protein synthesis in all of 11 examined patient's samples, and RP RNA content was lower in cases with deletion of respective RP gene. Strikingly, 89% (16/18) of evaluable deletion patients presented with at least one congenital anomaly Features not typical for classical DBA were often present, including neurostructural anomalies in 6/18 (33%) of evaluable cases, impaired mental or motor development in 9/18 (50%) of patients, and severe early onset anemia at birth in 9/18 (50%) of cases. In one case the deletion was inherited, while in all other index cases it occurred de novo and there were no silent carriers. *Conclusions.* Microdeletions occur frequently in DBA, therefore a test for CNVs should be included in the diagnostic process. RP gene deletions are commonly associated with non characteristic symptoms for DBA, most likely in context of a continuous gene syndrome.

014

PEARSON SYNDROME: MULTISYSTEM MITOCHONDRIAL DISORDER WITH BONE MARROW FAILURE

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Introduction. Pearson syndrome (PS) was originally reported as a fatal disease in infancy characterized by sideroblastic anemia with vacuolization of marrow precursors and exocrine pancreas dysfunction. This disease is caused by the single mitochondrial DNA (mtDNA) deletions. There are few systematic studies on PS because of its rarity. *Patients and Methods.* We retrospectively reviewed clinical and laboratory data of 20 patients (12 M/ 8 F) with PS diagnosed between 1987 and 2012 in Germany. *Results.* The median age of onset was 5 (23-31) months. Patients presented with anemia (n=20), failure to thrive (n=5), diarrhea (n=1), malformation (omphalocele and esophageal atresia: n=1) and mild hypotonia (n=3). Twelve patients had only anemia. The median hemoglobin level, neutrophil and platelet counts were 5.9 (2.2-9.8) g/dl, 0.9 (0.1-2.4) G/L and 116 (31-300) G/L, respectively. The MCV, hemoglobin F level and serum lactic acid were elevated in 4/12, 8/ 9 and 12/15 patients examined, respectively. All patients had vacuolization of erythroid/myeloid precursors in bone marrow, but ringed sideroblasts are observed only in 13/19 patients examined. The diagnosis of PS was confirmed by the detection of mtDNA deletion in all patients. The median age at the last follow-up was 48 months (7 months to 15 years). Ten patients showed hematological recovery at a median age of 27 (11 to 67) months. Various complications developed during clinical courses; failure to thrive (n=13), liver dysfunction (n=4), renal tubulopathy (n=5), pancreas insufficiency (n=6), cardiac disease (n=5), diabetes (n=1), other endocrine dysfunction (n=4), hearing loss (n=1), ophthalmoplegia (n=1), retinitis pigmentosa (n=1), cataract (n=3), muscle hypotonia (n=7), ataxia (n=2) and encephalopathy (n=1). Ten patients died at the median age of 49 (14-183) months. The main cause of the death was acute metabolic acidosis. Two long survivors died of arrhythmia. *Conclusions.* The findings in this largest study suggest that PS is a multisystem mitochondrial disorder in early childhood with bone marrow failure as the main presenting feature. Because anemia can be initially the only symptom, PS should be considered as a differential diagnosis in small children with anemia. Although hematological improvement can be expected in most long survivors, patients develop varied organ involvements. Therefore, intensive monitoring and managing of multisystem complications are crucial.

015

THE LONG NON-CODING RNA H19 IS REGULATED BY LIN28B

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Introduction. LIN28A and LIN28B are RNA-binding proteins highly active during embryonic development. Recently, we identified high LIN28B expression in a fetal-like subgroup of juvenile myelomonocytic leukemia (JMML), which was associated with higher HbF levels, worse overall survival, less monosomy 7 and a fetal stem cell-like gene expression signature. In order to further characterize the role of LIN28B in leukemic development, we modulated LIN28B expression in human and mouse hematopoietic cells. *Materials and Methods.* Stable cell line models for overexpression and knockdown of LIN28B were generated. To this end, Oci-AML3 cells without endogenous LIN28B expression were stably transduced with a retroviral plasmid that drives LIN28B expression or an empty vector control. In contrast, shRNA mediated knockdown was performed in K562, a myelogenous leukemia cell line with high endogeneous LIN28B expression levels. Both cell line models and their respective controls were hybridized in triplicate using a custom designed Agilent gene expression microarray. Leukemic cell RNA of 44 patients with JMML was previously profiled on the same platform. LIN28B and empty control vectors were inserted in murine embryonic stem (ES) cells and cultured in vitro to form embryoid bodies. Vav-Lin28b mice were generated having ectopic Lin28b overexpression in the hematopoietic compartment. *Results.* Gene expression profiling revealed 5065 RNA probes differentially expressed between LIN28B high (N=25) and low (N=19) patients, 4575 between the LIN28B overexpressing Oci-AML3 cells and control cells, and 6804 probes between the LIN28B knockdown K562 cells and control cells. The intersection of all three analyses contained 58 probes, of which 11 hybridized to the H19 long non-coding RNA (lncRNA). H19 showed a 4-fold up or down regulation after LIN28B overexpression or knock-

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down, respectively. Similarly, enforced expression of human LIN28B in murine ES cells resulted in up regulated H19 expression. Finally, Vav-Lin28b mice have higher H19 levels than their wild type littermates. *Conclusions.* Our results pinpoint to a role for LIN28B as a regulator of H19, further expanding the network of molecules regulated by LIN28B to lncRNAs. Noteworthy, H19 plays an essential role in fetal hematopoeisis, is involved in a negative feedback loop with let-7 (as is LIN28B) and was one of the first discovered lncRNAs. Currently, we are conducting further functional studies to decipher the role of H19 in JMML.

016

HSC-INDEPENDENT YOLK SAC PROGENITORS BEAR HALLMARKS OF JMML IN A PTPN11-D61Y MOUSE MODEL

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Background and Introduction. Juvenile Myelomonocytic Leukemia (JMML) is a fatal pediatric myeloproliferative neoplasm (MPN) that presents with monocytosis, anaemia, and hepatosplenomegaly. The only curative therapy for JMML is allogeneic hematopoietic stem cell (HSC) transplantation. Even so, 50% of treated patients will relapse and succumb to their disease. Recent studies have shown HSC-independent yolk sac progenitors persist in adulthood as tissue macrophages and are uniquely resistant to myeloablation. As such, their involvement in the pathogenesis of JMML could explain the high relapse rate following HSC transplantation. Given these findings, we assessed embryonic hematopoietic progenitors for hallmarks of MPN in a mouse model of JMML. *Materials and Methods.* Using the Vav1 Cre recombinase, we generated a mouse model of JMML that expresses the PTPN11-D61Y gain of function mutation in both embryonic and adult hematopoiesis. We used methylcellulose colony assays, western blotting, and flow cytometry to measure the two defining features of JMML: i) growth hypersensitivity to the cytokine GM-CSF, and ii) hyperactive Ras-Erk signaling. We performed our analyses using both HSC-dependent E14.5 fetal livers and HSC-independent E9.5 yolk sac samples. *Results.* PTPN11-D61Y/+; VavCre+ mice are viable, born at expected Mendelian ratios, and develop monocytosis as early as 4 weeks of age. HSC-dependent E14.5 fetal liver progenitors showed growth hypersensitivity at all measured doses of GM-CSF ($p<0.05$, n=7) and possessed hyperactive Ras-Erk pathway signaling. Intriguingly, HSC-independent E9.5 yolk sac progenitors from PTPN11D61Y/+; VavCre+ embryos also demonstrated marked GM-CSF hypersensitivity ($p<0.05$, n=8). Furthermore, macrophages derived from yolk sac progenitors possessed hyperactive Ras-Erk signaling by intracellular flow cytometry using antibodies against pErk and pSTAT5 ($p<0.05$, n=6). Finally, we purified erythro-myeloid progenitors (Ter119-, cKit+, CD41DIM) from yolk sacs and showed this population to be responsible for the observed MPN features. *Conclusions.* We have shown that HSC-independent yolk sac progenitors possess GM-CSF hypersensitivity and Ras-Erk pathway hyperactivation in a mouse model of JMML. These findings suggest that HSC-independent macrophages may be involved in the development of JMML in utero, and they highlight the potential need to develop therapeutics that can specifically target this myeloablation-resistant population.

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019

MUTATIONAL SPECTRUM OF JUVENILE MYELOMONOCYTIC LEUKEMIA

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Juvenile myelomonocytic leukemia (JMML) is a myeloproliferative neoplasm of early childhood. It is currently regarded as a RASopathy, as in 80-85% of the patients aberrations in RAS-associated genes can be found. The mutational background of the remaining 15-20% of JMML patients remains undefined. Here we investigate whether subclonal aberrations of RAS-pathway associated genes, or recurrently affected genes in de novo acute myeloid leukemia (AML), might be found in these patients. 73 JMML patients were included. From 31 patients, the mutational status of PTPN11, NRAS, KRAS, CBL and NF1 was known. From all patients, genomic DNA was isolated from the mononuclear cell fraction from either bone marrow (n=64) or peripheral blood (n=9), taken at time of diagnosis. Quality and quantity of the material was assessed using QuantIT. The DNA was subsequently sequenced using the TruSight Myeloid sequencing panel on the MiSeq platform. Aberrations were called and annotated with an in-house developed pipeline, and mutations found in more than 20% of the total reads were validated using Sanger sequencing. The MiSeq sequencing identified all previously reported mutations in the population of patients for which mutational status of PTPN11, NRAS, KRAS, CBL and NF1 was known. In addition, next generation sequencing allowed us to identify subclonal RAS aberrations in 42 patients for which Sanger sequencing did not reveal any mutations in RAS-associated genes. Interestingly, we also identified mutations in several de novo AML genes previously not described to be mutated JMML patients. . Correlation of the number of aberrations found and clinical outcome revealed that the presence of two or more aberrations is associated with an unfavorable prognosis. Taken together, next generation sequencing revealed mutations in around 90% of the sequenced patients. Thus, our study suggests that next generation sequencing should be the preferred technique over Sanger sequencing to assess the mutational burden of JMML patients at time of diagnosis. Furthermore, the occurrence of gene aberrations outside the RAS-pathway challenges the current idea of JMML as a RASopathy. These new findings might provide new therapeutic strategies in the future. Finally, the assessment of the mutational burden can provide insight into an eventual unfavorable outcome for the patient, and could be used to prevent relapse of the disease at an early stage.

020

IDENTIFICATION OF DEREGULATED MICRORNAS IN JUVENILE MYELOMONOCYTIC LEUKEMIA

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Introduction. Juvenile Myelomonocytic Leukemia (JMML) is an aggressive leukemic form of infancy/early childhood caused by gene mutations in the stem cell compartment, which lead to excessive proliferation of cells of the myeloid lineage. About 90% of patients show mutations of either PTPN-11, N/K-RAS, CBL, or NF1, which are mutually exclusive but converge on the Ras/MAPK signaling. Allogeneic hematopoietic stem cell transplantation (HSCT) is the treatment of choice for most JMML patients. microRNAs (miRNAs) are noncoding RNAs that govern stem cell differentiation in embryo and are often deregulated in leukemia. However, they have never been analyzed in JMML. Here we evaluate the expression levels of miRNAs in patients

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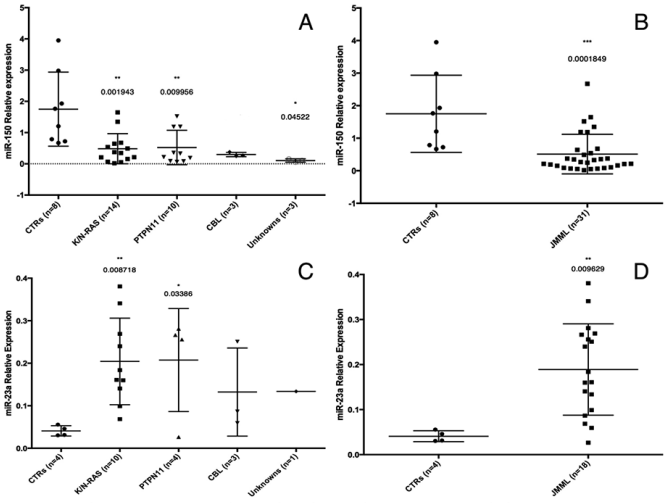
with JMML, and assess the modulation of putative target genes to identify novel potential therapeutic approaches. *Patients and Methods.* We analyzed miRNAs expression profile of 48 Bone Marrow samples (8 Healthy Controls and 40 Patients' Samples) using Nanostring technology (Ncounter Human v2 miRNA Expression Assay) covering almost 800 miRNA sequences. We included PTPN-11, N/K-RAS, CBL and Non-mutated patients' samples. We performed Hierarchical Clustering Analysis and built the distance matrix using Euclidean method. We validated the most relevant miRNAs with RT-qPCR and the expression of an identified target gene by western blot. *Results.* MiRNA profiling analysis showed several deregulated miRNA in JMML samples *vs* controls, among which miR-150-5p, miR-148a-3p, miR-26a-5p and miR-29b-3p as significantly downregulated (-2,4; -1,2; -1; -0,96 Fold-Change (Log2), respectively), and miR-23a-3p, miR-575 e miR-630 significantly upregulated (0.7, 1.4 e 2.3, Fold-Change (Log2), respectively) (P-Value <0,05). Validation with RT-qPCR confirmed miR-150-5p and miR-23a-3p deregulation (Figure 1), while the difference for the other miRNAs did not reach statistical significance. An in silico study for putative targets predictions of these miRNAs identified genes linked to leukemia such as MYB (already validated), FLT3 e STAT5 for miR-150-5p and MEIS1/2 for miR23a-3p. We then noticed the up-regulation of the total protein form of STAT5 in three JMML samples compared to control ones. *Discussion.* Our study identifies for the first time differentially expressed miRNAs in JMML, such as miR-150-5p and miR-23a-3p and suggest disease-specific putative new targets. Further studies are ongoing to identify the role of these miRNAs in this aggressive leukemic form.



miR-150 Relative Expression for each subset of JMML patients belonging to different mutational profiles and Healthy Controls (A) or for the pooled JMML patients' *vs* Healthy Controls (B). Relative expression is also reported for miR23a-3p in (C) and (D). *P*-values are on top of each JMML patient set (two tailed *t-test*) and are representative of every comparison against Healthy Controls.

Figure 1.

021

EXOME ANALYSIS IN JUVENILE MYELOMONOCYTIC LEUKEMIA

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Introduction and Background. Juvenile myelomonocytic leukemia (JMML) is a pediatric leukemia with poor prognosis. It is characterized by somatic or germline mutations in the PTPN11, NRAS, KRAS, NF1 and CBL genes of the Ras signaling pathway, but outside Ras signaling the molecular understanding of the disease is limited. Here we report on the ongoing Exome sequencing analysis of a large cohort of JMML patients. *Materials and Methods.* Tumor material of 75 JMML patients was collected and subjected to whole exome deep sequencing. In addition, for 31 patients of the cohort paired material from fibroblasts, hair follicles or buccal swab was collected and sequenced as germline controls under the same protocol as the tumor samples. A filtering scheme was employed in order to assess the importance of novel mutations. A network-based stratification that maps and diffuses out individual patient mutations allowed for a comparison of the mutational effects with respect to their similarity in pathway impact. *Results.* A total of ~22000 mutations were kept after filtering by checking against several human genetic variation databases and were identified as rare and of either somatic or inherited nature. All known Ras pathway mutations could be confirmed in the exome data. The patient-wise mutatomes were quite diverse and did not match up to the current mutational classification according to Ras-related genes. Most tumor samples were characterized by a notable sparsity of secondary mutations. Several identified novel mutations were assessed to have a deleterious effect on the protein level in both oncogenes and tumor suppressor genes. Network stratification identified highly linked mutation clusters around the Ras pathway being related to Notch and EGF signaling as well as cell adhesion and regulation of apoptosis. *Conclusions.* In conclusion, mining the recorded mutations will provide new insight into gene pathway associations. Further comparison with germline material will narrow down driver mutations and pathways and will possibly link the mutations closer to the epigenome and phenotype.

022

CBL SYNDROME AND THE ASSOCIATED CLINICAL COMPLICATIONS

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Introduction. Predisposition syndromes for juvenile myelomonocytic leukemia (JMML) include the recently described CBL syndrome. The CBL syndrome is caused by a heterozygous germline CBL mutation. Our aim was to systematically review CBL syndrome, the myeloproliferative disease (MPD) which can be associated with CBL syndrome and other associated complications. *Patients and Methods.* We retrospectively reviewed the clinical data of patients diagnosed with MPD and CBL mutation, registered to the EWOG-MDS studies between 1991 and 2015. Patients were classified into 4 groups: Germline CBL mutation with (A) and without (B) loss of heterozygosity (LOH) in somatic cells, (C) germline CBL mutation without LOH and a second mutation within the RAS/MAPK pathway, and (D) somatic CBL mutations. Patients of groups A (n=23) and B (n=5) were analyzed. *Results.* The 28 patients (15 M/13 F) had a median age at MPD diagnosis of 13 (1-61) months. Clinical and laboratory features at diagnosis were splenomegaly (n=27), leukocytosis (n=22), and monocytosis (n=28). The following Noonan-like phenotypic features were observed: Developmental delay (n=14), facial dysmorphic features (n=9), growth retardation/failure to thrive (n=9), café au lait spots/juvenile xanthogranuloma (n=9), bleeding complications (n=8), cardiac defects (n=3), hearing loss (n=1) and cryptorchidism (n=2). In group A with LOH (n=23), 10 patients received HSCT: 6 patients are alive and 4 of suffered treatment related mortality or died from progression of disease. There were no reports of vasculopathy after HSCT. Thirteen patients did not receive HSCT: 3 patients died of disease progression or other clinical complications; of the remaining 10 patients, 4 patients developed signs of vasculopathy: opticus atrophy (n=4), hypertension (n=2), acquired

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hypertrophic cardiomyopathy (n=2), one patient died of Takayashu arteritis. In group B without LOH all patients are alive (n=5), 4 patients received HSCT. *Conclusions.* This study shows that patients with CBL syndrome and MPD can have a stable clinical course in the absence of HSCT. However, our results suggest that patients with CBL syndrome, MPD and LOH are at risk of developing vasculopathy and may need long-term close monitoring. Interestingly, there were no patients with vasculopathy following HSCT. Further studies of the pathogenesis of vasculitis and the role of HSCT in patients with CBL syndrome and MPD are necessary.

023

FIRST ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR JUVENILE MYELOMONOCYTIC LEUKEMIA: DATA FROM THE JSHCT

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Background. Juvenile myelomonocytic leukemia (JMML) is a rare hematological malignancy in young children that is classified into the category of myelodysplastic/myeloproliferative neoplasm. Stem cell transplantation (SCT) is currently the only curative treatment for JMML, but few large studies on SCT exist because of rarity of the disease. *Patients and Methods.* We analyzed the outcomes of SCT for JMML using data from a nationwide transplantation registry of the JSHCT. From 1990 to 2011, 172 children (112 boys and 60 girls) with JMML received first SCT from a related (n=78) or an unrelated (n=94) donor with the stem cell source of bone marrow (n=124), peripheral blood (n=15), or cord blood (n=33). Median age at diagnosis was 20 months (range, 0-144 months). Cytogenetic abnormalities were found in 34% of patients, including 16% with monosomy 7. *Results.* The 5-year overall survival (OS) rate and cumulative incidence of relapse (RI) were 63% and 37%, respectively. Multivariate analysis revealed that chronic GVHD and normal karyotype were associated with lower relapse (hazard ratio [HR] 0.35; P=0.007, HR 0.46; P=0.014) and better survival (HR 0.20; P<0.001, HR 0.41; P=0.008). Regarding conditioning regimen, non-irradiation regimen was also identified as a favorable factor for survival (HR 0.37; P=0.006), and SCT following conditioning with busulfan, fludarabine and melphalan provided the best outcome (n=59, the 5-year OS and RI: 76% and 26%, respectively). Notably, all patients with chronic GVHD who received this regimen were alive (n=18). *Conclusions.* The current study reveal that a significant proportion of children with JMML can be cured with SCT, especially when receiving the conditioning regimen consisting of busulfan, fludarabine and melphalan. And an ongoing prospective study, JPLSG-JMML11, will confirm the favorable outcome. With lower relapse and better survival observed in patients with chronic GVHD, additional treatment strategies that focus on enhancing the graft-*versus*-leukemia effect may further improve survival.

024

OUTCOMES OF ALLOGENEIC STEM CELL TRANSPLANTATION IN PEDIATRIC PATIENTS WITH MYELODYSPLASTIC SYNDROME AND BONE MARROW FAILURE DUE TO GATA2 HAPLOINSUFFICIENCY

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Introduction. Germline mutations in GATA2 have been reported in a subset of pediatric myelodysplastic syndromes (pMDS) and bone marrow failure (BMF). Hematopoietic stem cell transplantation (HSCT) is the only curative therapy for pMDS, but studies on outcomes of patients with GATA2 mutations are limited. Here we characterize the unique phenotype of pMDS due to GATA2 mutations and describe outcomes after HSCT. *Methods.* We sequenced GATA2 on samples of 103 patients with molecularly undefined MDS and BMF. Data analysis included pathology review, clinical and laboratory data, pre-HSCT therapies, organ dysfunction, conditioning regimen, GVHD prophylaxis, transplant related toxicity (TRT) and mortality (TRM) and relapse. *Results.* We identified 14 patients (male 11, female 3) with a pathogenic GATA2 mutation. The median age at diagnosis was 15 years. The diagnoses were RCC (n=7), RAEB/AML (n=4) and ALL (n=1). We observed a spectrum of reported and novel phenotypes such as hydroceles, polyneuropathy, thrombosis, kidney abnormalities and tinea versicolor. A distinctive megakaryocytic dysplasia was observed. Monosomy 7 was the most common cytogenetic abnormality (n=7). Twelve patients underwent HSCT with a median follow up of 4 years. Three patients received AML therapy prior to HSCT. Conditioning regimens consisted of cyclophosphamide (CY)/total body irradiation (TBI) (n=7), CY/TBI/ATG (n=2), fludarabine/CY/TBI (n=2) and busulfan/CY (n=1). Donors were matched sibling (MSD) (n=1) and matched unrelated donors (MUD) (n=11) with cell sources from marrow (n=8) or umbilical cord blood (n=4). GVHD prophylaxis consisted of cyclosporine A (CSA)/MTX+/- prednisone (n=9) or CSA/ mycophenolate mofetil (n=3). Seven out of 12 patients are alive. One patient died from relapse and 4 had TRM due to respiratory failure, infections or chronic GVHD. Eight patients developed GVHD. Post-HSCT complications were necrotizing fasciitis, thrombotic events, recurrent clostridium difficile, leukencephalopathy, severe gastrointestinal bleeding, secondary cancers and lung transplant. *Conclusions.* MDS due to GATA2 mutation shows a heterogeneous presentation, unique pathology and high rate of monosomy 7. HSCT is a curative therapy and most successful prior to leukemic transformation. We saw a low relapse rate but moderate TRM, therefore a reduced intensity conditioning approach might be considered in future prospective trials. Given the implications GATA2 mutation testing should be performed on all patients with molecularly undefined MDS and BMF disorders and potential related donors.

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POSTERS

P01

EVALUATION OF BONE MARROW TREPHINE *VERSUS* BONE MARROW ASPIRATION SMEARS IN DIAGNOSIS OF PEDIATRIC MYELODYSPLASTIC SYNDROMES: PRELIMINARY RESULTS OF A STUDY FROM THE MORPHOLOGICAL BOARD OF THE AIEOP GROUP

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The diagnosis of MDS is primarily based on the presence of dysplasia in blood and marrow cells on smears. MDS in childhood is often characterized by hypoplastic bone marrow aspiration (BMA) and bone marrow trephine (BMT) can help distinguish MDS from non-clonal cytopenia. The diagnosis of MDS often is challeging requiring a multiple approach. Currently, there are no data regarding the use of the bone marrow aspiration and bone marrow trephine for MDS diagnosis. *Aims.* To improve the morphological/histological diagnosis of childhood MDS and to obtain criteria useful for differentiating hypoplastic forms of childhood MDS from non-clonal hypoplasia or aplastic anemia. In particular, we compared BMA and BMT on: 1) Evaluation of the grading of cellularity; 2) Dysplasia, with particular attention paid to the erythroid and megakaryocytic lineages; 3) Percentage of myeloblasts. *Methods and Results.* Between 2011 and 2014, we analyzed bone marrow smears and trephines of 32 patients (pts) with suspected MDS; the diagnoses were as follows: 5 cases of SDS, 15 RCC, 6 RAEB2, 3 cases of JMML and 3 unspecified MDS. The cases were centralized in the laboratories of Padua (morphology) and Rome (histology) to confirm the diagnosis. The aspects of dysplasia present in the bone marrow smears and bone marrow biopsy were evaluated according to the criteria proposed by the WHO classification (2008). The dysplastic features of each haematopoietic lineages were quantified from 0 to 4, while the cellularity was assessed as follows: aplastic (0), hypocellular (1) normocellular (2), hypercellular (3). Cellularity was reduced in 53% of cases both on morphological and histopathological evaluation. Reduced megakaryocytes were observed in 78% of pts, with concordant results between morphological and histological evaluation. Agreement on the percentage of blasts in bone marrow smears and bone marrow biopsy was found in RAEB. Moderate or severe dyserythropoiesis, was found in 31.2% of pts at morphological evaluation of smears, whereas it was found at the evaluation of bone marrow biopsy in all 32 cases analyzed, this finding confirming that dyserythropoiesis is the most relevant morphological criterion for performing a diagnosis of childhood MDS. *Conclusions.* Our study, although based on preliminary results, suggest that an integrated approach of both morphological smear evaluation and of the bone marrow trephine findings is instrumental for a reliable confirmation of a diagnosis of childhood MDS.

P02

p53 IS A USEFUL DISCRIMINATOR OF SEVERE APLASTIC ANEMIA FROM REFRACTORY CYTOPENIA OF CHILDHOOD

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The differentiation of aplastic anemia (AA) from refractory cytopenia of childhood (RCC) can be challenging. The aim of our study was to evaluate p53 protein expression as a diagnostic marker between different types of MDS and especially as a possible marker differentiating RCC from AA. The value of survivin expression was also evaluated. *Materials.* Bone marrow samples from 405 children suspected of MDS or JMML were analyzed. Samples were collected from the Nordic countries and the Czech republic. 188 patients were diagnosed with MDS according to the WHO classification. 47 patients were diagnosed with AA, 23 patients with acute myeloide leukemia and 147 patients with non neoplastic disorders. 28 patients were excluded with diagnosis including Down syndrome, Fanconi anemia and acute lymphoblastic leukemia. p53 and survivin expression was evaluated after immunohistochemical staining of trephine biopsies by two experienced hematopathologists. Spearman's rho was used to confirm the hypothesis of relation between p53 and survivin expression and diagnosis. Non-parametric Mann-Whitney was used to describe difference in marker between different diagnoses. *Results.* According to WHO classification 63 children were diagnosed with RCC, 75 children diagnosed as advanced MDS, and 50 children with JMML. The expression of p53 increased significantly in relation to type of disease (RCC, advanced MDS, and AML). No relationship was found in relation to survivin expression ($p > 0.4$) p53 expression was significantly lower in RCC as compared to patients diagnosed with advanced MDS ($p < 0.0001$) with a positive predictive value of having advanced MDS with more than 5% p53 positive cells of 70%. Figure 1 shows the median levels of p53 in the different diagnostic groups. No patient with AA was found to have more than 5% p53 positive cells in their bone marrow staining. A significantly lower p53 expression was found in AA compared with MDS (Figure 1) ($p < 0.000$). The positive predictive value of p53 expression was 88% with a sensitivity of 50% and a specificity of 84%. *Conclusions.* Our results suggest that p53 expression is a valuable tool in the discrimination of MDS diagnosis and especially in the differentiation between RCC and AA.

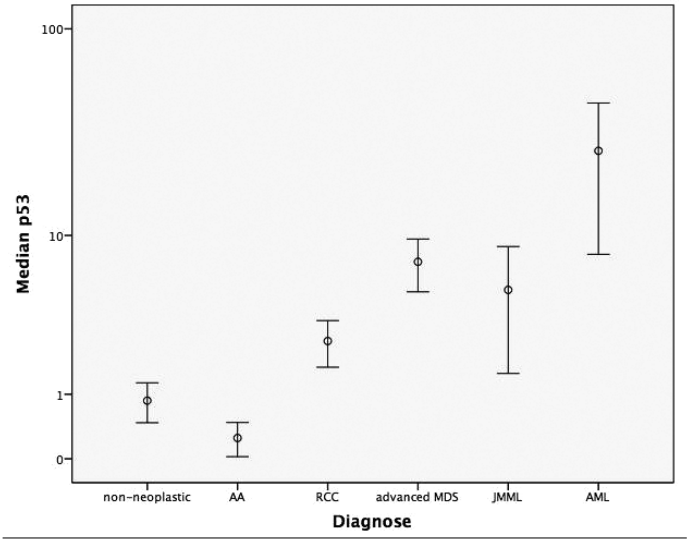

Figure 1.

P03

AML-ORIENTED CHEMOTHERAPY IS EFFECTIVE FOR RAEB AND RAEB-T IN CHILDREN

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The treatment of children with refractory anemia with excess blasts (RAEB), and RAEB in transformation (RAEB-T) remains a major challenge. Therapy of these disorders has been associated with severe treatment-related toxicity and a high risk of relapse. Previous reports suggested that conventional AML-type chemotherapy without hematopoietic stem cell transplantation (HSCT) resulted in survival rates below 30%. Kikuchi et al reported remission induction therapy with etoposide, cytarabine and mitoxantrone (ECM) brought high survival rates after HSCT in a Japanese study for MDS (MDS99) between 1999 and 2004. Thus, we retrospectively analyzed the role of chemotherapy prior to HSCT in RAEB and RAEB-T patients enrolled on a Japanese pediatric AML study. The JPLSG AML-05 is a nationwide clinical trial for children with de novo AML between November 2006 and December 2010 in Japan. A central review of diagnosis based on the 3rd WHO classification was prospectively performed. We deleted AML patients with MLL gene rearrangement or t(1;22), even if they showed the low blast counts. In 22 RAEB-T patients, karyotype analyses showed 8 patients with normal karyotype and 2 patients with -7 or +8, each. Patients with RAEB-T were all received induction chemotherapy of ECM and HCEI according to the AML-05 study and 18 of 22 (81.8%) achieved complete remission (CR). 12 of 22 (54.5%) received HSCT after median 5 months from the first diagnosis. Overall survival (OS) with (n=12) or without HSCT (n=10) was 58.3% *vs.* 80% (n.s). In 17 RAEB patients, karyotype analyses showed 7 patients with normal karyotype, 3 patients with -7 and one patient with +8. Fourteen patients received induction chemotherapy and 11 patients (11/14, 78.6%) achieved CR. Surprisingly, patients with chemotherapy alone (n=4) were all alive, however, OS of RAEB patients who received HSCT was 69.3%. Although, the ratio of patients who received HSCT in this study (RAEBT 54.5%, RAEB 76.5%) was lower than those of previous MDS99 study (RAEBT 91.7%, RAEB 100%), OS was superior than those with the previous MDS99 study, both (RAEBT 68.2% *vs* 58.3%, RAEB 76.5% *vs*, 71.5%). Our results suggested that AML-oriented chemotherapy would be beneficial for not only RAEB-T but also RAEB patients. Further large study will be needed.

P04

SEVERE CONGENITAL APLASTIC ANEMIA ASSOCIATED WITH PARTIAL DELETION OF THE HUMAN MECOM GENE AT CHROMOSOME 3q26.2

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Severe congenital aplastic anemia is a rare condition in neonates, and the genetics and mechanisms behind are largely obscure. Gene-targeting in murine systems revealed MECOM as a key player in normal hematopoiesis although its precise role and how this translates into humans remains elusive. Overexpression of MECOM is associated with human myeloid malignancies and solid tumors. Here we identified a de novo congenital submicroscopic deletion at chromosome 3q26.2 affecting exon 2 of MECOM with a maximal size of 175 kb in a neonate with progressive pancytopenia in the myeloid cell lineages. The neonate had no dysmorphic signs. The expression of several MECOM transcripts was significantly reduced. Whole exome sequencing revealed no pathogenic mutations in the MECOM gene or in other genes known to be associated with aplastic anemia. Sequencing of a segment containing a non-pathogenic variant of both parents and the child revealed a haplotype consistent with maternal origin of the deleted chromosome 3. Clinical cure was achieved with hematopoietic stem cell transplantation at 5 months of age. Reviewing the literature indicates that the present patient is the first human phenotype associated with constitutional deletion affecting a single exon of the MECOM gene. Our data show that MECOM is essential for normal myeloid hematopoiesis and dispensable for lymphoid differentiation. Moreover, MECOM seems to be essential for erythropoiesis, but not for granulopoiesis, in fetal life. We suggest that partial deletion in MECOM may be a primary event associated with congenital bone marrow failure syndrome.

P05

CHROMOSOME ANOMALIES AS CAUSE OF CONGENITAL BONE MARROW FAILURE SYNDROMES

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Background. Congenital bone marrow failure syndromes (BMFS) are a heterogeneous group of disorders characterized by the inability of the bone marrow (BM) to produce an adequate number of blood cells, with consequent peripheral blood (PB) cytopenia (anaemia, neutropenia and/or thrombocytopenia). A Mendelian pattern of inheritance is present in about 15-20% of the patients. The majority of the non-hereditary cases are considered idiopathic because their etiology is not known. A risk of transformation into a myelodysplastic syndrome (MDS) and/or an acute myeloid leukaemia (AML) is ascertained for inherited BMFS, and also in many patients with idiopathic BMFS or diagnosed as Aplastic Anaemia (AA). *Materials and Methods.* In the last 11 years we performed cytogenetic investigations, as part of the routine diagnostic procedures, in 97 paediatric patients with cytopenias. In this heterogeneous cohort of patients, 24 showed chromosome lesions in BM, which only contributed to the diagnosis in 18 cases, whereas in 6 of them the chromosome anomaly was the primary event leading to the BMFS. *Results.* In two patients a complex structural rearrangement of chromosome 21, constitutional in one and acquired in the BM of the other one, caused the disrupture or the loss of the gene RUNX1, which was therefore hypo-expressed and led to a Severe AA (SAA) in the first patient, and to a congenital thrombocytopenia in the other one. Another patient showed as acquired chromosome anomaly a paracentric inversion of chromosome 1 in the BM, with a position effect on the gene MPL, severely hypo-expressed, with a final diagnosis of Congenital Amegakaryocytic Thrombocytopenia (CAMT). The clonal anomaly in the BM of the fourth patient was a complex translocation with partial monosomy of the long arms of chromosome 11 including the FLI1 gene, consequently hypo-expressed and leading to Paris-Trousseau type thrombocytopenia. In two further patients, with diagnosis of SAA and pancytopenia, two different constitutional structural complex anomalies involving chromosome 8 led to the disorder due to effects on the RUNX1T1 gene, either hypo- or hyper-expressed in the BM. *Conclusions.* In our cohort of 97 patients with BMFS, 6 showed a chromosome anomaly which was the primary event leading to the disorder, through effects on specific genes. We postulate that a chromosome change is the primary cause of BMFS in a possibly small but not negligible proportion of cases.

P06

RETROSPECTIVE ANALYSIS OF HEMATOPOIETIC TRANSPLANTATION FOR NON MALIGNANT HEMATOLOGICAL DISEASES. A SINGLE CENTER STUDY

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Objectives. To review patients diagnosed with non-malignant blood disorder: congenital bone marrow failure (CBMF), acquired bone marrow failure (ABMF), or congenital anemia (CA) undergoing hematopoietic stem cell transplantation between 1999 and 2014 in our institution. Material and *Methods.* This was a retrospective study. Patients diagnosed with CBMF were those with the following diagnoses: Diamond-Blackfan anemia, congenital aplastic anemia, Fanconi anemia, congenital neutropenia, dyskeratosis congenita, and idiopathic aplastic anemia associated with immune deficiency. Within the ABMF were those patients diagnosed with idiopathic aplastic anemia. Finally, patients within the congenital anemia (CA) group were those diagnosed with sickle cell anemia, beta-thalassemia, sideroblastic anemia and pyruvate

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kinase deficiency. Type of transplant, donor, source, used of fludarabine, and total body irradiation were analyzed. Development or graft-*versus*-host disease, overall survival (OS), event-free survival (EFS) and causes of death were analyzed. *Results.* 45 patients were included, and a total of 49 transplants were performed. CBMF accounted for 55.6%, ABMF 24.4% and CA 20%. Overall survival was 65.8% (73% when excluding haploidentical donors), and the event free survival was 58.8% (mean follow-up 40 months). The best overall survival was observed in the group of CA (80%) and for CBMF was 59.4%. Donor type were: 28 HLA identical family donors, 15 unrelated HLA matched, and 5 haploidentical. Stem cell source was: 44.9% bone marrow (BM), 42.9% peripheral blood (T cell depleted) (PBSC), and only 12.2% of cord blood (CB). Overall survival was equal using HLA identical family donors, or unrelated HLA matched donors (73%). Overall survival was higher using BM stem cells than PBSC (71% *vs* 50%). Most PBSC transplants were haploidentical. Overall survival for those patients transplanted after 2010 is 77.6% *vs* 59.6% for those transplanted before. 42.2% of patients developed acute graft against host disease and 26.7% chronic graft-*versus*-host. Of the 45 patients 14 died (31.1%). The most frequent cause of death was sepsis. *Conclusions.* HSCT is the only curative treatment for non malignant hematological diseases. The morbidity and mortality of allogeneic HSCT is still high often associated with infectious complications, although over the last years is improving. In this series the prognosis is equal using related or unrelated donors.

P07

TURKISH NATIONAL SEVERE CONGENITAL NEUTROPENIA REGISTRY

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Introduction. Severe congenital nötropenia (SCN) is a very rare disease. Genetic mutation in neutrophil elastase gene (ELANE) is the most frequent mutation in European and North American registries. However, differences could be expected in the countries where consanguineous marriages are common. It is important to find out whether the approach for genetic typing shall be the same in western Europe, eastern Europe and middle East. We aimed to establish a national neutropenia registry in Turkey, a country with an extraordinary mixed population of Caucasian and Asian decent and the proportion of consanguineous families being higher than in most other parts of Europe. *Patients and Methods.* In this study, establishment of a national registry for severe congenital neutropenia (SCNR) and national bone marrow failure (BMFR) was aimed. Clinical and laboratory findings of 476 patients with BMF including 152 patients (31.9%) with SCN were entered into Turkish National BMFR. *Results.* The median age of the children with SCN (male/ female: 0.91) was 12.3 years (range 1 month to 35 years). The median follow up period for patients were 9.7 years (range 3 months to 34 years). Consanguinity between the parents of SCN patients was 49.2%. HAX1 mutation was the most frequently seen mutation among the patients entered into national registry (n=47, 33.8%). The same mutation, homozygote c130-131insA, p.W44X, was detected in 43 of 46 patients (93.5%) with HAX-1 mutation. ELA2 mutation was detected in 13 patients (9.6%) and it was the second most common mutation for SCN. In this series 7 patients (5.1%) had a mutation in G6PC3 gene. CSF3R and JAGN1 mutations were seen in 3 (2.2%) and 2 (1.5%) patients respectively. No mutation was found in 34 (22.4%) tested patients (All tested for ELANE and HAX1 and 20 of them were also tested for G6PC3). Twenty eight patients (18.4%) were not tested for SCN. Sixty percent of the patients were given GCSF. The median dose was 5 mcg/kg for 5 days a week. Two patients died with infectious complications and five developed MDS/ AML. *Conclusions.* In Turkey SCN mostly resulted from the p W44X mutation in HAX1 gene. Therefore in Turkey mutation analysis should be started with HAX1 and if it is negative ELANE and G6PC3 should be checked. Rare mutations should be tested in mutation negative patients because of very high percentage of consanguineous marriage.

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P08

INSIGHTS FROM THE NATIONAL DUTCH FANCONI ANEMIA COHORT STUDY: EPIDEMIOLOGY, PHENOTYPING AND SCT RESULTS

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Introduction. Fanconi anemia (FA) is a rare, inherited bone marrow failure (BMF) syndrome characterized by congenital abnormalities, BMF and a high risk of cancer, mainly AML and squamous cell carcinoma (SCC). Stem cell transplantation (SCT) is the only curative treatment for the hematological manifestations of FA. The c.67delG mutation in the FANCC gene is a common mutation in Dutch FA patients. Patients with this Dutch mutation are considered to have a mild phenotype. However, data on phenotype and course of disease in this patient group are scarce. *Patients and Methods.* In 2007 Dutch FA treatment guidelines, including a FA patient registry, were implemented. After informed consent, clinical data at diagnosis and yearly follow-up were collected and stored anonymously in a dedicated database. We analyzed the epidemiology of FA, described the phenotype of patients with homozygous c.67delG mutations and studied the characteristics and results of SCT for Dutch FA patients. *Results.* To date, 125 Dutch FA patients are known of whom 74 are currently alive (prevalence 1:229.000). On average, 2.9 children with FA are born in The Netherlands each year (range 0-6, birth incidence 1:63.000). Mutation analysis was performed in 108 patients. Homozygous c.67delG mutations were found in 28 (26%) patients. In these patients none or mild congenital malformations (thumb abnormalities, mild congenital heart defects, café au lait spots) were reported. Sixteen of 28 patients (57%) developed progressive BMF and/or MDS of whom 11 received a SCT at a median age of 10.8 years (range 4.3-22.4). Five of 17 (29%) patients ≥18 years developed cancer at a median age of 36 years (range 25-40). Seven of 28 patients died at a median age of 26.1 years (range 23.5-38.6). Cause of death was SCC (n=4), SCT-related (n=2) and BMF (n=1). Since 1972, 69 FA patients received a SCT of whom 22 were treated with the current Dutch conditioning regimen (CY30/FLU150±ATG). Stable engraftment was achieved in 19/22 (86%) patients, 2/19 (11%) patients at risk developed aGVHD ≥grade 2 and no patient developed cGVHD, 5-year overall survival was 91% (SE 6.4%). *Conclusions.* FA patients with homozygous c.67delG FANCC mutations have in general no major congenital abnormalities, but they do develop BMF and SCC underlining the critical importance of lifelong and stringent screening also in this patient group. With a non-irradiation conditioning regimen SCT can be performed safely and effectively in most cases of FA.

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P09

THE USE OF AZACITIDINE AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION TO PREVENT RELAPSE IN CHILDREN WITH ADVANCED MYELODYSPLASTIC SYNDROMES AND JUVENILE MYELOMONOCYTIC LEUKEMIA: EXPERIENCE OF THE BRAZILIAN PEDIATRIC MYELODYSPLASTIC SYNDROMES STUDY GROUP

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Introduction. Myelodysplastic syndromes (MDS) and Juvenile Myelomonocytic Leukemia (JMML) are rare and aggressive pediatric diseases and the only current curative therapy is allogeneic hematopoietic stem cell transplantation (HSCT). Nevertheless, relapse after HSCT is the most important cause of treatment failure and it is associated with a dismal prognosis. In adults, low-dose azacitidine, given after allogeneic HSCT as maintenance therapy for patients with high-risk MDS and acute myelogenous leukemia (AML), is well tolerated and appears to prolong event-free and overall survival. We report here the description of 6 children with very advanced MDS (N=5) or JMML (N=1) who received azacitidine after HSCT to prevent relapse. *Methods.* Six cycles of post-HSCT azacitidine were administered at 75mg/m/day for 7 days, every 4 weeks, intravenously or subcutaneously. All transplants used myeloablative conditioning and all children were in hematologic remission with complete donor chimerism before starting azacitidine. *Results.* The 6 patients had a median age of 12.7 years (range 5.8-16.8). Azacitidine was started after a median of 53 days post-HSCT (17-60), immediately upon recovery from transplant-related toxicities and with adequate engraftment. One patient with AML evolving from MDS received donor lymphocyte infusions after the 5th and 6th cycles of azacitidine, due to positive minimal residual disease by flow cytometry. Two patients had also used azacitidine before HSCT. All patients developed grade 4 hematological toxicity, but no severe infections or bleeding were observed. No patient was admitted to the hospital. Two patients had moderate nausea, vomiting and diarrhea and 2 had pain in sites of subcutaneous injection. Two children have moderate chronic graft *versus* host disease and no graft rejection was observed. One patient relapsed and died 14 months after HSCT, already off azacitidine and 5/6 are in remission with complete donor chimerism, with a median follow-up of 24 months (6-38) after the end of azacitidine. *Conclusions.* The use of azacitidine in conventional treatment doses after HSCT seems to be safe and well tolerated in children, may prevent relapse of advanced MDS and JMML and should be prospectively studied.

P10

COMPREHENSIVE GENETIC ANALYSIS OF BLAST CRISIS IN JUVENILE MYELOMONOCYTIC LEUKEMIA

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Juvenile myelomonocytic leukemia (JMML) is a rare but aggressive myeloproliferative neoplasm in childhood. It is characterized by excessive proliferation of myelomonocytic cells and hypersensitivity to granulocyte-macrophage colony-stimulating factor. Somatic and germline mutations in RAS pathway genes (NRAS, KRAS, NF1, PTPN11, and CBL) are identified in nearly 80% of children with JMML Some children JMML may experience spontaneous resolution of hematological abnormalities; however, hematopoietic stem cell transplantation is the only curative therapy for most of the children with JMML. Our recent whole-exome analysis of JMML revealed causative somatic/germline RAS pathway mutations affecting PTPN11, NRAS, KRAS, NF1, and CBL, and secondary acquired SETBP1 and JAK3 mutations. These secondary mutations were involved in poor clinical outcome. Another prognostic factor includes a blast crisis of JMML, which is only seen in about 10% of children with JMML, and its nature has not been defined yet. To further clarify genetic events in JMML, we performed whole-exome sequencing-based genetic analysis of a blast crisis in JMML. Three cases were studied at multiple time points. We identified a uniparental disomy (UPD) event in a patient with JMML which progressed to form a sarcoma in an orbit. The UPD resulted in homozygous activating NRAS mutation. In another patient whose disease progressed to mature B cell lymphoma, a chromosomal translocation was detected which formed an oncogenic IGH-MYC fusion gene. In the third patient whose disease progressed to acute lymphoblastic leukemia, we could not detect any genetic events newly acquired at the time of a blast crisis. Our genetic analysis of blast crises in JMML identified driver genetic events, which might contribute to a better understanding of the nature of JMML and a discovery of novel therapeutic approaches.

P11

METHYLATION CHANGES UNDER AZACITIDINE TREATMENT DIFFER IN CELL SUBSETS OF JUVENILE MYELOMONOCYTIC LEUKEMIA

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Juvenile myelomonocytic leukemia (JMML) is rare myeloproliferative/myelodysplastic disease of early childhood characterized by the excessive production of myelomonocytic cells. Genetic lesions affecting members of the Ras signalling pathway have been involved in the pathogenesis of JMML. However, the molecular pathology underlying this disease might be even more complex. Recent evidence implicates aberrant DNA methylation in the malignant biology of JMML with possible consequences on clinical course, prognosis and therapy of this disease. Clinical use of demethylating agents has already shown promising results. Herein we use array technology to assess methylation gene status in JMML MNCs, CD34 and CD14 blast subsets from a patient with JMML at diagnosis and after relapse after azacitidine treatment. After data processing we observe a decrease in global methylation in the MNCs and the CD14+ subset collected after treatment with azaciditine. Unexpected, however, the CD34+ subset demonstrated increased global methylation at disease progression compared to initial diagnosis. Further analyses of the top 200 methylation sites in the CD34+ cells were performed and show that these sites include genes already known to be involved in the regulation of apoptosis such as HRK protein, CAMK2A and members of the SOX protein family. Our observations put forward a mechanism in which genes that are involved in apoptosis become hypermethylated in the CD34+ population under azaciditine therapy. This culminates in increased MNC/blast counts and disease progression, probably as a consequence of inactive apoptotic pathways. Such a mechanism of resistance against demethylating treatment that particularly targets the CD34+ population might prove to be a bottleneck for this promising therapeutic strategy. This shift in methylation from diagnosis to disease progression affects genes involved in the apoptosis pathway.

P12

ALTERNATIVE CONDITIONING REGIMEN IN A PATIENT WITH JMML AND KRAS MUTATION

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For patients with juvenile myelomonocytic leukemia (JMML) and germline NF1 or somatic PTPN11, KRAS or NRAS mutation, allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative treatment. According to the EWOG-MDS consensus guidelines preparative regimens are based on busulfan, cyclophosphamide and melphalan. Lately, NRAS and KRAS mutations came into focus since somatic mutations in these genes have not only been found in JMML but also in ALPS-like disease characterized by lymphoproliferation and autoimmune cytopenia. In order to embrace the broad and overlapping phenotype of the two types of disease, different authors suggested introducing a new disease entity of RAS-associated ALPS-like disease (RALD). Another puz-

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zling observation was that selected JMML patients with NRAS mutation show a favorable clinical outcome even in the absence of HSCT. Less data are available on JMML with KRAS mutation. Within the prospective studies EWOG-MDS 98 and 2006 43 patients with JMML and KRAS mutation were included. We observed a strong correlation of JMML with KRAS mutation and monosomy 7. In this group of patients HSCT following a conditioning regimen with busulfan, cyclophosphamide and melphalan was characterized by a surprisingly low rate of relapse (4%) compared to all other patients with JMML (20-38%). In contrast, transplantation-related mortality was comparable between all groups. Thus, JMML patients with KRAS mutation might be candidates for more intensive GVHD prophylaxis and/or a less intensive preparative regimen. We here show a casuistic of a one year old girl suffering from JMML with KRAS mutation that was subjected to a matched unrelated donor transplantation following a conditioning regimen consisting of treosulfan based regimen. The course of HSCT was uneventful with timely hematological engraftment and full donor chimerism.

P13

MUTATION ANALYSIS IN JUVENILE MYELOMONOCITIC LEUKEMIA: A REPORT FROM THE BRAZILIAN COOPERATIVE GROUP ON PEDIATRIC MYELODYSPLASTIC SYNDROME

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Introduction. Juvenile myelomonocitic leukemia (JMML) is a rare but aggressive form of myeloproliferative neoplasm (MPN) frequently observed in children <2 years. The disorder is mainly characterized by an exacerbated proliferation of monocytic cells. Bone marrow transplantation (BMT) is the unique alternative of cure for JMML. Several efforts to define genomic landscape of JMML identified mutations in NF1, PTPN11, KRAS, NRAS and CBL in >90% of the patients. The identification of these mutations is important to support diagnosis and therapeutic approach for JMML. We report here the analysis of mutations in these genes for 14 JMML patients of the Brazilian Cooperative Group on Pediatric MDS (BCG-MDS-PED). *Materials and Methods.* Genomic DNA was extracted from the bone marrow at diagnosis of 14 patients with JMML, and submitted to gene mutation analysis using PCR followed by direct sequencing. *Results.* Five patients (35.7%) presented mutations in PTPN11, 3 (21.4%) in KRAS, 2 (14.3%) in NRAS and 2 (14.3%) in NF1. No mutations were observed for CBL. One patient (7.2%) presented mutations for PTPN11 and NRAS and 1 (7.2%) presented no mutations. Nine patients started azacytine treatment while waiting unrelated donor for BMT. For 4 of them, that presented PTNP11 mutations, we collected bone marrow samples during azacytidine treatment, prior BMT. The mutation analysis revealed the persistence of mutated PTPN11 clone after 6th (2 patients) and 8th (2 patients) azacytidine cycle. For one patient, we no longer detected PTNP11 mutation post BMT and for 3 patients the BMT is ongoing. *Conclusions.* The frequency of mutations in NF1, PTPN11, KRAS, NRAS and CBL from the JMML Brazilian patients seems to be similar according to literature worldwide. A higher number of patients should be assessed to evaluate the clinical impact of these mutations for Brazilian patients. However, the development of the molecular analysis by the BCG-MDS-PED through the Molecular Diagnostic Center of Barretos Cancer Hospital can support a better clinical management of the patients, resulting in a significant change in the natural history of JMML patients in Brazil.

P14

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION USING A REDUCED-INTENSITY CONDITIONING REGIMEN FOR DYSKERATOSIS CONGENITA: EXPERIENCE OF OUR INSTITUTES

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Introduction. Bone marrow failure (BMF) is a major cause of mortality for dyskeratosis congenita (DC) patients. Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative approach for BMF. However, transplantation-related toxicity is a serious challenge in DC; therefore, a reduced-intensity conditioning (RIC) regimen is recommended. Herein, we present three consecutive cases of DC treated by HSCT with a RIC regimen. *Patients and Methods.* BMF progressed in all cases before HSCT and required transfusion. Telomere length was shortened in all cases. One patient had Revesz syndrome caused by TINF2 mutation without pulmonary complications. The other patients were siblings with RTEL1 mutation, and the elder brother was complicated with hepatic fibrosis and mild portal hypertension. Age at HSCT ranged from 2 to 16 years. The patient with Revesz syndrome underwent bone marrow transplantation (BMT) from his HLA -matched healthy sibling. Both patients with RTEL1 mutation underwent unrelated cord blood transplantation (CBT) because HLA-matched bone marrow donors were not available. Conditioning regimens consisted of fludarabine (100 mg/m^2), cyclophosphamide (3 g/m^2), and anti-thymocyte globulin with or without low-dose (3 Gy) irradiation. Written informed consent was obtained before HSCT from the guardians of all cases. *Results.* All cases exhibited sustained donor engraftment and improved hematopoiesis without transfusion for 29–54 months, although one case who underwent CBT experienced engraftment failure and required a second CBT. Regimen-related toxicity included bacteremia in two patients and stomatitis was mild in all patients. One patient had steroid-responsible acute GVHD , and no cases developed chronic GVHD. Respiratory distress with unknown etiology appeared in the case with Revesz syndrome 41 months after BMT. He died 54 months after BMT due to respiratory failure. The elder brother with RTEL1 mutation suffered progression of portal hypertension after CBT, leading to recurrent bleeding of gastric varices, which necessitated vascular interventions. *Conclusions.* HSCT for BMF of DC patients with an RIC regimen was feasible even when using CBT. However, non-hematological complications due to DC might be accelerated by HSCT, despite using this regimen. Deciding whether HSCT is performed for DC patients requires careful consideration. For patients undergoing HSCT, careful follow-up for non-hematological complications is mandatory.

P15

GATA-2 DEFICIENCY IN CHILDREN IN THE CZECH REPUBLIC: FLOW CYTOMETRY FINDINGS AND PREVALENCE WITHIN MYELODYSPLASTIC SYNDROME

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Introduction. Germline mutation in GATA2 was recently identified in patients with immunodeficiency, familiar and sporadic myelodysplastic syndrome (MDS) and lymphoedema. It represents the most frequent genetic cause of MDS among children. Published data on flow cytometry (FC) findings concerned mostly adult patients. Detailed prevalence of GATA2 mutation among pediatric patients with MDS or aplastic anemia (AA) was not so far estimated. *Patients and Methods.* In total 11 patients

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were found to harbor GATA2 mutation in the Czech Republic. Eight were diagnosed with MDS in childhood: 3 with advanced MDS ("non RCC", *i.e.* RAEB, RAEBt or MDR-AML) and 5 with MDS RCC. Another 2 immunodeficient patients developed MDS diagnosis in early adulthood, the last patient without MDS was found by family search. We compared these patients with GATA2 wild type MDS RCC (n=31), non RCC (n=22) and AA patients (n=38). Peripheral blood (PB) and bone marrow (BM) were examined by FC immunophenotyping and evaluated for level of intronRSS-Kde recombination excision circles (KREC) to estimate the level of B cell production. *Results.* The prevalence of GATA2 mutation among pediatric patients with MDS RCC, MDS non RCC and AA is 17%, 14% and 0%, respectively. The prevalence within pediatric MDS patients with monosomy 7 and trisomy 8 is 41% and 17%, respectively. One patient with MDS did not have any cytogenetic aberration, although her father died of MDS with monosomy 7. Patients with GATA2 mutation had significantly decreased B cell and B cell progenitors in BM, B cells in PB are shifted to mature phenotype (decreased transitional B cells and naive forms). Level of KREC is very low in both PB and BM. Analyzed 4 newborn blood spots showed almost normal value in 3 patients, one patient with decreased KRECs at birth was diagnosed with MDS at 4y and is the youngest patient in our cohort. Absolute monocyte count in PB is often decreased, relative count was steadily decreased in 2 patients. Two patients with GATA2 mutation died: one with CMV pneumonitis, another one in progression to acute leukemia complicated by mycotic infection. *Conclusions.* We show the prevalence of GATA2 mutation within Czech pediatric MDS patients. No patient was classified as aplastic anemia, which is in contrast to published data. Cytogenetic aberrations could be absent and therefore flow cytometric findings can help to identify candidates for GATA2 screening.

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P16

PERICARDIAL AND PLEURAL EFFUSIONS AS AN INITIAL PRESENTATION OF PEDIATRIC SECONDARY ACUTE MYELOGENOUS LEUKEMIA AFTER APLASTIC ANEMIA

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Introduction. Secondary myelodysplastic syndrome and acute myeloid leukemia (sMDS/sAML) are the most serious secondary events occurring after immunosuppressive therapy (IST) in patients with aplastic anemia (AA). The frequency of clonal evolution in pediatric patients ranging from 2% to 26% and is associated with a very poor prognosis. We report a case of pediatric patient with pericardial and pleural effusions as an unusual manifestation of sAML. *Case Report.* A 14-year-old girl was admitted in 2004, with the diagnosis of acquired aplastic anemia without matched sibling donor available. She received IST (antithymocyte globulin and cyclosporine) with partial response. In 2010, the patient relapsed and failed to the second immunosuppression. Clinical support (transfusions) was offered as needed. In 2014, she developed acute respiratory failure, Hb 8,3 g/dL, WBC 61.49 10^9/L (65% neutrophils, 17% monocytes, 12% lymphocytes, 4% blast cells), plt 15X10^{10}/L, normal abdominal ultrasonography and an echocardiogram showed effusions affecting pleural and pericardial spaces, with cardiac tamponade. Both effusions: 20% of myeloid blasts cells with CD117+, CD13+ and CD45+. BM biopsy: hypercellular with immature granulocytes and monocytosis; BM aspiration (BMA): 7% of blastoid cells positive for CD13, CD33, CD34, CD117, CD36, CD64 and 24% of dysplastic monocytes. karyotype: 46,XX,der(19)t(1;19)(q23;p13) [18]/46,XX[2] by the G-banding analysis. Inv 16, BCR-ABL, TEL-AML 1, AML1-ETO, PML/RARα and MLL-AF4 fusion gene were not detected. Fetal hemoglobin was inferior to 0.8%. PDRGFa negative. Despite the probable evolution to AML and the clinical condition, we started her on low-dose Ara-C (40 mg/m /day x 10 days) followed by 5-azacitidine (75 mg/m^2/d x7 days, Q 28d). After 4 cycles of Aza, she developed an AML picture, BMA: 49% of blasts and increased myelomonocytic population. Kariotype: 50 XX, +12, +18, +der (19)t (1,19) (q23;p13),+mar(20). T (1;19)(E2A-PBX1) was negative. After sAML diagnosis she had no clinical stability for chemotherapy or stem cell transplantation. Palliative clinical care was given and she died in 2015 (CNS bleeding). *Discussion.* Pericardial and pleural effusions in patients with AML and MDS are a rare occurrence and the possible mechanisms involved are: extramedullary proliferation of a quiescent leukaemic clone with subsequent metastasis to the BM or a subclinical marrow relapse, undetected by standard methods with consequent seeding to extramedullary sites. Prospective studies are warranted to better characterize the incidence and outcomes of leukemic pleural and pericardial effusions from AML.

P17

TWO UNIQUE CASES OF MYELOPROLIFERATIVE SYNDROME IN CHILDREN CONNECTED BY TOUCHPOINTS IN PATHOGENESIS

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Myelodysplastic/myeloproliferative syndromes (MDS/MPNs) in children are rare clonal myeloid neoplasms characterized by overlapped myelodysplasia and myeloproliferation at the time of presentation and high propensity to AML transformation. Except for well defined JMML and malignancies associated with germline mutations of RAS/MAPK pathway genes, a cathegory of MDS/MPN unclassifiable is established in the WHO 2008 classification. Provisional diagnosis of MDS/MPN-U is based on clinical and morphological findings and exclusion of JMML. Genetic abnormalities, if any, are nonspecific. We report on two unusual cases of MPS, unclassifiable by common diagnostic methods, finally clarified as RAS/MAPK dysregulation origine. *Case 1.* 3-years-old boy with juvenile xanthogranuloma (JXG) presented with fever, rash, hepatosplenomegaly and pancytopenia. Significant macrophage proliferation and activation was observed, MAS assumed as consequence of systemic progression of JXG. However, bone marrow evaluation didn't show histiocytic infiltration but hypercellular hematopoiesis with multilineage dysplasia and hyperdiploid clone 50,XY[11],+11,+12,+21,+22. During 8 weeks window to MUD-SCT and despite salvage LCH and MAS-directed therapy, progression to RAEB-t (29% blasts in BM) was evident. Molecular analyses focused on clonal transformation driver finally revealed somatic KRAS mutation (c36G>A) and iMLL/PTD amplification in hematopoietic cells, assumed as causative. *Case 2.* 11-years old girl presented with fever, lumbalgia, generalised lymphadenopathy and splenomegaly. Blood (leukocytosis 35x10.9/l, polyglobulia, eosinophilia, myeloid precursors) and bone marrow analysis showed chronic phase of atypical myeloproliferative neoplasm, Ph chromosome and JAK2V617 negative. mFISH analysis confirmed specific translocation t(8;13) (p11;q12) involving FGFR1 gene, genetic abnormality consistently associated with extremely rare, aggressive myeloid malignancy, 8p11 MPS, defined as independent entity. Myeloproliferation is driven from constitutive activation of tyrosin kinases affecting RAS/MAPK pathway. Overlap MDS/MPN syndrome, except for JMML, are extremely rare in children. Detection of involved genetic abnormalities is necessary for correct diagnosis and treatment. As demonstated, somatic mutations in genes regulating RAS/MAPK pathway may be the pathogenetic driver, that should be searched for.

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P18

EOSINOPHILIA DRIVEN BY ABNORMAL T LYMPHOID POPULATION IN CHILDHOOD. ROLE OF FLOW CYTOMETRY IN DIAGNOSTIC ALGORITHM

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Eosinophils and their products play an important role in the pathogenesis of various reactive and neoplastic disorders. Hypereosinophilia can be divided into various categories based on underlying etiology: primary (neoplastic) hypereosinophilia with an underlying clonal myeloid or stem cell disorder or reactive condition characterized by expansion of non clonal eosinophils. The lymphoid variant represents specific situation and exhibits several features of reactive hypereosinophilic syndrome (L-HES). The lymphocytes from patients with L-HES show an aberrant phenotype CD3negCD4pos and clonality of the population is frequently proven by identification by monoclonal T cell receptor rearrangement. In 2006 we diagnosed L-HES in a 13 year old girl with severe form of eczema gradually worsening since her fifth year of life. She had hyperleucocytosis ($50.1x10^9$/L) and hypereosinophilia ($25.1x10^9$/L). Atypical T lymphocytes were CD3negCD4posCD45ROposHLA DRpos and they covered majority of lymphocytes present in her peripheral blood (74%). Molecular genetics identified monoclonal T cell receptor rearrangement (incomplete D 1-J 1.2). After therapeutic attempts with corticosteroids (transient clinical improvement), hydroxyurea (no effect) and anti-CD52 antibody (clinical improvement) she was transplanted from unrelated donor and doing well. Inspired by this case we introduced in 2006 6-color flow cytometry screening using markers HLA DR, CD4, CD45, CD8, CD14 and CD3) for identification of L-HES. In 2012 we diagnosed L-HES in 3 year old girl with eczema, leucocytosis ($25x10^9$/L) and eosinophilia $12x10^9$/L. By FACs we identified a population CD3negCD4pos at level of 1.5% out of all lymphocytes. Detailed phenotyping revealed CD45RO and CD5 bright positivity on this population. Despite intensive search using material from FACsorting we did not find clonality by TCR gene rearrangement. Patient is treated intermittently by corticosteroids with clinical effect. So far she is one of the youngest known patients with L-HES. *Conclusions.* L-HES in childhood is very rare. Flow cytometry can help in early establishment of the diagnosis. Identification of the immunological origin of the atypical T cells remains challenge.

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P19

THE CLINICAL PHENOTYPE OF 2 SIBLINGS WITH JMML WITH HETEROGENEOUS c-CBL MUTATIONS

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About 15% of all JMML cases present with c-CBL mutations. We describe a family with two siblings carrying non synonymous c-CBL mutations. The eldest child, without dysmorphic features, was diagnosed with JMML at the age of 3 months in 2008. By that time, no known RAS pathway related mutations were identified. He received an allograft of a matched unrelated donor. He received a second graft because of graft failure. Currently, he is in complete remission, 4 years after transplant, without any signs of graft *versus* host disease donor and complete donor chimerism. After identifying the relevance of c-CBL retrospectively screening for c-CBL mutations was found and a somatic mutation was identified: c.1201T>A. Three years later a sister presented at the age of 1 month with JMML. Mutational screening revealed also ac-CBL mutation (c.1201T>A). Based on the assumption that c-CBL mutated JMML cases may not require SCT, we followed a watch and wait strategy. Nevertheless, after three months extramedullary hematopoiesis occurred, resulting in skull hyperplasia due to extra bone formation which resulted in a peripheral facialis paresis with the presence of leukocytosis, monocytosis and hepatosplenomegaly. For that reason we started treatment with 6-mercaptopurine, which resulted in clinical complete remission (cCR). Subsequently 6-mercaptopurine dose was tapered over nine months. Three months after discontinuation of treatment she presented with a recurrence of JMML signs and symptoms with an excess of blasts (morphological 85%, 0,3% monoclonal population in the BM, again accompanied by extramedullary hematopoiesis resulting in excess bone formation of the skull in the weeks thereafter. As there was no response on 6-mercaptopurine nor cytarabine, we bridged the time to SCT with two courses of intensive chemotherapy (FLAG) according to EWOG policy, but could not prevent disease progression during the conditioning procedure. This was accompanied again, by extra bone formation and even resulted in paraplegia. At day 15 after transplant she died of progressive disease with multi-organ failure, obviously not yet engrafted. These siblings illustrate the heterogenety of the JMML-phenotype, which may be related to the nature of the c-CBL mutations. Large JMML cohort studies will be necessary to unravel the genotype-phenotype correlations in order to be able to provide evidence based guidelines for clinical management according to genotype.

P20

SUCCESSFUL UNMANIPULATED HAPLO-IDENTICAL BONE MARROW TRANSPLANTATION FOR A CHILD WITH BLAST CRISIS OF JUVENILE MYELOMONOCYTIC LEUKEMIA

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Background. Juvenile myelomonocytic leukemia (JMML) is an intractable pediatric leukemia classified into the category of myelodysplastic/myeloproliferative neoplasm by the World Health Organization. Although hematopoietic stem cell transplantation (HSCT) is the only curative procedure for JMML, high relapse rate is still a matter of great concern. Some studies emphasize the importance of graft-*versus*-leukemia (GVL) effect against JMML, and strategies that focus on enhancing the GVL effect may improve the outcome of high-risk patients with JMML. *Patients.* We report a case of a 7-month-old boy who presented with leukocytosis (59% of blasts in peripheral blood), splenomegaly, pulmonary infiltration, and elevated HbF level up to 30.5% at his first visit. An initial diagnosis of acute erythroid leukemia was made by bone marrow (BM) examination according to the French-American-British classification. Although neither spontaneous colony formation nor gene mutations in the Ras pathway were identified, his clinical features were enough to convince us the diagnosis of JMML in blast crisis (BC) phase. *Results.* He achieved partial remission after cytarabine based chemotherapy, and received bone marrow transplantation (BMT) from a matched sibling following busulfan based myeloablative conditioning (MAC) regimen. Neutrophil engraftment was achieved on day +18. Complete remission (CR) with full donor chimerism was confirmed on day +28. However, the disease relapsed on day +62. After acute myelogenous leukemia-oriented chemotherapy, he achieved second CR and underwent unmanipulated haplo-identical BMT from his mother with total body irradiation based MAC regimen. Graft-*versus*-host disease (GVHD) prophylaxis consisted of post-transplant cyclophosphamide, tacrolimus, and mycophenolate mofetil. Neutrophil engraftment was achieved on day +12 and complete donor chimerism was confirmed on day +28. Tacrolimus was discontinued on day +28 because of intestinal thrombotic microangiopathy folllowed by grade 3 acute GVHD, but steroid administration resolved it. He is now alive in CR with chronic GVHD over 9 months after the second BMT. *Conclusions.* Current patient with JMML was successfully treated with haplo-identical BMT in spite of his high-risk status, suggesting that the presence of chronic GVHD was associated with his favorable outcome. Haplo-identical BMT aiming GVL effect could be a hopeful treatment option even in high-risk JMML cases.

P21

SUCCESSFUL SECOND HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) FOR A CHILD WITH JUVENILE MYELOMONOCYTIC LEUKEMIA WITH GRAFT FAILURE AFTER FIRST HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Introduction. The only current curative therapy for Juvenile Myelomono-

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cytic Leukemia (JMML) is allogeneic hematopoietic stem cell transplantation (HSCT). However, the inability to identify or quickly secure an HLA-matched donor has been an important obstacle. HLA-haploidentical HSCT is a promising therapy for high-risk hematological malignancies. We report here a child with JMML who was successfully treated with a second haploidentical HSCT from a different family donor after first haploidentical HSCT with graft failure. *Case report.* A 8-year-old boy was diagnosed with JMML (with monosomy 7) in March 2014. He was refractory to 3 cycles of azacitidine (with cytarabine and mercaptopurine) and evolving with rapid clinical deterioration. Owing to the lack of HLA-matched donor, he received a non-T-cell-depleted (TCD) haploidentical HSCT from his mother in May 2014. The conditioning regimen consisted of IV busulfan 15,2 mg/kg and melphalan 140 mg/m. Graft-*versus*-host disease (GVHD) prophylaxis consisted of cyclophosphamide (50 mg/kg IV, days +3 and +4), mycophenolate mofetil (MMF) and tacrolimus (FK). Peripheral blood stem cells (PBSC) were harvested from his mother, and 27,92x106/kg CD34+ cells were infused. Hematopoietic recovery was rapid and full donor chimerism was observed on day 16. However, he had become pancytopenic, and showed 24% donor chimerism on day 33. At this time, he developed aspergillosis treated with voriconazole and granulocyte transfusions. On day 51 he had JMML relapse and underwent splenectomy followed by one cycle of azacitidine and cytarabine. Then he received the second non-TCD haploidentical HSCT from his sister, on day 62 after the first HSCT. The conditioning regimen consisted of fludarabine 125 mg/m, melphalan 140 mg/m and and 400 cGy total body irradiation. GVHD prophylaxis consisted of MMF and FK. PBSC were harvested from his sister, and 10,22x10^6/kg CD34+ cells were infused. Hematopoietic recovery was on day 15, without signs of acute GVHD. So far, he has been in complete remission and with full donor chimerism. He received 6 cycles of post-HSCT azacitidine to prevent relapse. After immunosuppression withdrawal, he had an overlap GVHD with good response to combination therapy with corticosteroid and methotrexate. *Conclusions.* This is a report of a child with JMML successfully salvaged with a second non-TCD haploidentical HSCT after a first non-TCD haploidentical HSCT with graft failure and early relapse of the disease.

P22

ROLE OF FLOW CYTOMETRY IN DIAGNOSTICS OF EXTRAMEDULLARY RELAPSE OF JUVENILE MYELOMONOCYTIC LEUKEMIA IN STOMACH

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Juvenile myelomonocytic leukemia (JMML) is an aggressive myeloid neoplasm, which is characterized by overproduction of monocytic cells, which can infiltrate organs, including spleen and gastrointestinal tract. Until now almost 90% of driver mutations in JMML were identified, all of which thus far affect Ras signalling pathway. So far only anecdotal reports of extramedullary relapses can be found in the literature. Here we describe a patient with retrospectively identified K ras mutation, who experienced extramedullary relapse in stomach. Patient born as a monozygotic twin suffered from JMML at age of 7 years. Cytogenetics revealed aberrant karyotype with additional chromosome X, trisomy 8 and 19. Patient was transplanted from HLA identical twin unfortunately he relapsed three months after SCT. Patient underwent 2nd SCT from an unrelated donor, he experienced two hematological relapses (d+78 and 1 year after SCT) confirmed by the presence of autologous signal and reappearence of the cytogenetic aberrancies. Hematological relapses were successfully treated by withdrawal of immunosuppression and DLI respectively. More than 4 years after 2nd SCT he was admitted to hospital with anorexia and abdominal pain. Ultrasound investigation revealed gastric wall thickening and enlargement of adjacent lymph nodes. Clinically lymphoma was suspected. Biopsy was taken from gastric wall and material was analyzed by standard histopathology, flow cytometry and molecular genetics. Histopathological analysis failed to find the origin of infiltrating tumor cells. The only positive marker in immunohistochemistry was CD45 (LCA), the specific markers for B, T and myeloid (MPX, CHAE) lineage, were negative on atypical cells. By flow cytometry infiltration of atypical CD34 positive cells was found. Atypical cells were characterized by co-expression of CD7, CD13 and CD33 markers. These cells had been also present at diagnosis of JMML in bone marrow and spleen. Despite intensive chemotherapy and transient response patient progressed to overt hematological relapse and died. *Conclusions.* According to our knowledge this is the first description of JMML stomach relapse. Flow cytometry has a limited role in establishment of JMML diagnosis. However, in this patient flow cytometry helped in diagnosis of relapse through identification of identical aberrant myeloid cells at diagnosis and relapse of the disease.

P23

LONG-TERM PERSISTENCE OF SOMATIC HETEROZYGOUS CBL MUTATION IN JUVENILE MYELOMONOCYTIC LEUKEMIA

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Introduction. CBL mutation is identified in up to 12% of total Juvenile myelomonocytic leukemia (JMML) cases. In most cases, germline CBL mutation occurs on one allele and LOH on the other allele also occurs. Spontaneous regression of hematological abnormality is frequently seen in patients with CBL mutations despite the persistence of acquired somatic mutations. Therefore, HSCT is not recommended immediately after diagnosis in patients with germline CBL mutations. In addition, there are some reports describing patients with somatic heterozygous CBL mutation without germline mutation. However, detailed characterization has not been clarified yet. We describe such a case with a long term follow up. Patient/*Methods.* 12-month-old boy was referred because of massive hepatosplenomegaly and leukocytosis (48,300/L, blast 1.8%, monocyte 8.0%). Bone marrow smear showed hypercellularity and 3% of blasts. Karyotype was 46, XY. From the consensus criteria of JMML and centralized morphological review of bone marrow and blood smears, JMML was most compatible. Clinically, he had no anomalies. Colony assay and molecular genetic analysis (NRAS, KRAS, PTPN11 and CBL) were performed. Nail and buccal swab samples for confirmation of germline status were also obtained additionally. *Results.* 6-MP was administered in total for four weeks to control hepatosplenomegaly and lung infiltration. Hepatosplenomegaly was gradually improved in two years from diagnosis. CD34 positive cells showed hypersensitivity to granulocyte-macrophage colony-stimulating factor in colony assay. Heterozygous CBL mutation (c.1106 del 66bp) was detected in blood sample. This mutation is located in the cluster site and it disrupt the RING finger domain. The absence of LOH was confirmed by sequencing DNA of individual colonies from PBMCs. No identical mutation was detected in nail or buccal swab. The disease status has been now in remission for more than 10 years and no any other events such as vasculitis that is frequently observed in long term survivor of germline CBL mutation. Heterozygous CBL mutation is still detected in blood samples. *Conclusions.* There are no precise reports describing difference in clinical course between JMML with somatic heterozygous CBL mutation and JMML with germline CBL mutation. Somatic heterozygous CBL mutation itself causes JMML-like disease in infancy. Deletion mutation instead of missense mutation may have a key event in such somatic heterozygous CBL mutations.

P24

DETECTION OF MONOSOMY 7 BY G-BANDING AND FISH IN A PEDIATRIC PATIENT WITH JUVENILE MYELOMONOCYTIC LEUKEMIA SUBMITTED TO BONE MARROW TRANSPLANT: A CASE REPORT

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Introduction. Juvenile myelomonocytic leukemia (JMML) is a rare dis-

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ease that presents myeloproliferative and dysplastic features, accounting for 2-3% of malignant blood disorders in children. The survival rate is very low in patients not undergoing allogeneic bone marrow transplantation, the overall survival is around 10 months after diagnosis. Changes involving chromosome 7 are observed in approximately 10% of the cases. *Objectives.* To describe a case of a 8-year-old, male patient, who had appetite loss, weight loss, leg pain and difficulty walking. Due to the unusual age for JMML, the clinical and laboratory diagnosis could be strengthened by the presence of monosomy 7. The patient had a significant disease progression, even after 1 haploidentical transplantation (mother). However, he went into remission after the second haploidentical transplantation (sister). *Methods.* The bone marrow aspirations were subjected to a 24-hour cell culture and karyotyping and later they were subjected to the fluorescent in situ hybridization technique for research on the monosomy 7. *Results.* The karyotype revealed monosomy of chromosome 7 in 50% of the cells and was confirmed by FISH in 98.2% of interphase cells. After having been submitted and accepted the second transplant, the patient presented three karyotypes {//46, XX [20]} evidencing complete chimerism in the bone marrow and peripheral blood and three FISH exams {nuc ish (CENx2) [150]} revealing a higher cytogenetic response over the past nine months. *Conclusions.* This case highlights the importance of classical cytogenetic and molecular monitoring to monitor disease progression and response of the patient to the treatment regimen proposed.

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AN HLA-IDENTICAL TWIN WITH JUVENILE MYELOMONOCYTIC LEUKEMIA

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Introduction. Juvenile myelomonocytic leukemia (JMML) is a rare myeloproliferative disorder. Children typically present at young age. Till now only few data are published about familial cases of JMML, and especially reports of twins affected by JMML are sparse. *Case.* We report a case of a monozygotic twin pair affected by JMML. They presented at the age of 3.8 and 4.3 years with recurrent infections, erythematous dermatitis and progressive splenomegaly. Based on blood cell counts and morphology they were both diagnosed as JMML. Cytogenetics revealed the presence of a partial duplication of chromosome 3 (q13.3q28) in one of the first twin, whereas the second twin had a normal karyotype. Mutation analysis of RAS pathway related genes resulted in a heterozygeous PTPN11 aberration in both twins, which could not be confirmed in the analysis of fibroblasts. Both twins were transplanted at the age of 4 years with bone marrow of a 5/6-HLA-matched family donor. Both twins developed Graft-*versus*-Host disease, treated with anti-thymocyte globulin (ATG) and methylprednisolone. The first twin developed died three months after transplant due to a pneumocystis carinii pneumonia. The second twin is still in complete hematological remission without serious sequelae, now 18 years after SCT. *Discussion.* JMML in monozygotic twins is very rare. This case illustrates that both twins carry the same somatic aberration in the hematopoietic systems, without a germline aberration, which suggest that the somatic aberration occurred in the early development of the embryos.

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JUVENILE MYELOMONOCYTIC LEUKEMIA OR RAS-ASSOCIATED AUTOIMMUNE LYMPHOPROLIFERATIVE DISORDER? A JAPANESE INFANT PRESENTED A DIFFICULT DISEASE ENTITY

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Introduction. Ras-associated autoimmune lymphoproliferative disorder (RALD) is a rare genetic disorder characterized by somatic RAS mutations. Their symptoms and laboratory findings overlap with those of juvenile myelomonocytic leukemia (JMML). Both conditions are sometimes difficult to distinguish when diagnostic tests including genotyping are negative. Here, we report a Japanese boy who had clinical aspects of both RALD and JMML. Since he had no RAS mutations, we couldn't determine the diagnosis. *Patients.* This was a case of a preterm infant boy with a birth weight of 3.2kg and was referred to our department because of respiratory distress. No particular family history was reported and no risk of perinatal infection was identified. He had presented hepatosplenomegaly and skin rash at birth. His condition got worse and he showed high fever with pancytopenia. Prednisolone (PSL) was administered after he was diagnosed as having hemophagocytic syndrome. PSL was apparently effective, however, dose of PSL couldn't be decreased as the fever and rash continued. Then, cyclosporine was started at the age of 2 months. At the age of 3 months, he was diagnosed with inflammatory bowel disease (IBD). He continued to be administered immunosuppressive therapy, however, his clinical symptoms didn't disappear and he did not gained weight sufficiently (5 kg at 24 months). At the age of 29 months, he underwent reduced intensity conditioned allogeneic cord blood transplantation. A complete chimerism was achieved, however, he died of intracranial hemorrhage 1 month later. His clinical course and laboratory data were inconsistent. Familial hemophagocytic lymphohistiocytosis, Wiskott-Aldrich syndrome and primary autoinflammatory disorders such as cryopyrin-associated periodic syndrome were denied by genetic testing. The bone marrow was hypercellular with dysplasia. Spontaneous granulocyte/macrophage-colony formation and hypersensitivity to GM-CSF were confirmed by in vitro culture. These findings fulfilled most of the diagnostic criteria for JMML. However, IBD did not fit the criteria for JMML and he required immunosuppressive therapy for a long time, which suggest that he might have had RALD. However, mutations in the PTPN11, RAS, and CBL genes were not detected. *Conclusions.* More clinical and laboratory data of patients with similar symptoms should be accumulated and analyzed to clarify the disease entity our patient was involved.

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MYELOFIBROSIS AS AN EARLY PRESENTING SYMPTOM OF PERIPHERAL T-CELL LYMPHOMA

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Here we present a twelve year old girl that presented with pancytopenia and bone pain, but absent hepatosplenomegaly. A performed PETscan showed only bone marrow activity. The diagnosis of leukemia could not be confirmed by immunophenotyping nor morphology. Consecutive bone marrow biopsies showed a hypercellular bone marrow with excessive myelofibrosis (MF2-3), maximum of 15% blasts and extensive invasion of T-cells. Monoclonality coud not be demonstrated with gene rearrangement studies. No clonal cytogenetic aberrations or JAK2 mutations were present. Before transplantation, treatment with steroids did result in temporary decrease of symptoms. She was transplanted with a haplo-identical transplant (mother) with progressive pancytopenia and intractable pain. She was conditioned with anti-thymocyteglobulin, fludarabine, treosulfan and thiothepa. After the first ATG she developed respiratory insufficiency. Chest-CT revealed infiltrative abnormalities and widespread lymphadenopathy, including mediastinal and paraortic lymphnode enlargement. A bronchial alveolar lavage confirmed aspergillus and CMV infection for which she was treated with micafungin, liposomal amphotericin and ganciclovir. Inguinal lymphe node excision biopsy revealed a high-grade monoclonal peripheral T-cell lymphoma (PTCL), positive for TIA-1 and CD2. Other T-cell markers were negative, as were TCRB and TCRG. Granzyme B was positive in parts of the lesion and Ki67 showed a high proliferation index (>95%). B-cell lineage markers (CD20 and CD79a) were only positive in a small percentage of cells. CD30 was positive in less than 30% of the lesional cells, whereas CD10, TdT, CD56, ALK-1 and LMP-1 were all negative. Treatment for the PTCL was not possible, because of rapid dissemination of the PTLC, aplasia, and concurrent apergillosis and CMV pneumonitis. She died at day +8 after transplant

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from progressive respiratory failure. In retrospect, the diagnosis of this malignancy was not made, also because of lack aberrant T-cell markers in the first bone marrow trephine and the lack of monoclonality in subsequent bone marrow investigations. Myelofibrosis is very rare in children and PTCL account for less than 1% of non-hodgkin lymphomas in children. Myelofibrosis is described as an auto-immune phenomenon in elderly people preceding the diagnosis of PTCL as in our case. This case illustrates that in exceptional pediatric myelofibrosis can also be an early presenting symptom of PTCL.

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MOLECULAR AND CYTOGENETIC ANALYSIS IN CHILDHOOD ACUTE MYELOID LEUKEMIA WITH t(10;11)(p12;q23): CASE REPORT

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Abstract. The clinical, hematological and cytogenetic data for a child with an acquired abnormality of 11q23 and 10p have been described at this work. Patient was diagnosed with AML M5 and the cytogenetic finding was a translocation between 11q and 10p. He started the treatment with BFM 2004 protocol for AML. This translocation subgroup t(10;11) defined a subset of younger 11q23 patients, most of whom achieve a first complete remission despite the differing treatment regimens. *Materials and Methods.* Bone marrow cells were cultured and harvested according to standard procedures RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO) with 20% fetal bovine serum (Sigma-Aldrich), 1% L-glutamine (Sigma-Aldrich), 1% antibiotics for 24 and 48 hours. Karyotypic analysis was performed on G-banded metaphases. After harvesting and slide aging the metaphase chromosomes were banding by conventional trypsine Giemsa banding technique and karyotyped according to ISCN 1995.8 At least 20 metaphases were analyzed.The molecular biology analysis was performed using primers and probes designed to identify four different breakpoints for the fusion between the genes KMT2A (MLL) located at 11q23 and MLLT10 located at 10p12. *Results.* Cytogenetic analysis at diagnosis revealed a chromosome translocation (10;11)(p12;q23) in all 20 cell analysed. Subsequent the presence of MLL-MLLT10 fusion was confirmed with RT-PCR analysis. *Conclusions.* The t(10;11)(p12;q23) translocation is a rare recurrent translocation, most commonly seen in young adult AND pediatric AML, and are seen a high incidence of early morbidity from leukocytosis-related complications. Rearrangements involving MLL gene on chromosome 11q23 are typically associated with an overall poor prognosis. Is difficult to identify by G-banding alone and the number of cases with t(10;11)(p12;q23) is small, so additional studies are needed. We are doing more molecular studies to better understand the rearrangement and the breakpoints involved.

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CHILBLAIN LUPUS-LIKE AUTOIMMUNE SYNDROME WITH STEROID-RESPONSIVE PANCYTOPENIA PRECEDING NRAS- AND MONOSOMY 7-ASSOCIATED MDS-RAEB/AML

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Introduction. Paraneoplastic phenomena may occur in hematologic malignancies including myelodysplastic syndrome (MDS). However, a lupus-like syndrome in NRAS-associated 7- MDS has not been described yet. *Patients and Methods.* A 3yo boy was diagnosed with Chilblain lupus based on recurring painful livid acral nodules resembling perniones, relapsing perichondritis, aphthous oral lesions, and a maculopapular rash, often accompanied by fever. The family history of hematologic, immunologic, or rheumatoid diseases was negative. *Results.* Dermathopathology was compatible with histiocytic Sweet syndrome. IgG levels were increased; ANA, APLA, Coombs test, and platelet antibodies were borderline positive. Pancytopenia developed (356/µL ANC, 1335/µL ALC; 79/µl monocytes, 1164/µL T cells, 92/µL B cells, 105/µL NK cells, 71.000/µL platelets) with transfusion-dependent anemia. A bone marrow aspiration and trephine biopsy showed mild MDS with excess of about 15% CD34+CD33dim CD117+CD7+CD11b+CD123+ blasts and 6% CD14-negative monocytes. However, repeated follow-up examinations within 6 months showed a diminishing proportion of 10%, later 3,7-7,3% immature cells, furthermore, progressive fibrosis and nest-like accumulation of lymphocytes without cytogenetic aberrations, compatible with Systemic Lupus Erythematosus. TREX1 and NOD2 mutation analyses were negative. B cell hypoplasia and altered dendritic cell subpopulations warranted GATA-2 exon sequencing, which was normal. High-dose i.v. immunoglobulins and oral hydroxychloroquin had no effect. In contrast, prednisolone (2mg/kg/d) induced rapid remission of pancytopenia and autoimmunity. However, when prednisolone was tapered after 7 months, pancytopenia recurred and monosomy-7-associated MDS-AML (FAB M5) with the previously noted immune phenotype was diagnosed. A somatic NRAS mutation (c.181C>A; p.Q61K) was detected in bone marrow (but not in DNA from oral mucosa). The boy was treated with AML-BFM2004 induction (cytarabin, daunoxome, etoposide) and allogeneic peripheral blood stem cell transplantation from his HLA-identical brother (alpha-betaTCR/CD19-depleted) after conditioning with busulfan, cyclophosphamide and melphalan two years after his first paraneoplastic symptoms. He is now in excellent condition and hematological remission at day+180 after HSCT, albeit with mixed T cell chimerism. *Conclusions.* Lupus-like autoimmunity and steroid-responsive pancytopenia may precede and conceal NRAS-MDS-RAEB for years.

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AZACYTIDINE THERAPY IN CHILDREN

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Introduction. 5-azacytidine (5-AZA) is a DNA methyltransferase inhibitor that induces DNA hypomethylation. Administration of the azacytidine, has been shown to delay progression to AML in MDS patients. So azacytidine is an attractive alternative to traditional induction chemotherapy as a pre-HSCT cytoreductive modality. *Methods.* From February 2009 to February 2015, children (n=7) with MDS (n=5), AML(n=1) and JMML (n=1) , were treated with 5-azacytidine prior to HCT at the Department of Pediatric Hematology, Ege University Faculty of Medicine. Clinical charts were reviewed retrospectively. Patients received 5-AZA at a fixed dose of 75 mg/m2 subcutaneous daily for 7 days (days 1-7). Courses of therapy were repeated not earlier than every 4 weeks. *Results.* A total of 7 patients were included in the analysis (4 girls, 3 boys). Seven patients with a median age of 7,5 years (range 1-15) received a total of 25 cycles of azacytidine (median 3,5 cycles, range 1-8). Five patients had a unfavourable cytogenetics including monosomy 7 (n=2), 7q deletion (n=1), complex karyotype (n=1) and hypodiploid (n=1). Two of them had normal cytogenetic. After azacytidine therapy six patients achieved hematologic response and three patients achieved molecular response. Four patients (one with JMML, one with AML and the others with MDS) underwent allogeneic SCT following treatment with azacytidine. No problem occurred in these patients during posttransplant follow-up of 7-22 months. Two patients with MDS who had hematologic and cytogenetic response to treatment developed AML afterward and died, after 5-4 cycles of therapy respectively. One of the MDS patients did not continue treatment after first course, and developed AML three months after. The most frequently observed toxicities were nausea, vomiting, mild-moderate myelosuppression and febril neutropenia. No severe fungal and bacterial infections or treatment-related deaths were observed. *Conclusions.* Despite recent developments in our country, appropriate unrelated donor for HSCT can not be found within the desired time. The only known curative treatment modality for MDS is allogeneic hematopietic SCT but in order to keep patients in remission until transplantation, alternative treatment options are required. Azacytidine have a very favorable toxicity profile and allow for outpatient treatment for these group of pediatric patients.

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AN ASSOCIATION OF VASCULITIS, MYELODYSPLASIA AND ETV6 DELETION IN A CHILD WITH REFRACTORY CYTOPENIA OF CHILDHOOD

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Myelodysplastic syndromes (MDS) are rare in children and characterized by peripheral cytopenias, hypocellularity of the bone marrow (BM), clonal cytogenetic abnormalities and a propensity to transform into Acute Myeloid Leukemia (AML). MDS coexisting with autoimmune disorders are generally diseases of adults. ETV6, an ETS family transcription factor maps to 12p13 and frequently acts as a tumor suppressor. Abnormalities of 12p have been noted in childhood AML. There have been no reports in literature of children with ETV6 deletion, MDS and vasculitis. We illustrate the association of childhood MDS and vasculitis in a child who was found to have a refractory cytopenia and somatic chromosome 12 abnormality with deletion of the ETV6 gene. A previously healthy 10 year boy presented with a 1mo h/o weight loss, fatigue and progressive non pruritic acral rash involving his extremities and pinnae. Skin biopsy revealed perivascular 3+ IgM deposits. He had an ANC of 500/μL, normal Hgb and platelets with no macrocytosis. Serum cryoglobulins were normal on 2 occasions. A thorough workup for infectious and rheumatological diseases was negative. Bone Marrow cellularity was 20% with no blasts, but had dysplastic appearing megakaryocytes and erythroid precursors. Fluorescent In Situ Hybridization (FISH) for abnormalities of chromosomes 5, 7, 8 and 20 was normal. BM karyotype was 46 XY but a clone with t (12; 15) was noted. FISH confirmed deletion of ETV6 with der (12) t (12; 15). FISH for ETV6-RUNX1 was negative for fusion products. Studies for IBMFS were negative and telomere lengths were normal. Sequential BM exam showed fluctuations in the size of the clone without increase in blasts; but his vasculitic rash appeared to wax and wane with the size of the clone. He underwent a well matched unrelated BM transplant and maintained his graft for 5 months. Recurrence of his vasculitic rash and cytopenias prompted a BM exam which confirmed relapse of myelodysplasia and recurrence of the ETV6 silenced clone. Subtle cytopenias in children with vasculitis may represent an underlying MDS and should prompt deeper investigation. Abnormal bone marrow cytogenetic abnormalities that can wax and wane have been noted in children – but this case represents an association of a Vasculitis, Myelodysplastic Syndrome and t (12; 15) not previously reported.

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A RARE CAUSE OF SECONDARY HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS: MYELODYSPLASTIC SYNDROME

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Introduction. Hemophagocytic lymphohistiocytosis (HLH) is an uncommon, hyperinflammatory syndrome caused by severe hypercytokinemia with excessive activation of lymphocytes and macrophages. HLH may represent as a primary congenital immune disorder, or it may develop secondary to infections, autoimmune diseases or malignancies. Here, we describe a case of HLH associated with myelodysplastic syndrome (MDS). *Case Report.* A 3 year-old boy presented with a fever, hepatosplenomegaly and pancytopenia. Peripheral blood smear showed 3% of normoblast, microcytic hypochromic anemia, anisopoikilocytosis and thrombocytopenia. Bone marrow aspiration revealed erythroid predominance with marked dyserythropoietic changes and limited lymphoid and myeloid dysplasia. Bone marrow biopsy showed 100% cellularity and increased number of histiocytes with hemophagocytosis. Natural killer (NK) cytolytic activity was measured as 7.3% (normal value >10%). Hair analysis for Griscelli sydrome and SAP/XIAP genes related with X-linked lymphoproliferative syndrome were normal. Testing for mutations in the genes associated with familial HLH, including PRF1, UNC13D, STX11 and STXPB2 (munc18-2) revealed no abnormality. Extensive workup for the secondary forms of HLH was done; but no cause was found. Cytognetic was normal and there was no evidence of chromosomal, klonal by FISH. HLH-2004 protocol was initiated. So that he had a complete clinical and laboratuary response to the treatment, the protocol was terminated at thirteen weeks. Four months later, he again presented with fever, hepatosplenomegly and pancytopenia. Bone marrow examination revealed increased number of severely dysplastic erythroid precursors (56%) and myeloblasts (10%). Deletion of 7q and monosomy 7 were detected 31% by FISH and it was confimed by cytogenetic. It is thought that, the patient developed HLH secondary to MDS at first admission and clonality was developed soon later. *Conclusions.* It is difficult to diagnose MDS in childhood because of the interference of signs and symptoms with many diseases. Hence non-clonal myelodysplasia may mimic many disorders, cytogenetic studies play a pivotal role in the diagnosis. The cytogenetic changes may be observed at the onset of symptoms or evolve over time as the disease progresses. This report highlights the importance of considering the diagnosis of Myelodysplastic Syndrome in patients with HLH with unusual features.

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HEMATOLOGICAL IMPROVEMENT OF RELAPSED CHILDHOOD ACUTE MYELOID LEUKEMIA WITH MULTILINEAGE DYSPLASIA BY AZACITIDINE AND DONOR LYMPHOCYTE INFUSIONS

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Background. Patients with acute myeloid leukemia (AML) or advanced myelodysplastic syndrome (MDS) who relapse early after allogeneic stem cell transplantation have a poor prognosis. Intensive chemotherapy may result in severe toxicities, prolonged cytopenia, and life-threatening infections. Some clinical studies of adults have shown that azacitidine (AZA) and donor lymphocyte infusions (DLI) is a tolerable and effective approach for relapsed MDS or AML after allogeneic stem cell transplantation. *Patients and Methods.* A three-year-old girl was diagnosed as having AML M4 with a FLT3-ITD mutation. She achieved complete remission after induction therapy consisted of fludarabine, cytarabine, granulocyte colony-stimulating factor (G-CSF), and idarubicin (FLAG-IDA) after unsuccessful two different induction regimens. She received allogeneic bone marrow transplantation (BMT) from a matched unrelated donor following a myeloablative conditioning regimen consisting of total body irradiation (TBI) 12Gy and melphalan. Four months after BMT she suffered from hematologic relapse with 1.5% blasts in peripheral blood and 24.0% blasts in bone marrow with multilineage dysplasia. Cytogenetic analysis revealed a complex karyotype, and, interestingly, the FLT3-ITD mutation was negative. Since intensive chemotherapy might cause severe toxicities with relatively low response rate, we then decided to treat her with AZA, a DNA methyltransferase inhibitor with proven antileukemic activity, and DLI as first salvage therapy. AZA was administered daily for 7 days and repeated every 4 weeks at doses of 75 mg/m^2/day followed by DLI (from 2 10^6 to 1 10^8 CD3+cells/kg) after every second AZA cycle. *Results*. After four cycles of AZA and two doses of DLI, bone marrow aspiration revealed a decrease in the percentage of blast cells from 30.4% to 10.0%, after seven cycles of AZA and three doses of DLI without requirement of platelet transfusion. During twelve cycles of AZA treatment, neither graft-*versus*-host disease nor obvious worsening of cytopenia was observed without any clear change of blood transfusion frequency. FLT3-ITD mutation remained negative throughout the treatment course. *Conclusions.* AZA and DLI was effective at least in restraining the disease progression for one year without any serious complications. This approach could be one therapeutic option for children with AML or advanced MDS relapsed early after allogeneic stem cell transplantation.

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AN UNUSUAL ASSOCIATION OF THERAPY RELATED CHRONIC MYELOMONOCYTIC LEUKEMIA AND MLL REARRANGEMENT IN A CHILD TREATED FOR ACUTE MYELOID LEUKEMIA

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Introduction. Therapy related Myelodysplastic Syndromes/AML (t-MDS/AML) are amongst the most devastating complications after cancer treatment that have dismal outcome. Abnormalities at 11q23 involving MLL gene are frequently found in t-MDS/AML following use of topoisomerase-II inhibitors, but anecdotal cases of t-CMML in childhood have been reported. *Aims.* To report an unusual presentation of t-CMML with MLL rearrangement (MLL+/t-CMML) in a child previously treated for AML. *Case.* A three-year-old girl was referred to our institution due to leukocytosis and presence of blasts in peripheral blood and was diagnosed with acute myelomonocytic leukemia. Cytogenetic analysis detected 51,X,t(X;11)(?q24;p15) and trisomy of chromosomes 6, 17, 19, 21 & 22. By FISH, MLL rearrangement and BCR-ABL fusion gene were ruled out. The patient followed treatment according to SHOP-LANL-07 protocol and received two induction courses with idarubicin, etoposide and cytarabine. She achieved complete remission and underwent consolidation therapy with two cycles of mitoxantrone and cytarabine. She had not siblings and underwent autologous hematopoietic stem cell transplantation (auto-HSCT) as per our national protocol and was conditioned with busulfan, cyclophosphamide and etoposide. Five months after auto-HSCT she presented with monocytosis and severe dysplastic features in peripheral blood. The bone marrow aspirate was hypercellular with global dyshemopoiesis. Karyotype showed 46,XX,t(9;11)(p22;q23),t(9;22)?(q34;q11)[2]/46,XX,t(9;11)(p22;q23)[18]. MLL rearrangement was confirmed by FISH, while no BCR-ABL fusion gene was detected by FISH nor PCR. N and K-RAS mutations were ruled out. She was diagnosed with MLL+/t-CMML and an unrelated umbilical cord blood HSCT was performed two months later. She is in clinical remission 10 months after HSCT. Discussion: CMML is very rare in children and is considered a secondary malignancy in childhood. Although new and more intensive chemotherapeutic regimens have resulted in higher cure rates in pediatric oncology patients, secondary malignancies are severe late-effects in cancer survivors. HSCT is currently the only curative treatment and some authors have suggested that the addition of hypomethylating agents may benefit pediatric t-MDS/AML patients. Here we report a rare association of MLL+/t-CMML presenting with short latency after treatment with etoposide in a child. Very few cases of such combination have been described in children.

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