10th International Symposium on MDS and SAA in Childhood

28-30 SEPTEMBER 2023 | LISBON

PROGRAM and BOOK OF ABSTRACTS

ORGANIZER

EWOG-SAA

EWOG-MDS

SOCIEDADE DE HEMATOLOGIA E ONCOLOGIA PEDIÁTRICA
DA SOCIEDADE PORTUGUESA DE PEDIATRIA

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WELCOME

Dear Colleagues and Friends,

It is our great pleasure to welcome you to the **10th International Symposium of the European Working Group on Myelodysplastic Syndrome (EWOG-MDS) and Severe Aplastic Anemia (SAA) in Childhood** in **Lisbon**.

From basic science to clinical care, we have an excellent program highlighting the novelties in SAA, MDS and predisposition syndromes, JMML and related disorders in children, adolescents, and young adults.

We invited young researchers and clinicians to share their work and become an active part of the group and had amazing feedback, with a growing number of international participants in all available formats.

With the “no child left behind” challenge and keeping the spirit of the last meeting in Athens, we hope to have an excellent meeting promoting growth and development in these areas.

With all that in mind, welcome to Lisbon!

Paula Kjollerstrom
Chair of the Local Organizing Committee

Charlotte Niemeyer
Chair of EWOG-MDS

Brigitte Strahm
Chair of EWOG-SAA

We are very grateful to the sponsors of the **Travel** and **Remote** grants:

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Hybrid 10th International Symposium
on MDS and SAA in Childhood

PROGRAM

Thursday, 28th SEPTEMBER 2023

09.00 – 12.00	**Introductory Session for young EWOG**	
12.00 – 12.45	**Registration for the Symposium**	
12.45 – 13.00	**Welcome to the Symposium** Paula Kjöllerström – Chair of the Local Committee Pierre Goncalves – Local Committee Charlotte Niemeyer – Chair of EWOG-MDS Brigitte Strahm – Chair of EWOG-SAA	
SESSION I: SEVERE APLASTIC ANEMIA Chairs: Ayami Yoshimi, Nuno Reis Farinha		
13.00 – 13.30		**RECENT ADVANCES AND LONG-TERM RESULTS OF MEDICAL TREATMENT OF ACQUIRED APLASTIC ANEMIA** Phillip Scheinberg, São Paulo, Brazil
13.30 – 14.00	**OC1**	**THE ROLE OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN APLASTIC ANEMIA** Brigitte Strahm, Freiburg, Germany
14.00 – 14.15	**OC2**	**ACCELERATED CLEARANCE OF ANTI-THYMOCYTE GLOBULIN IN CHILDREN TRANSPLANTED FOR REFRACTORY CYTOPENIA OF CHILDHOOD VERSUS APLASTIC ANEMIA IS ASSOCIATED WITH INCREASED RISK OF GVHD** Eva Koopman-Coenen*; Joyce Meesters-Ensing; Stefan Nierkens; Ireen Kal; Konradin Müskens; Birgitta Versluys; Caroline Lindemans; Marc Bierings; Rick Admiraal, Mirjam Belderbos *Utrecht, The Netherlands
14.15 – 14.30	**OC3**	**VARIABLE CLINICAL COURSES OF VARICELLA ZOSTER VIRUS INFECTION - OR VACCINATION-RELATED BONE MARROW FAILURE** Vasil Toskov*; Annamaria Cseh; Alexander Claviez; Natalia Rotari; Beatrice Drextler; Stephan Schwarz-Furlan; Matthias Braun; Peter Bader; Peter Lang; Rita Beier; Bernhard Erdlenbruch; Monika Führer; Miriam Erlacher; Charlotte M Niemeyer; Brigitte Strahm; Ayami Yoshimi *Freiburg, Germany

14.30 – 15.00		**SOMATIC MUTATIONS AND DYNAMICS OF CLONAL HEMATOPOIESIS IN ACQUIRED APLASTIC ANEMIA** Austin Kulasekararaj, London, UK
15.00 – 15.15	**OC4**	**NEXT GENERATION SEQUENCING APPROACH TO BONE MARROW FAILURE SYNDROMES – PORTUGUESE EXPERIENCE** Margarida Coucelo*; Joana Azevedo; Isabel Bogalho; Ana Teresa Simões; Ana Catarina Oliveira; Sara Batalha; Conceição Constanço; Teresa Melo; José Carlos Almeida; Joana Desterro; Patricia Ribeiro; Emilia Costa; Anabela Ferrão; Paula Kjöllerström; Catarina Geraldes *Coimbra, Portugal
15.15 – 15.30	**Coffee break**	

SESSION II: JUVENILE MYELOMONOCYTIC LEUKEMIA (I) Chairs: Tim Lammens, Valerie de Haas		
15.30 – 16.00	**OC5**	**NOVEL APPROACHES TARGETING THE RAS PATHWAY IN JMML** Eliot Stieglitz, San Francisco, USA
16.00 – 16.15	**OC6**	**THE INTERFACE OF EPIGENETICS AND ENERGY METABOLISM IN JUVENILE MYELOMONOCYTIC LEUKEMIA** Zoé Wehbe*, Ruba Hammad; Toni Cathomen; Sheila Bohler; Jovana Rajak; Miriam Erlacher; Foued Ghanjati; Charlotte Niemeyer; Christian Flotho *Freiburg, Germany
16.15 – 16.45	**OC7**	**CLONAL ARCHITECTURE IN JMML** Christian Flotho, Freiburg, Germany
16.45 – 17.00	**OC8**	**GENOMIC LANDSCAPE AND SINGLE BASE SUBSTITUTION MUTATIONAL SIGNATURES IN JUVENILE MYELOMONOCYTIC LEUKEMIA: A NEW JMML PORTRAIT** Alberto Peloso*; Andrea Binatti; Alice Cani; Concetta Micalizzi; Simone Cesaro; Laura Sainati; Franco Locatelli; Stefania Bortoluzzi; Riccardo Masetti; Silvia Bresolin *Padua, Italy

17.00 – 17.15	**OC9**	**HOMOZYGOUS CBL MUTATION IN B LYMPHOCYTES AFTER CBL-DRIVEN JMML IMPAIRS B CELL MATURATION, FUNCTION AND ANTIBACTERIAL IMMUNITY** Jonathan Bohlen*; Marine Michelet; Federica Barzaghi; Francesco Saettini; Francesca Vendemini; Albert Catala; Laia Alsina; Francesca Conti; Fillippo Consonni; Davide Learndini; Riccardo Masetti; Edoardo Muratore; Francesco Baccelli; Ivan Bagaric; Taja Vatovec; Feroj Seyed; Isabelle Andre; Lori Buetow; Eric Delabesse; Laetitia Largeaud; Cindy Ma; Laurent Abel; Steicy Sobrino; Masato Ogishi; Boris Bessot; Cecile Rouillon; Christine Bole; Yoann Seeleuthner; Tom Le Voyer; Darawan Rinchai; Jeremie Rosain; Peng Zhang; Matthieu Chaldebas; Anna-Lena Neehus; Lucia Erazo; Zarah Janda; Camille Soudee; Chantal Lagrese; Emmanuelle Six; Danny Huang; Stuart Tangye; Vivien Beziat; Eleonora Gambineri; Marinella Veltroni; Miriam Erlacher; Alessandro Aiuti; Marlene Pasquet; Jean-Laurent Casanova; Jacinta Bustamante *New York, USA
17.15 – 17.30	**OC10**	**BCR:ABL-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS IN CHILDREN AND ADOLESCENTS: INCREASED DISEASE BURDEN IN PATIENTS WITH JAK2 MUTATION, UNMET DIAGNOSTIC NEEDS AND OPTIMAL TREATMENT OPTIONS** Charikleia Kelaidi*; Kondylia Antoniadi; Loizos Petrikkos; Vassilios Papadakis; Maria Ampatzidou; Maria Kourti; Vasiliki Tzotzola; Aikaterini Bountali; Kalliopi Manola; Kalliopi Stefanaki; Sophia Polychronopoulou *Athens, Greece
17.30 – 18.00	**Official opening of the symposium** Nuno Reis Farinha - President of the Portuguese Society of Hematology and Oncology Paula Kjöllerström – Chair of the Local Committee Charlotte Niemeyer – Chair of EWOG-MDS Brigitte Strahm – Chair of EWOG-SAA	

PROGRAM

Friday, 29th September 2023

08.30 – 09.30	**Young EWOG welcome breakfast**	
SESSION III: JSPHO-EWOG SPEED SESSION AND SELECTED POSTERS Chairs: Brigitte Strahm, Yoshiyuki Takahashi		
08.00 – 08.30	**Methylation, SETBP1/JAK3, and CBL** Manabu Wakamutsu, Nagoya, Japan	
08:30 – 09:00	**Predisposition in JMML** Christian Flotho, Freiburg, Germany	
09:00 – 09:30	**Selected Posters**	
SESSION IV: JUVENILE MYELOMONOCYTIC LEUKEMIA (II) Chairs: Albert Catala, Jean-Pierre Gonçalves		
09.30 – 09.45	**OC11**	**SH2B3 GERMLINE MUTATION CAUSE A MULTISYSTEM DISORDER WITH PREDISPOSITION TO MYELOPROLIFERATIVE NEOPLASMS** Davide Leardini*; Sara Cerasi; Francesco Baccelli; Francesca Gottardi; Edoardo Muratore; Krisztián Miklós Kállay; Paula Kjollerstrom; Sara Batalha; Elisa Rumi; Valeria Santini; Marco Gabriele Raddi; Anupama Rao; Ana Rio-Machin; Riccardo Masetti *Bologna, Italy
09.45 – 10.00	**OC12**	**EARLY RELAPSE DETECTION IN JMML PATIENTS FOLLOWING HAEMATOPOIETIC STEM CELL TRANSPLANT** Susanne Kricke*; Anupama Rao; Eleni Louka; Katharine Patrick; Stuart Adams; Owen Williams; Elaine Cloutman-Green *London,UK
10.00 – 10.15	**OC 13**	**HOW TO DESIGN THE NEXT CLINICAL STUDY IN JMML?** Charlotte Niemeyer, Freiburg, Germany
10.15 – 10.30	**Coffee break**	
10.30 – 11.30	**Poster session I with Poster Walk (see list of the posters)**	
SESSION V: PATHOPHYSIOLOGY OF MYELODYSPLASTIC SYNDROME AND ACUTE MYELOID LEUKEMIA Chairs: Martina Rudelius, Charlotte Niemeyer		

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11.30 – 11.50	**OC14**	**IMPLICATIONS OF NOVEL CLASSIFICATION OF MYELOID NEOPLASMS FOR CHILDHOOD MDS** Katherine Calvo, Bethesda, USA
11.50 – 12.10	**OC15**	**WHAT CAN WE DEDUCE FROM GENETIC CLASSIFICATION OF MYELOID NEOPLASIA FOR THERAPY IN PEDIATRICS?** Henrik Hasle, Aarhus, Denmark
12.10 – 12.30	**Discussion**	
12.30 – 12.45	**OC16**	**THE NEW KID ON THE BLOCK: UBTF-TD** Miriam Erlacher, Freiburg, Germany
12.45 – 13.00	**OC17**	**IMPACT PROGNOSIS OF CYTOGENETIC ALTERATIONS IN BRAZILIAN CHILDREN WITH MYELODYSPLASTIC NEOPLASM** Viviane Lovatel*; Eliane Rodrigues; Beatriz Da Silva; Rita De Cássia Tavares; Amanda Fonte; Ana Paula Bueno; Teresa Fernandez *Rio de Janeiro, Brazil
13.00 – 13.30	**Lunch**	
13.30 – 14.30	**Poster session II with Poster Walk (see list of the posters)**	
SESSION VI: BONE MARROW FAILURE AND GERMLINE PREDISPOSITION Chairs: Valeria Santini, Petr Sedlacek		
14.30 – 15.00	**OC18**	**CLONAL HEMATOPOIESIS IN SHWACHMAN-DIAMOND SYNDROME: MECHANISM AND CLINICAL IMPLICATIONS** Akiko Shimamura, Boston, USA
15.00 – 15.15	**OC19**	**FUNCTIONAL ANALYSES OF RUNX1 VARIANTS IN THE CONTEXT OF FAMILIAL PLATELET DISORDER WITH PREDISPOSITION TO HEMATOLOGIC MALIGNANCIES** Melanie Decker*; Alisa Förster; Alina Prüne; Anne Seebacher; Alena Wittstock; Thomas Illig; Brigitte Schlegelberger; Tim Ripperger *Hannover, Germany

15.15 – 15.30	**OC20**	**GENOTYPE/PHENOTYPE ASSOCIATIONS IN 174 INDIVIDUALS WITH GERMLINE GATA2 MUTATIONS** Lili Kotmayer*; Emilia Kozyra; Maximilian Kaiser; Michael Dworzak; Barbara De Moerloose; Jan Starý; Henrik Hasle; Kirsi Jahnukainen; Sophia Polychronopoulou; Krisztián Kállay; Owen Smith; Shlomit Barzilai; Riccardo Masetti; Jochen Buechner; Marek Ussowicz; Paula Kjollerstrom; Ivana Boďová; Marko Kavcic; Albert Catala; Dominik Turkiewicz; Markus Schmugge; Valérie De Haas; Rebecca Voss; Anna Bigas; Damia Romero; Csaba Bödör; Miriam Erlacher; Alessandra Giorgetti; Charlotte Niemeyer; Marcin Wlodarski *Budapest, Hungary
15.30 – 15.45	**OC21**	**LOSS OF HSC STEMNESS IDENTITY IS ASSOCIATED WITH EXHAUSTION AND HYPORESPONSIVENESS IN GATA2 DEFICIENCY SYNDROME** Laëtitia Largeaud*; Vincent Fregona; Laura Jamrog; Camille Hamelle; Stephanie Dufrechou; Naïs Prade; Esmaa Sellam; Pauline Enfedaque; Manon Bayet; Sylvie Hebrard; Christine Didier; Eric Delabesse; Bastien Gerby; Marlène Pasquet; Cyril Broccardo *Toulouse, France
15.45 – 16.00	**OC22**	**GERMLINE LOSS-OF-FUNCTION MUTATIONS IN MDM4 CAUSE A NEW BONE MARROW FAILURE SYNDROME WITH TP53-DEPENDENT HEMATOPOIETIC CELL DEATH** Richa Sharma*; Senthil Velan Bhoopalan; Robert Meyer; Lei Han; Shondra M. Pruett-Miller; Claudia Khurana; Miriam Erlacher; Jean Soulier; Fabian Beier; Marcin Wlodarski *Memphis, USA
16.00 – 16.15	**Coffee break**	

SESSION VI: BONE MARROW FAILURE AND GERMLINE PREDISPOSITION (continued)
Chairs: Hannah Tamary, Kristián Kállay

16.15 – 16.45	**OC23**	**GENOME SEQUENCING APPROACHES FOR DIAGNOSIS AND DISCOVERY OF BMF/MDS SPECIFIC GENETIC ALTERATIONS** Marcin Wlodarski, Memphis, USA

16.45 – 17.00	**OC24**	**MYELODYSPLASTIC SYNDROME AND LEUKEMIA IS A SECONDARY EVENT AFTER BONE MARROW FAILURE IN GATA2 HAPLOINSUFFICIENT MICE** Cansu Koyunlar; Juncal Fernandez-Orth; Julia Weiss; Emanuele Gioacchino; Hans De Looper; Geoffrey Andrieux; Mariette Ter Borg; Baris Yigit; Joke Zink; Remco Hoogenboezem; Irene Gonzalez-Mendez; Eric Bindels; Mathijs Sanders; Ivo Touw; Miriam Erlacher; Emma De Pater* *Rotterdam, The Netherlands
17.00 – 17.15	**OC25**	**OPTICAL GENOME MAPPING: A NEW TOOL TO OVERCOME CONVENTIONAL CYTOGENETICS' LIMITATIONS IN PATIENTS WITH BONE MARROW FAILURE** Josune Zubicaray*; Ana Gomez; June Iriondo; Reyes Gimenez; Lorea Abad; Carmen Matasans; Elena Sebastian; Alejandro Sanz; Jesus Gonzalez De Pablo; Manuel Ramirez; Julian Sevilla *Madrid, Spain
17.15 – 17.30	**OC26**	**A COUNTRYWIDE STUDY OF GATA2 DEFICIENCY IN ITALY REVEALS NOVEL SYMPTOMS AND GENOTYPE-PHENOTYPE CORRELATION** Samuele Roncareggi*; Katia Girardi; Francesca Fioredda; Lucia Pedace; Luca Arcuri; Raffaele Badolato; Sonia Bonanomi; Erika Borlenghi; Emilia Cirillo; Tiziana Coliva; Filippo Consonni; Francesca Conti; Piero Farruggia; Eleonora Gambineri; Fabiola Guerra; Gaia Mancuso; Antonio Marzollo; Riccardo Masetti; Concetta Micalizzi; Daniela Onofrillo; Claudio Pignata; Valeria Santini; Francesca Vendemini; Andrea Biondi; Francesco Saettini *Monza, Italy
17.30 – 17.45	**OC27**	**CHEK2 GERMLINE VARIANTS AND ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT OUTCOMES** Atte K Lahtinen; Jessica Koski; Jarmo Ritari; Kati Hyvärinen; Satu Koskela; Jukka Partanen; Kim Vettenranta; Minna Koskenvuo; Riitta Niittyvuopio; Urpu Salmenniemi; Maija Itälä-Remes; Kirsi Jahnukainen; Outi Kilpivaara; Ulla Wartiovaara-Kautto; Maarja Karu* *Helsinki, Finland

PROGRAM

Saturday, 30th September 2023

SESSION VII: INNOVATIVE THERAPIES IN MYELODYSPLASTIC SYNDROME, ACUTE MYELOID LEUKEMIA AND JUVENILE MYELOMONOCYTIC LEUKEMIA Chairs: Anupama Rao, Henrik Hasle		
09.00-09.30		**NOVEL APPROACHES OF IMMUNE AND CELLULAR THERAPIES FOR HIGH RISK PEDIATRIC MYELOID MALIGNANCIES** Franco Locatelli, Rome, Italy
09.30-09.45	**OC28**	**PEDIATRIC MDS IN GATA2-DEFICIENCY: ENHANCED HISTONE TRIMETHYLATION AND DEREGULATED APOPTOSIS AS DRIVER?** Franziska Schreiber*; Guido Piontek; Yuki Schneider-Kimoto; Stephan Schwarz-Furlan; Rita De Vito; Franco Locatelli; Carole Gengler; Charlotte M. Niemeyer; Miriam Erlacher; Martina Rudelius *Munich, Germany
09.45-10.00	**OC29**	**VENETOCLAX-BASED THERAPIES IN PEDIATRIC ADVANCED MDS AND RELAPSED /REFRACTORY AML: A MULTICENTER RETROSPECTIVE ANALYSIS** Riccardo Masetti*; Francesco Baccelli; Davide Leardini; Francesca Gottardi; Francesca Vendemini; Alessandro Digangi; Marco Becilli; Mariachiara Lodi; Manuela Tumino; Luca Vinci; Miriam Erlacher; Brigitte Strahm; Charlotte M. Niemeyer; Franco Locatelli *Bologna, Italy
10.00-10.15	**OC30**	**JUVENILE MYELOMONOCYTIC LEUKEMIA (JMML) CELLS ESCAPE IMMUNE SURVEILLANCE BY MULTIPLE MECHANISMS** Jun Wang*; Jovana Rajak; Naile Koleci; Hui Xiao; Anton Niels Wehner; Bertram Bengsch; Juncal Fernandez-Orth; Charlotte Niemeyer; Sheila Bohler; Miriam Erlacher *Freiburg, Germany
10.15 – 10.30	**Coffee break**	

SESSION VII: INNOVATIVE THERAPIES IN MYELODYSPLASTIC SYNDROME, ACUTE MYELOID LEUKEMIA AND JUVENILE MYELOMONOCYTIC LEUKEMIA (continued) Chairs: Anupama Rao, Henrik Hasle		
10.30 - 11.00	**OC31**	**PATHWAY-DIRECTED THERAPY APPROACH IN PEDIATRIC MDS AND AML** Barbara De Moerloose, Ghent, Belgium
11.00-11.15	**OC32**	**DEVELOPMENT OF NEW GENE EDITING APPROACHES FOR BONE MARROW FAILURE AND MDS PREDISPOSITION SYNDROMES** Damian Krzyzanowski*; Mjad Khiami; Lei Han; Sushree Sahoo; Shondra Miller; Shengdar Tsai; Senthil Bhoopalan; Jonathan Yen; Marcin Wlodarski *Memphis, USA
11.15 – 11.30	**OC33**	**ILLUSTRATION OF CLONAL ARCHITECTURE IN JUVENILE MYELOMONOCYTIC LEUKEMIA BY TARGETED SINGLE-CELL DNA SEQUENCING** Foued Ghanjati*; Miriam Erlacher; Dirk Lebrecht; Peter Nöllke; Franco Locatelli; European Working Group Of Myelodysplastic Syndromes In Childhood; Charlotte Niemeyer; Christian Flotho *Freiburg, Germany
11.30-11.45	**OC34**	**ONCO-FETAL REPROGRAMMING DRIVES HIGH-RISK JUVENILE MYELOMONOCYTIC LEUKEMIA, WHICH CAN BE TARGETED BY ANTI-CD52 TREATMENT** Mark Hartmann; Maximilian Schönung; Jovana Rajak; Joschka Hey; Valentin Maurer; Ling Hai; Sina Staeble; Jens Langstein; Katharina Bauer; Mariam Hakobyan; Laura Jardine; Sheila Bohler; Dominik Vonficht; Abdul-Habib Maag; Dirk Lebrecht; Katrin M. Bernt; Roland Roelz; Tobias Boch; Eleonora Khabirova; Pavlo Lutsik; Simon Haas; Muzlifah Haniffa; Sam Behjati; Jan-Philipp Mallm; Christian Buske; Michael D. Milsom; Stefan Fröhling; Marc-Jan Bonder; Charlotte Niemeyer; Christian Flotho; Christoph Plass; Miriam Erlacher; Matthias Schlesner; Daniel B. Lipka* *Heidelberg, Germany
11.45 – 12.45	**Closing remarks and presentation of poster prizes**	

OC1

THE ROLE OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN APLASTIC ANEMIA

Authors: **Brigitte Strahm**[1]

Affiliations: 1 - Division of Pediatric Hematology and Oncology, Department of Pediatrics and Adolescent Medicine, Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany

Background and aims

Hematopoietic stem cell transplantation (HSCT) is a well established curative treatment for patients with severe aplastic anemia (SAA). Improved outcomes with excellent survival probabilities have resulted in the application of HSCT in an increasing number of patients (incl. treatment naïve or older individuals), the increased use of unrelated and haploidentical donors and the growing need to assess long-term effects.

Methods

In EWOG-SAA upfront HSCT of pediatric patients from a matched sibling donor peformed after conditioning with cyclophosphamide
(20) or cyclophosphamide/anti-thymocyte-globuline (29) has resulted in an excellent outcome with a probability of overall survival (pOS) of 95% and 97%, respectively. The rate of primary and secondary graft failure was 2% and 8%, respectively and chronic GvHD was documented in 4% of patients.

Results

Patients transplanted after failure of immunesuppressive therapy (IST) from a matched (48) or mismatched (32) unrelated donor had an inferior outcome with a pOS of 82%. Interestingly, failure of IST with thymoglobuline® was associated with a lower pOS compared to failure following Atgam® (62% vs 89%, p=0.08). The selected treatment naïve patients who received a MUD-HSCT (21) had an excellent outcome with no graft failure, two patients with chronic GvHD (1 mild, 1 severe) and all but one patients being alive after a median FUP of 2.0 (0.3-5.9) years.

Conclusions

In view of these results, EWOG-SAA has changed the recommendations for HSCT in pediatric patients with SAA. To reduce the potential late effects of high dose cyclophosphamide MSD-HSCT is now performed following a fludarabine containing regimen with a lower dose of cyclophosphamide. In addition, selected patients are eligible for upfront MUD-HSCT if a donor is available and HSCT can be performed in a timely manner. Patients following failure of IST with no matched donor are eligible for haploidentical HSCT.
However, the best approach for these challenging HSCT needs to be determined.

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OC2

ACCELERATED CLEARANCE OF ANTI-THYMOCYTE GLOBULIN IN CHILDREN TRANSPLANTED FOR REFRACTORY CYTOPENIA OF CHILDHOOD VERSUS APLASTIC ANEMIA IS ASSOCIATED WITH INCREASED RISK OF GVHD.

Authors: **Eva Koopman-Coenen**[1]; Joyce Meesters-Ensing[1]; Stefan Nierkens[1,3]; Ireen Kal[1]; Konradin Müskens[1]; Birgitta Versluys[1]; Caroline Lindemans[1,2]; Marc Bierings[1,2]; Rick Admiraal[1]; Mirjam Belderbos[1]

Affiliations: 1 - Princess Máxima Center of Pediatric Oncology, Utrecht, the Netherlands; 2 - Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, the Netherlands; 3 - Center for Translational Immunology, University Medical Center Utrecht, Utrecht, the Netherlands

Key words: MDS-RCC, SAA, graft-versus-host-disease, anti-thymocyte globulin, conditioning, transplantation

Background and aims

Myelodysplastic syndrome–refractory cytopenia of childhood (MDS-RCC) and severe aplastic anemia (SAA) are two forms of pediatric bone marrow failure that share similar clinical features but have different pathophysiology. Hematopoietic cell transplantation (HCT) is a curative therapy for both conditions. While the outcome of HCT for SAA is excellent, there is limited knowledge on the outcome of HCT for MDS-RCC. This study aims to compare HCT outcomes for MDS-RCC and SAA and explore the impact of anti-thymocyte globulin (ATG) exposure on these outcomes.

Methods

Consecutive patients with MDS-RCC and SAA, diagnosed according to EWOG guidelines and treated between January 1994 and December 2021 in our institute, were included. Primary outcome was grade 2-4 acute graft-versus-host-disease (aGvHD)-free, event- free survival (GEFS). HCT outcome was related to diagnosis (MDS-RCC versus SAA) and to measured ATG exposure using a pharmacokinetic model.

Results

25 MDS-RCC and 24 SAA patients were included. The median age at HCT was 13.4 versus 10.2 years, respectively. ATG exposures were available for 13 versus 15 patients. Conditioning was mainly busulfan/treosulfan-based in MDS-RCC (64%), and cyclophosphamide/fludarabine-based in SAA (92%). GEFS in MDS-RCC was 34%, compared to 70% in SAA (hazard ratio (HR) 0.42, p=0.064). Overall survival was 79% versus 96% (p=0.12). The incidence of aGvHD was significantly higher in MDS-RCC compared to SAA (44% versus 8%, HR 0.14, p=0.015). Despite similar dose ranges, MDS-RCC patients had accelerated ATG clearance, resulting in lower post-HCT exposure compared to SAA (median 2.5 AU*day/L versus 31.9 AU*day/L, p=0.002). Importantly, low post-HCT ATG exposure was strongly correlated with aGVHD (HR 0.90, p=0.013).

Conclusions

HCT for MDS-RCC is associated with accelerated clearance of ATG and a higher incidence of GvHD compared to SAA. Insight into the differential mechanisms that affect ATG clearance in bone marrow failure patients is needed to define the optimal dosing regimen and to improve HCT outcome.

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OC3

VARIABLE CLINICAL COURSES OF VARICELLA ZOSTER VIRUS INFECTION- OR VACCINATION-RELATED BONE MARROW FAILURE

Authors: Vasil Toskov[1]; Annamaria Cseh[2]; Alexander Claviez[7]; Natalia Rotari[1]; Beatrice Drextler[1,3]; Stephan Schwarz-Furlan[4,5]; Matthias Braun[6]; Peter Bader[8]; Peter Lang[9]; Rita Beier[10]; Bernhard Erdlenbruch[11]; Monika Führer[12]; Miriam Erlacher[1]; Charlotte M Niemeyer[1,13]; Brigitte Strahm[1]; Ayami Yoshimi[1]

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Key words: varicella zoster virus, pancytopenia, aplastic anemia

Background and aims

Although mild hematological complications such as isolated neutropenia, anemia or thrombocytopenia are relatively common after primary varicella zoster virus (VZV) infection, severe pancytopenia after VZV infection or vaccination is extremely rare.

Methods

We report five children, who developed severe pancytopenia with bone marrow hypoplasia after VZV infection (n=4) or vaccination (n=1) between 2004 and 2022 in Germany. We summarized the clinical course of these patients (1 male, 4 female).

Results

The median age at diagnosis was 5 (1-6) years. Four patients developed pancytopenia a median of 3 weeks (5 days-4 weeks) after primary VZV infection and one patient 3 month after VZV vaccination. The median neutrophil count, the platelet count and hemoglobin level were 0.2 (0.02-0.65) G/L, 85 (0-97) G/L and 8.6 (5.1-9.0) g/dl, respectively. The histological diagnosis was aplastic anemia in two patients, while it was more compatible with refractory

cytopenia of childhood in remaining patients. Two patients were treated with intravenous immunoglobulins (IVIGs): one patient showed slow hematological recovery, whereas the other patient was rescued by allogenic hematopoietic stem cell transplantation (HSCT) due to progressive cytopenia 6 months after diagnosis. Immunosuppressive therapy (IST) with anti-thymocyte globulin and cyclosporine (n=2) led to complete response in one patient and non-response and subsequent HSCT 5 months after IST in another patient. One patient received primary HSCT. All patients are alive at the median follow-up of 3 (0.5-7) years after diagnosis.

Conclusions

This study highlights the variable clinical courses of VZV-associated bone marrow failure. Although some cases with spontaneous recovery and response to IVIGs have been reported in literature, most patients require intensive therapy including allogeneic HSCT, which points towards a persisting immune-mediated disease triggered by infection/vaccination.

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OC4

NEXT GENERATION SEQUENCING APPROACH TO BONE MARROW FAILURE SYNDROMES – PORTUGUESE EXPERIENCE

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Key words: Bone Marrow Failure Syndromes, Genetics, Telomeres, Diagnosis, NGS

Background and aims

Bone marrow failure syndromes (BMF) are a heterogeneous group of inherited or acquired diseases that share similar clinical features and high genetic heterogeneity. The differential diagnosis between these entities is essential for an accurate diagnosis and therapeutic adequacy. The aim was analyse the results of a national BMF panel and to test a qPCR-Assay to Telomere Length quantification.

Methods

251 patients were evaluated (2019-2023) using a customized NGS panel including 101 genes, excluding Fanconi Anemia. qPCR Assay for Telomere Length quantification was performed according instructions (ScienCell). Patients were grouped: classic BMF (cBMF,n=58); non-classic BMF/cytopenias (uBMF,n=110); MDS/AL/JMML (n=38); AA(n=45).

Results

A total of 63 germline variants were identified: 32 pathogenic, 31 VUS; and 2 somatic mutations. In the cBMF group the diagnosis was clarified in 20.7% of patients: 4/12 with suspected DBA (2 RPS19,RPL35,RPL5), 6/22 with neutropenia/suspected SBDS (4 SBDS,SRP54,GATA2); 2/24 thrombocytopenia (WAS,GATA2). In the uBMF group, 15 VUS and 6 pathogenic variants were identified allowing a diagnosis in 5.5% of patients: 2 predisposition syndromes (SAMD9L,DDX41), telomeropathy (RTEL1), DBA-like (GATA1), immunodeficiency (LIG4) and Cartilage-hair hypoplasia (RMRP).In MDS/LA/LMMJ group, 11 pathogenic variants were identified: 8 associated with a predisposition syndrome (GATA2,2RUNX1,2 DDX41,ERCCL2,TP53,NF1), 1 Shwachman-Diamond syndrome and 2 immunodeficiency (STK4,RAB27A); 2 patients had only somatic mutations (RUNX1,NF1). In the AA group, TERT (2) and MPL variants were identified in 3 patients, reclassifying them. In 4% of patients (10/251) we found VUS in telomeropathies-related genes, 4 of them with telomere length <1st percentile for age, strengthening the

pathogenicity of the variant and supporting a diagnosis.

Conclusions

NGS approach clarified the diagnosis in 12.7% of patients involving 22 different genes. Six uBMF and 3 AA had a genetic diagnosis, and patients were reclassified. Telomere Length is a useful complementary assay in patients with VUS in telomeropathies-related genes, allowing to support the diagnosis in 4 other patients.

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OC5

NOVEL APPROACHES TARGETING THE RAS PATHWAY IN JMML

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Key words: JMML, DNA methylation, Azacitidine, Trametinib, Risk stratification

Background and aims

Juvenile myelomonocytic leukemia (JMML) is an aggressive myeloproliferative disorder of childhood. The biochemical hallmark of JMML is aberrant signaling through the Ras pathway caused by initiating mutations in NF1, NRAS, KRAS, RRAS, RRAS2, SH2B3, PTPN11 and CBL. While hematopoietic stem cell transplantation (HSCT) can be curative, short- and long-term toxicity remains substantial. Recently, several studies have identified mutational burden and DNA methylation as being predictive of outcome in JMML. This trial sponsored by the Therapeutic Advances in Childhood Leukemia (TACL) consortium aims to risk stratify newly diagnosed patients to receive optimal intensities of therapy.

Methods

In this trial, lower-risk patients are defined those with one somatic alteration AND low DNA methylation while high-risk patients are defined as those with more than one somatic alteration OR intermediate/high DNA methylation. Lower-risk patients will receive trametinib and azacitidine for up to 12 courses and will only proceed to HSCT in the event of progressive disease. High-risk patients will receive trametinib and azacitidine (Aza) in combination with cytarabine and fludarabine for up to two courses and then will proceed to off-protocol HSCT.

Results

The primary objectives of this study are to determine the safety of combining trametinib with Aza for patients with newly diagnosed lower-risk JMML and to determine the safety of combining trametinib with azacitidine, fludarabine (FLA) and cytarabine for patients with newly diagnosed high-risk JMML.

Secondary objectives are to describe the event free survival (EFS) in patients with newly diagnosed lower-risk JMML treated with trametinib and azacitidine who do not proceed to stem cell transplant within 1 year and to determine the rate of molecular response pre-transplant using trametinib plus Aza-FLA in patients with newly diagnosed high-risk JMML.

Conclusions

This trial sponsored by TACL will enroll newly diagnosed JMML patients and risk stratify patients based on mutational burden and DNA methylation for the first time.

OC 6

THE INTERFACE OF EPIGENETICS AND ENERGY METABOLISM IN JUVENILE MYELOMONOCYTIC LEUKEMIA

Authors: **Zoé Wehbe**[1]; Ruba Hammad[2]; Toni Cathomen[2]; Sheila Bohler[1]; Jovana Rajak[1]; Miriam Erlacher[1]; Foued Ghanjati[1]; Charlotte Niemeyer[1]; Christian Flotho[1]

Affiliations: 1 - Department of Pediatrics and Adolescent Medicine, Division of Pediatric Hematology and Oncology, Freiburg, Germany; 2 - Institute for Transfusion Medicine and Gene Therapy & Center for Chronic Immunodeficiency, Freiburg, Germany Key words: JMML, IPSC, Mitochondrial respiration, Epigenetics

Background and aims

Juvenile myelomonocytic leukemia (JMML) is a hematopoietic neoplasm that results in excessive proliferation of leukemic monocytes and granulocytes and presents as clinically distinct subtypes depending on genetic alterations. Previous studies have identified DNA methylation signatures that correlate with clinical, genetic, and prognostic features. However, no metabolic studies have been published to date. The aim of this study is to investigate the metabolic flexibility of JMML-initiating cells and the role of energy metabolism for the risk of relapse after hematopoietic stem cell transplantation (HSCT).

Methods

The gene and protein expression of metabolic pathways in leukemic cells from 18 patients with JMML were investigated using RNA sequencing (N=10), proteomics (N=10), and Seahorse technology (N=8). Induced pluripotent stem cells (IPSCs) were generated from bone marrow cells of 7 patients, to be used as preclinical model.

Results

Preliminary data suggest an association of DNA methylation category and energy metabolism in JMML cells, as evident from the expression levels of oxidative phosphorylation genes and proteins, and mitochondrial respiration measured in culture. Cases with high DNA methylation, known to be at increased risk of relapse after HSCT, exhibited low energy metabolism in leukemic cells. Conversely, low DNA methylation was associated with higher energy consumption and a more favorable disease course after HSCT. Interestingly, 4 of 5 patients with a PTPN11 mutation and high or intermediate methylation belonged to the low energy group. Seahorse analysis performed on IPSCs showed a relatively similar energetic value between cells, regardless of the mutation of origin.

Conclusions

Taken together, the results suggest that JMML-initiating cells at a low metabolic state may be more resistant to myeloablative chemotherapy. Our next step will be to extend the study to metabolic states in myelomonocytic cell populations differentiated from JMML-derived IPSC.

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OC7

"CLONAL ARCHITECTURE IN JUVENILE MYELOMONOCYTIC LEUKEMIA"

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Background and aims

Juvenile myelomonocytic leukemia results from the acquisition of genetic and epigenetic alterations in a hematopoietic stem/progenitor cell population, sometimes in the context of a predisposing congenital condition. The significance of founding driver mutations in one of five genes of the Ras signal transduction pathway has long been a paradigm in the understanding of the pathogenesis of JMML; however, advances in high-throughput sequencing have led to a more complex picture, describing secondary alterations in defined target genes with prognostic relevance and evolution of such secondary lesions during the longitudinal time course of the disease. In addition, the subclonal allelic frequencies of variants indicate a previously unrecognized intratumoral diversity of leukemic cells. Recent single-cell DNA sequencing technologies allow the mapping of existing subclones with various genetic alterations and, unlike previous bulk sequencing approaches, have the capability of resolving not only linear but also branching architectures of clonal evolution. This presentation will review the current knowledge of clonal origin and clonal dynamics in JMML and discuss the potential significance of genetic and epigenetic diversity of leukemic cells for the disease course and outcome.

OC 8

GENOMIC LANDSCAPE AND SINGLE BASE SUBSTITUTION MUTATIONAL SIGNATURES IN JUVENILE MYELOMONOCYTIC LEUKEMIA: A NEW JMML PORTRAIT

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Key words: JMML, WES, Mutational Signature

Background and aims

Juvenile myelomonocytic leukemia (JMML) is a rare myeloproliferative/myelodysplastic neoplasm of early childhood, showing a high heterogeneity and poor prognosis. Next Generation Sequencing (NGS) allowed to enhance the knowledge of the molecular mechanisms driving JMML and to implement risk stratification towards an improvement of diagnostic and therapeutic precision. Despite the definition of several JMML driver mutations, the entire somatic mutational burden has never been considered to identify the underlying mutational processes leading to tumor development.

Methods

To characterize the genomic landscape of JMML, we performed Whole Exome Sequencing (WES) from the bone marrow of 20 patients at diagnosis and the corresponding normal matched samples; in some patients, relapses were also available to assess clonal dynamicity. iWhale pipeline was used to identify variants. We generated 96-context single base substitution (SBS) mutational catalogs and used SigProfiler to extract the relative mutational signatures.

Results

Although the most variable genes were related to the already known RAS pathway mutations, new clonal mutations were identified by WES analysis. Moreover, coexistence of secondary mutations in RAS pathways genes with driver genes has been found in 2 patients. To investigate the hidden mutational processes of JMML disease, we took advantage of WES data and described JMML mutational signatures. Two mutational signatures were identified and decomposed mainly into COSMIC SBS5, SBS15, SBS42 and SBS1. Interestingly, in a recent study, clock-like signatures SBS1 and SBS5 have been found across multiple pediatric cancers, showing a correlation with age. The SBS15 signature is associated with defective DNA mismatch repair and was already found in pediatric acute lymphoblastic leukemia.

Conclusions

Together these findings confirm the coexistence of RAS pathway mutations in JMML patients while the identification of multiple mutational signatures, highlight the importance of considering the whole mutational burden, to better dissect the pathogenesis of JMML, suggesting a possible mutational process underlying JMML disease.

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OC 9

HOMOZYGOUS CBL MUTATION IN B LYMPHOCYTES AFTER CBL-DRIVEN JMML IMPAIRS B CELL MATURATION, FUNCTION AND ANTIBACTERIAL IMMUNITY

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Key words: CBL Syndrome, B cells, Bacterial Infection, Inborn error of immunity, IEI

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Background and aims

Germline monoallelic Casitas B-Lineage Lymphoma Proto-Oncogene (CBL) mutations underlie a RASopathy called CBL Syndrome, and predispose to juvenile monomyelocytic leukemia (JMML). Typically, this JMML is driven by loss-of-heterozygosity (LOH) of CBL by uniparental isodisomy (UPD). CBL-driven JMML usually regresses spontaneously, but renders the hematopoietic system homozygous for mutated CBL. Whether and how this genetic transformation affects the adaptive immune system is unclear.

Methods

We studied humoral immunity and B cell function in 9 patients with CBL mutation and LOH. They suffered from life-threatening bacterial infections (n = 5), autoinflammation (n = 3) and autoimmunity (n = 2). We quantified immunoglobin levels, humoral responses to vaccines and infections. Furthermore, we studied B cell function in terms of immunoglobulin production and isotype switching in vitro. We characterized the mosaicism for LOH in mature leukocyte subsets by amplicon sequencing.

Results

We detect monocytosis (1397+/-322 monocytes/µl) and B cell lymphocytosis (1107+/-122 B cells/µl) in all pediatric patients. Immature transitional B cells are particularly overrepresented compared to age-matched children (409 +/- 169 cells/µl). Five patients have hypergammaglobulinemia (17,2+/-1,6mg/dl IgG) and two produce antibodies targeting auto-antigens (ANA, LAC, actin, smooth muscle). Sorted B cells of all tested patients produce reduced levels of immunoglobulins in vitro in response to B cell receptor and TLR activation (5to10-fold decreased) but normal levels upon IL-21. We quantified the allele burdens in sorted B-cell subsets. We find that the frequency of B cells lacking LOH is lowest in transitional B cells compared to naïve and memory, where heterozygous cells are 5-fold and 18-fold more frequent, respectively. This suggests that in vivo, maturation and differentiation of LOH B cells is significantly impaired.

Conclusions

In summary, patients that recover from CBL-driven JMML have impaired B cell function and anti-bacterial immunity. A B cell intrinsic role of CBL in the maturation and differentiation pathway can be assumed.

OC 10

BCR:ABL-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS IN CHILDREN AND ADOLESCENTS: INCREASED DISEASE BURDEN IN PATIENTS WITH JAK2 MUTATION, UNMET DIAGNOSTIC NEEDS AND OPTIMAL TREATMENT OPTIONS

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Key words: Myeloproliferative, thrombocytosis, JAK2

Background and aims

Background: Due to their rarity, BCR::ABL negative myeloproliferative neoplasms (MPN) in children and adolescents, should be analyzed by cooperative groups.

Methods

Methods: We performed a combined retrospective, from 1986 and prospective from 2017 onward, cohort study of children and adolescents with MPN diagnosed/referred to our Department of Pediatric Hematology-Oncology. Patients included in 2022 had biobanking according to EWOG-MDS-SAA standards.

Results

Results: Twenty-three patients were included, 10/13 boys/girls, of whom 15, 8 and 1 presented with thrombocytosis, erythrocytosis and the Budd-Chiari syndrome, respectively. Classification was essential thrombocytosis (ET), polycythemia vera (PV) and MPN-NOS, in 10, 8 and 5 patients, respectively. Median age in ET, MPN-NOS and PV was 11.8, 14 and 9.6 years, respectively. $JAK2^{mut}$ was found in 7 cases (V617F in 6, exon12 in 1) among those tested for the mutation: 4/9 patients with ET, 2/4 with MPN-NOS, 1/4 with PV. Ten patients were triple negative for JAK2/CALR/MPL^{mut} in ET/MPN-NOS (N=7), and for JAK2V617F/exon12/$EPO\text{-}R^{mut}$ in PV (N=3). Three of 7 $JAK2^{mut}$patients were diagnosed in 2022. With a median follow-up of 6 years, 10-y EFS and OS were 66% and 100%, respectively. Four $JAK2^{mut}$ patients had 6 major thrombotic events (cardiovascular N=3, cerebral vein N=1, Budd-Chiari N=2) and 1 episode of preeclampsia. $JAK2^{mut}$ patients had lower EFS than triple-negative patients (10-y EFS 21% vs. 100%, resp., P=0.05). Treatment included hydroxyurea (N=5/response rate (RR)=3/5), PEG-interferon alpha (N=4/RR=2/4), anagrelide (N=4/RR=0), acetylsalicylic acid and phlebotomy. Two triple-negative patients had an ET-like disease with spontaneous remission after ≥2y from diagnosis.

Conclusions

Children and adolescents with a triple-negative MPN had an unmet diagnostic need, although with a low risk of events. In contrast, disease burden was high in $JAK2^{mut}$, and treatment options were unclear. Collaborative efforts could unveil the origin and trajectories of MPN in children, and issue treatment recommendations.

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OC 11

SH2B3 GERMLINE MUTATION CAUSE A MULTISYSTEM DISORDER WITH PREDISPOSITION TO MYELOPROLIFERATIVE NEOPLASMS

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Key words: SH2B3, myeloproliferative neoplasms, cancer predisposing symdromes, thrombocytosis, JMML-like neoplasm

Background and aims

The SH2B adaptor protein 3 (SH2B3) gene encode for an adapter protein that regulates multiple signaling pathways in hematopoietic cells. Somatic SH2B3 mutations have been described in myeloproliferative neoplasms and in acute lymphoblastic leukemia. Little evidence is available on the role of germline mutations in development and disease, particularly in children.

Methods

We conducted a multicenter, international study to collect clinical information on patients harboring germline homozygous or heterozygous mutations in SH2B3. Data was gathered using an electronic case report form.

Results

Eleven patients from 6 families from 3 countries (Italy, Hungary, and Portugal) were included. Eight patients presented heterozygous mutation (c.1697G>A, c.1A>G, c.622G>C) while 3 homozygous (c.1174C>T, c.1A>G). Notably, two patients with heterozygous mutations harbored a germline heterozygous CBL mutation (c.1141T>C). Nine patients were studied for a hematological phenotype, while 2 as relatives of affected patients. Among those with hematological alterations, 3 patients presented with severe thrombocytosis (>1,000,000/µL) which manifested at various ages, ranging from birth to 15 years and spontaneously resolved. Two patients were diagnosed with polycythemia vera with a co-occurring somatic mutation in JAK2 (V617F) at 42 and 67 years of age, respectively. Two sisters developed a JMML-like neoplasm in the first months of life, with 2% and 7% of blast, respectively, and they both underwent hematopoietic stem cell transplantation. The two remaining patients with hematological alterations exhibited immune cytopenia, and neutropenia with mild dysplastic features. Homozygous patients presented an earlier onset of the phenotype. Other clinical manifestations included splenomegaly (n=5), dysmorphisms (n=4), cognitive defects (n=3), kidney alterations (n=2), growth retardation (n=2), vitiligo (n=1), Crohn's disease (n=1), idiopathic juvenile arthritis (n=1).

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Conclusions

SH2B3 germline mutation is associated with a multisystemic disorder with hematological involvement consisting of thrombocytosis and JMML-like neoplasm in children. More extensive studies are warranted to provide a better characterization of the disease's clinical phenotype and natural progression.

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OC 12

EARLY RELAPSE DETECTION IN JMML PATIENTS FOLLOWING HAEMATOPOIETIC STEM CELL TRANSPLANT

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Key words: Juvenile Myelomonocytic Leukaemia, Haematopoietic stem cell transplant, Chimerism, Microchimerism, Mutation burden, Minimal Residual Disease

Background and aims

JMML is a rare and aggressive childhood leukaemia, with haematopoietic stem cell transplantation (HSCT) as the only curative treatment available. Approximately 30% of children with JMML relapse following HSCT requiring a second HSCT. Due to the lack of robust markers for minimal residual disease detection, it is often very difficult to detect disease relapse early. Chimerism analysis is currently one of very few indicators of disease relapse but standard methods detect >1% recipient cells, hence methods with greater sensitivity would be beneficial detecting as low as 0.01% cells (microchimerism). Detectable recipient chimerism and mutation burden usually precede leukaemic relapse. This study assesses the role of microchimerism and mutation burden in blood and bone marrow in detecting early relapse using hyper-sensitive laboratory methods. Further, the study assesses whether blood analysis alone can indicate early relapse, leading to a reduction in bone marrow sampling.

Methods

The project studies these predictive relapse indicators in a retrospective cohort (transplanted 2018-2022 UK-wide, n=13). The retrospective cohort's samples are bio-banked. Paired blood and bone marrow samples from this cohort are analysed using qPCR and ddPCR methods to assess microchimerism in individual cell lines, and JMML-associated mutations within the same cell lines.

Results

Preliminary results indicate that recipient microchimerism is indeed present at <1% in patients initially observed to be fully engrafted by standard chimerism testing, who go on to relapse within the first 3 months post-BMT.

Conclusions

The project builds a detailed picture of relapse kinetics in patients with JMML across the mutation subgroups. It is expected that blood analysis has a sufficient predictive quality leading to a reduction in bone marrow sampling. Using data from the retrospective cohort, we aim to validate a risk stratification algorithm on a prospective cohort of JMML patients, identifying early relapse and the impact of immunomodulatory therapies on relapse kinetics.

OC13

HOW TO DESIGN THE NEXT CLINICAL TRIAL IN JMML

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Background and aims

Therapy decisions for a child with juvenile myelomonocytic leukemia (JMML) require a detailed understanding of JMML pathobiology and range from observation to allogeneic hematopoietic stem cell transplantation (HSCT). Recently, DNA methylation categories augmented clinical, hematological and genetic risk criteria for inferior outcome. While the cross-continental development of a common molecular classifier allows prospective assignment of DNA methylation classes in clinical trials, the analysis is not readily available in all countries. In this presentation, I will consider requirements for a molecularly driven risk-stratification in future clinical trials.

Methods

EWOG-MDS registry data of patients with JMML transplanted over a 20-year period were analysed for risk parameters predictive of outcome.

Results

Between genetic subtypes, the clinical and hematological genotype-phenotype correlation is poor with the exception of syndromic features. Within the five major genetic JMML subgoups (PTPN11-, NRAS-, KRAS-, NF1-, CLB-mutated JMML) all risk criteria including hemoglobin F (HbF) and methylation class stratify for outcome. Applying HbF and methylation class for risk criteria results in patient groups with similar characteristics and clinical outcome.

Conclusions

Improved risk stratification moved JMML from a rare disease to a group of ultra-rare genetic distinct entities. To ensure the best possible care for every child affected by these entities, word wide clinical studies are needed. Depending on availability and ressources, study design may have to take in to accout both, HbF and methylation class for risk stratification.

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OC14

IMPLICATIONS OF NOVEL CLASSIFICATIONS OF MYELOID NEOPLASMS FOR CHILDHOOD MDS

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Background and aims

Classification of pediatric myeloid neoplasms by expert panels of pathologists and clinicians is critical for international standardization of diagnostic criteria, unification of disease terminology, informing therapy, clinical trials, and research. Overall MDS in children has important differences from MDS in adults including hypocellular marrow, presentation with neutropenia and/or thrombocytopenia, different mutational landscape, and a higher proportion of germline predisposition warranting separate classification. The WHO Classification of Tumours of the Haematopoietic and Lymphoid Tissues (WHO 2008 and WHO4thR 2017 editions) introduced the provisional entity of refractory cytopenia of childhood (RCC) which encompassed histopathology, morphologic, cytologic, cytogenetic and molecular features of the most common subtype of MDS in children, recognizing that differentiation from bone marrow failure syndromes and acquired aplastic anemia may be challenging. In 2022, two new updated classifications emerged: International Consensus Classification (ICC) and WHO 5th edition (WHO5th).

Methods

The WHO5th and ICC classifications were reviewed in detail for comparison of differences, similarities, and potential impact.

Results

The two new classifications have important changes from the WHO4thR, and differ in the approach to pediatric MDS diagnosis. WHO5th: 1) eliminates RCC replacing it with childhood MDS with low blasts, hypocellular and childhood MDS with low blasts, NOS, and 2) excludes MDS cases with germline pathogenic variants, which are classified under secondary MDS along with therapy related MDS. In contrast, ICC: 1) retains RCC, 2) expands RCC to include manifestations of MDS in patients with germline predispositionsyndromes and 3) hematologic neoplasms with germline predisposition is a distinct category separate from other secondary myeloid neoplasms.

Conclusions

Fundamental differences in the ICC and WHO5th classifications have implications for diagnosis, treatment, clinical trials, and research of pediatric MDS within the international community. Unification of the classifications in the future is critical for patient care and standardization of diagnostic criteria.

OC15

WHAT CAN WE DEDUCE FROM MOLECULAR CLASSIFICATION OF MYELOID NEOPLASIA FOR THERAPY IN PEDIATRICS?

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Key words: MDS JMML AML classification therapy

Background and aims

The classification of myeloid malignancy has evolved from being purely morphology-based (FAB) to be more cytogenetic oriented (WHO) and lately including more genetic subgroups.

Results

A number of disease-defining specific genetic abnormalities has been included in the recent classification neglecting blast count and morphology. However, not all patients with the same genetic aberrations share the same prognosis. Classification of myeloid neoplasms with specific rearrangements irrespective of blast count constitutes useful entities and the mutational profile may provide a more detailed stratification of the patients. Driver mutations have been incorporated in the classification as disease defining but additional genetic aberrations may be more important for the prognosis, whereas some aberrations keep the prognostic impact regardless of the additional aberrations. Presenting features like WBC and morphology may still contribute to the prognosis. Targeting specific genetic aberrations has long been a declared aim with the unravelling of new genetic entities. A dramatic success was seen with introduction of ATRA and arsenic trioxide for PML::RARA in APL but improvements in targeted therapy for other subtypes of myeloid malignancies has been limited. Recent years have shown significant improvements using FLT3 inhibitors and more recently menin inhibitors giving hope for a new era of therapy designed for molecular subtypes. However, therapeutic benefits have mainly been for AML patients with limited improvement in genetically based therapy in MDS.

Despite specific aberrations leading to hyperactivation of the RAS/MAPK pathway in the large majority of JMML patients, the results of targeted therapy in JMML are in the early stage, although promising compounds have been tested.

Conclusions

Progresses in understanding the genetic landscape of myeloid malignancies have led to better understanding but finding the optimal classification incorporating molecular findings is still challenging. Although we have seen significant progress in therapy of molecular defined AML, advances in MDS and JMML are still limited.

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OC16

THE NEW KID ON THE BLOCK: UBTF-TD

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Background and aims

Recurrent tandem duplications (TD) in exon 13 of the upstream binding transcription factor gene (UBTF) have recently been identified in 4% of pediatric AML and associated with adolescent age, normal karyotype, trisomy 8, as well as FLT3-ITD and WT1 mutations. Response to chemotherapy was poor and probability of overall survival (OS) at 5 years only 44%. The recent analysis of UBTF in 235 patients with primary MDS-EB enrolled in the prospective studies 98 or 2006 of the European Working Group of MDS in Childhood (EWOG-MDS) revealed the presence of a TD in 26% of cases. UBTF-TD was associated with hypercellular bone marrow, pronounced dysplasia of all hematopoietic lineages, normal karyotype and trisomy 8. WT1 and RAS pathway mutations were frequent. Of note, UBTF-TD was not found in patients with germline predisposition to MDS (i.e. GATA2 or RUNX1 deficiency, SAMD9/9L germline disorder). Probability of OS at 5 years was significantly inferior to that of patients without TD. The presentation will outline the clinical and hematological presentation of UBTF-TD related myeloid neoplasia and discuss the impact of concomitant somatic aberrations and possible therapeutic approaches.

OC 17

IMPACT PROGNOSIS OF CYTOGENETIC ALTERATIONS IN BRAZILIAN CHILDHOOD MYELODYSPLASTIC NEOPLASM

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Key words: Childhood MDS, cytogenetic alterations, leukemia evolution

Background and aims

Childhood myelodysplastic neoplasm (cMDS) is a rare disease characterized by dysplasias, inefficient hematopoiesis, and cytopenias in peripheral blood. Its clinical course is variable; however, around 10-40% of cases progress to acute myeloid leukemia (AML). The only curative therapy for these patients is hematopoietic stem cell transplantation (HSCT). Cytogenetic analysis is essential for the diagnosis, prognosis, and for clinical decision-making as the indication for HSCT. Clonal cytogenetic alterations can be detected in approximately 50% of cMDS, mainly in advanced subtypes. The prognostic value of cytogenetics has been studied extensively in adult patients with MDS, however, the knowledge of cytogenetics in cMDS is still limited, except for alterations involving
chromosome 7. The aim of this study was to analyze the frequency of chromosomal alterations in cMDS patients and their impact on the disease evolution.

Methods

The cytogenetic analysis was performed in bone marrow samples from 200 cMDS patients. The karyotype pattern was characterized by G-banding and fluorescence in situ hybridization (FISH).

Results

Cytogenetic alterations were detected in 50.5% (101/200) of patients. These alterations were more frequent in patients with more advanced subtypes representing 86.4% (57/66). Among the initial subtype, the abnormal karyotype was observed in 31.3% (42/134). The most frequent alterations were -7 (22%), del(11)(q23) (10%), +8 (9%), and complex karyotype (10%). Rare chromosome alterations such as biclonal chromosomal alteration, hyperdiploid karyotype, and chromosomal translocations were present at 2%, 2.5%, and 1% respectively. There was no association between a specific cytogenetic alteration and a cMDS subtype. Leukemia evolution was present in 29% of the patients, being associated with -7, +8, del(11) (q23), complex karyotypes, and rare chromosome alterations.

Conclusions

This study provides new information on the role of common and rare cytogenetic abnormalities in cMDS with important clinical implications. This study was supported by FAPERJ(E-26/201.2018/2022).

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CLONAL HEMATOPOIESIS IN SHWACHMAN DIAMOND SYNDROME: BIOLOGY AND CLINICAL IMPLICATIONS

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Key words: Clonal hematopoiesis, clinical implications, Shwachman Diamond syndrome

Background and aims

Shwachman Diamond syndrome is a genetic condition characterized by bone marrow failure and increased risk of leukemia. Biallelic mutations in the SBDS gene, which results in impaired ribosome assembly, account for the majority of cases of SDS. Severe marrow failure is more common at younger ages, while the risk of myeloid malignancy increases with age. Neutrophil counts tended to rise with increasing age. The major cause of mortality for patients with SDS is myeloid malignancy due to both relapsed/refractory disease and treatment-related toxicities. To understand the molecular pathways driving bone marrow failure and malignant transformation, we examined the patterns of clonal hematopoiesis together with their biological and clinical consequences. Clonal hematopoiesis was detectable from a young age and was universal by early adulthood. We identified two pathways with distinct clinical implications: one pathway driven by TP53 mutations promoted leukemia development, while the other pathway driven by EIF6 mutations resulted in somatic rescue of the ribosomal impairment. EIF6-mutated clones did not progress to leukemia. Clones with heterozygous TP53 mutations could persist at stable VAF for many years; however malignancies harbored biallelic TP53 mutations. Integrated assessment of clinical, pathological, cytogenetic, and genomic analyses may identify patients at high risk for malignant transformation. Implications for risk stratification and leukemia interception will be discussed.

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OC 19

FUNCTIONAL ANALYSES OF RUNX1 VARIANTS IN THE CONTEXT OF FAMILIAL PLATELET DISORDER WIT PREDISPOSITION TO HEMATOLOGIC MALIGNANCIES

Authors: **Melanie Decker**[1]; Förster Alisa[1]; Prüne Alina[1]; Anne Seebacher[1]; Alena Wittstock[1]; Thomas Illig[1,2]; Brigitte Schlegelberger[1]; Tim Ripperger[1]

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Key words: familial leukemia, RUNX1, RUNX1-FPD

Background and aims

Pathogenic germline variants in RUNX1 cause familial platelet disorder with predisposition to hematologic malignancies (RUNX1- FPD). Since RUNX1 variants are often reported in individual families and no functional data is available at the time of identification, its missense variants are often classified as variants of uncertain significance (VUS) impeding clinical actions. To support variant classification, we established a functional analysis platform for the characterization of RUNX1 variants.

Methods

To characterize RUNX1 variants in HEK293T and HEL cell models, we analyzed i) their ability to heterodimerize with CBFB via FRET assay, ii) their phosphorylation by western blot, and iii) their ability to regulate transcription of target genes as well as their effect on different signaling pathways using luciferase reporter assays.

Results

Besides 10 known pathogenic and one benign control, 45 RUNX1 germline variants were functionally characterized. Based on our functional data, 18 out of 45 variants (40%) could be finally curated. Five RUNX1 variants are classified as likely benign and 13 RUNX1 variants are classified as (likely) pathogenic. In addition, our data suggested that there are apparently different pathomechanism of N- and C-terminal variants of RUNX1.

Conclusions

The clinical utility of our applications could be demonstrated in the context of different clinical questions including isolated thrombocytopenias, MDS/AML and incidental findings. Our functional analysis platform is suitable to support variant classification and thereby improves diagnostics and patient care. The analysis of additional RUNX1 germline variants (n=14) is ongoing. Additional and future attempts encompass the investigation of cooperating leukemic effects of RUNX1 and other somatic gene variants frequently identified in patients with RUNX1-FPD. Additionally, multiplexed assays for variant effects (MAVE) are currently established to allow high-throughput screening even before they are identified in patients.

OC 20

GENOTYPE/PHENOTYPE ASSOCIATIONS IN 174 INDIVIDUALS WITH GERMLINE GATA2 MUTATIONS

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Key words: GATA2, MDS, gremline predisposition, genotype/phenotype correlations

Background and aims

Germline GATA2 variants are associated with a pleiotropic Mendelian disorder predisposing to myelodysplastic syndrome (MDS) with variable penetrance and atypical presentations. Uncommon phenotypes continue to emerge, rendering a clear "from phenotype to genotype" diagnostic pathway difficult. Our aim was to describe the clinicopathological presentations of GATA2-deficient cases in the EWOG-MDS and GATA2-HuMo cohorts and reconcile the findings with 408 GATA2 cases from literature.

Methods

Cases presenting with MDS or cytopenia with germline GATA2 variants and family members were included. Our cohort is a subset of patients registered 1986-2021 in the EWOG-MDS patient registry and GATA2-HuMo consortium. We developed 26 clinicopathological groups to ascertain genotype/phenotype correlations. Four diagnostic categories were established: i) high-risk MDS (HR-MDS) defined by blast expansion and/or with monosomy 7, del(7q) and der(1;7), ii) low-risk MDS (LR-MDS), iii) cytopenia insufficient for MDS diagnosis, and iv) asymptomatic carriers.

Results

We identified 91 unique GATA2 variants of which 57 were null mutations (nonsense/frameshift/stop-gained), 28 missense, 4 intron 4, and 2 in-frame deletions. Of the 174 cases, 164 symptomatic were diagnosed with HR-MDS (n=96), LR-MDS (n=55), cytopenia (n=12) and MDS unclassified (n=1) at a median age of 13.7 (0.4-75) years. The 10 asymptomatic carriers (family members) had a median age of 23 (5-60) years. Patients with HR-MDS had significantly lower number of missense mutations compared to other groups (p=0.0245). Presence of asymptomatic status correlated with intron 4 regulatory mutations (p=0). Analysis of extra-hematopoietic manifestations revealed a statistically significant correlation between sensorineural deafness and null mutations (p=0.0186) and a trend for the association of lymphatic anomalies with GATA2 null mutations (p=0.0546). Finally, deep sequencing identified recurrent somatic mutations in 61% (72/118) of patients in SETBP1, ASXL1, STAG2, RUNX1 and EZH2 genes, with expected association of SETBP1 mutations and monosomy 7.

Conclusions

We expand the genotype/phenotype spectrum and identify new potential genotype/phenotype associations.

OC 21

LOSS OF HSC STEMNESS IDENTITY IS ASSOCIATED WITH EXHAUSTION AND HYPORESPONSIVENESS IN GATA2 DEFICIENCY SYNDROME

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Key words: GATA2 deficiency, Hematopoietic Stem cells, RNA-Seq, ATAC-Seq

Background and aims

Germline GATA2 mutations are identified in a complex disorder termed GATA2 deficiency syndrome. Heterogeneous clinical manifestations are associated with a wide diversity of molecular alterations (missense, frameshift, nonsense, intronic or splicing mutations). Truncating mutations decrease GATA2 expression suggesting a haploinsufficiency mechanism while molecular consequences of missense mutations are not known. We focus our research on one of the most recurrent GATA2 mutation, GATA2 R396Q.

Methods

In vivo approaches have considerable potential for modeling GATA2-linked disease phenotype so we modelized a knock-in mouse model with constitutive expression of Gata2 R396Q mutation.

Results

Our results revealed an abnormal distribution of Hematopoietic stem and progenitor cells (HSPCs) in this model, with increased HSCs but decreased self-renewal potential and inability to respond to acute inflammatory stimuli. Moreover, combining chromatin accessibility and gene expression approaches, we have highlighted that mutated HSPCs were primed for hyporesponsiveness, as evidenced by lower interferon signaling and enrichment of inflammatory stress signatures. Further analysis revealed an allelic imbalance in Gata2 correlating with molecular and functional heterogeneity in the mutated LT-HSC (Long-Term-HSC) population.
This mechanism was also identified in leukemic cells from a GATA2 deficient patient who had developed AML.

Conclusions

Taken together, these findings suggest that Gata2 plays a crucial role in HSCs' ability to perceive and respond to their environment, and its deficiency contributes to the decline in HSC functionality in the presence of GATA2 mutations. The mechanism of allelic balance raises questions about the initiation of hematological diseases, as well as the identity and selection of somatic abnormalities in the context of GATA2 deficiency.

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GERMLINE LOSS-OF-FUNCTION MUTATIONS IN MDM4 CAUSE A NEW BONE MARROW FAILURE SYNDROME WITH TP53- DEPENDENT HEMATOPOIETIC CELL DEATH

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Background and aims

The mouse double minute 4 (MDM4) negatively regulates TP53 through the TP53/MDM2/MDM4 network. Toufektchan et al reported MDM4 p.T454M mutation in a single patient with cytopenia who also had short telomeres with pathogenic TERT mutation. Here, we describe five unrelated patients with unique germline heterozygous loss-of-function (LOF) MDM4 variants, presenting with variable bone marrow (BM) failure phenotypes ranging from erythroid hypoplasia to pancytopenia. We model the mutations in vitro and in vivo to understand their impact on hematopoiesis.

Methods

Genome/RNA sequencing, western blot, CRISPR/Cas9-guided knock-in of MDM4 mutations in induced pluripotent stem cell (iPSC) lines, iPSC hematopoietic differentiation, deletion of MDM4 in $CD34^+$ cells and mouse xenotransplantation studies.

Results

Patient 1 (c.78+1G>A) presented at 10 years old with thrombocytopenia and megakaryocytic dysplasia and developed portal hypertension; Patient 2 (c.90_91insA/p.Leu31Thrfs*15) was 11 months at diagnosis of red cell aplasia requiring chronic red blood cell transfusions; patient 3 (c.1333C>T/p.Arg445*) presented at 6 years old with reduced granulopoiesis and megakaryocytic dysplasia, had telomere length <1st percentile, and liver fibrosis. Patient 4 (c.1334 G>A/p.Arg445Gln) presented at 53 years old with pancytopenia, BM dysplasia and del7q31 with telomere length <1st percentile. Patient 5 (c.1361C>T/p.T454M) presented at age of 4 weeks with neutropenia, hypoplastic myelopoiesis and multilineage dysplasia. When modeled in iPSCs, all interrogated MDM4 variants resulted in lower protein levels and yielded reduced erythroid and myeloid differentiation compared to controls. Deletion of MDM4 in healthy CD34+ cells resulted in increased TP53 activity and fewer blast-forming erythroid and myeloid colonies which was rescued by TP53 gene knock-out. MDM4-deleted CD34+ cells transplanted into immunodeficient mice resulted in reduced engraftment capacity. MDM4 deletion and complementation studies are currently ongoing to define distinct functional

domains of MDM4.

Conclusions

We establish MDM4 as a new BM failure syndrome with LOF MDM4 mutations resulting in TP53 hyperactivity and variable hematopoietic manifestations.

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OC23

GENOME SEQUENCING APPROACHES FOR DIAGNOSIS AND DISCOVERY OF BMF/MDS SPECIFIC GENETIC ALTERATIONS

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Background and aims

Accurate genetic diagnosis is essential for inherited bone marrow failure (IBMF) and myelodysplastic syndromes (MDS) because the treatment approach is very dependent on the underlying genetic cause. The genetic diagnosis must be reached quickly in some, for example in patients with severe IBMF. The management is individually tailored based on the genetic information, which includes both germline and somatic events. Timely genetic diagnosis might improve outcomes. Finally, the surveillance is dictated by the underlying genetic findings.

This presentation will first outline the genetic classification of inherited BMF/MDS syndromes and review several studies with large cohorts of BMF/MDS patients in which the diagnostic utility of NGS or whole exome sequencing has been demonstrated. We also show our data from the St. Jude whole genome sequencing (WGS) pilot study for IBMF/MDS. The purpose of this study was to assess the utility of WGS as an all-in-one diagnostic platform to identify germline and somatic alterations and telomere length. We performed WGS, followed by blinded analysis by computational biologist in blood DNA from 67 BMF/MDS patients who were divided into three groups. Group 1 consisted of 44 patients with known molecular diagnosis - in this group WGS correctly resolved all germline lesions as well as acquired chromosomal alterations. In group 2 (17 patients with unknown genetic diagnosis), WGS identified previously missed molecular cause in 5/17 patients. Finally, group 3 (6 aplastic anemia patients with small clones) WGS detected clones in only 3/6 patients. The telomere length assessed by WGS did not correlate with Southern blot measurements. In conclusion, WGS has proven its diagnostic utility as an all-in-one platform to detect germline alterations and chromosomal changes. Main limitation is the low sensitivity (precluding detection of small somatic clones) thus emphasizing the need for complementary high-depth targeted NGS.

OC 24

MYELODYSPLASTIC SYNDROME AND LEUKEMIA IS A SECONDARY EVENT AFTER BONE MARROW FAILURE IN GATA2 HAPLOINSUFFICIENT MICE

Authors: Cansu Koyunlar[2]; Juncal Fernandez-Orth[3]; Julia Weiss[3]; Emanuele Gioacchino[2]; HansDe Looper[2]; Geoffrey Andrieux[4]; Mariette Ter Borg[2]; Baris Yigit[3]; Joke Zink[2]; Remco Hoogenboezem[2]; Irene Gonzalez-Mendez[5]; Eric Bindels[2]; Mathijs Sanders[2]; Ivo Touw[2]; Miriam Erlacher[2]; **Emma De Pater**[1]

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Key words: GATA2, Mouse model, MDS/AML

Background and aims

GATA2 is a transcription factor essential for the generation and differentiation of hematopoietic stem and progenitor cells (HSPCs), controlling gene transcription of multiple target genes. In the adult hematopoietic system, GATA2 is crucial for the proliferation and maintenance of HSPCs. Heterozygous germline GATA2 mutations were associated with different syndromes nowadays subsumed as "GATA2 deficiency". The most consistent findings are variable cytopenias, immunodeficiency and an 80% risk of progression to myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Unfortunately, the underlying mechanisms remain elusive. With this study we aim to identify the molecular and pathophysiological mechanisms underlying leukemic transformation upon GATA2 deficiency.

Methods

To investigate these mechanisms we have modeled GATA2 deficiency in mice. One model made use of aging and serial transplantation of germline GATA2 heterozygous BM, and in the other, we transplanted limited numbers of Vav-CRE;Gata2$^{fl/+}$HSPCs into lethally irradiated recipients.

Results

Serial transplantation of GATA2$^{+/-}$ BM after aging resulted in monocytopenia, B-cell cytopenia as well as reduced HSC fitness. Transplantation of limited numbers of Vav-CRE;Gata2$^{fl/+}$HSPCs into lethally irradiated WT recipients led to lethal bone marrow failure in approximately 40% of recipients. Histochemical analyses showed bone marrow aplasia in all deceased recipients with significant alterations in B cell and myeloid differentiation. Importantly, 30% of bone marrow failure mice progressed to acute leukemia characterized by both the acquisition of somatic mutations and chromosomal aberrations. Recipients not succumbing to bone marrow failure presented a highly dysplastic normocellular bone marrow, although being phenotypically healthy. Transcriptome analysis showed a marked increase in proliferative signatures and upregulation of G2-M checkpoints in Gata2 haploinsufficient HSCs compared to WT HSCs after transplantation. Furthermore we found, using a human cell model of GATA2 haploinsufficiency, increases in chromosome mis-segregation.

Conclusions

Our results suggest that the combination of replicative stress and genomic instability drive bone marrow failure and leukemia in Gata2$^{+/-}$ HSCs.

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OPTICAL GENOME MAPPING: A NEW TOOL TO OVERCOME CONVENTIONAL CYTOGENETICS' LIMITATIONS IN PATIENTS WITH BONE MARROW FAILURE.

Authors: **Josune Zubicaray**[1]; Ana Gomez[1]; June Iriondo[1]; Reyes Gimenez[1]; Lorea Abad[1]; Carmen Matasans[1]; Elena Sebastian[1]; Alejandro Sanz[1]; Jesus Gonzalez De Pablo[1]; Manuel Ramirez[1]; Julian Sevilla[1]

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Key words: Optical genome mapping, Cytogenetics, Bone marrow failure

Background and aims

Cytogenetic studies are essential in the diagnosis and follow-up of patients with bone marrow failure syndromes (BMFS). Unfortunately, obtaining enough metaphases for G banding is often challenging. Optical Genome Mapping (OGM) is a novel technology capable of detecting all types of chromosomal structural variants (SVs) at much higher resolution than standard cytogenetics. However, most reported results are on hematologic malignancies and to our knowledge there are no reports about its applicability in BMFS.

Methods

We compared findings of conventional cytogenetic techniques (chromosome banding analysis –CBA- and FISH) to those obtained by OGM (Bionano Saphyr platform from Bionano Genomics®) in bone marrow samples of patients with suspected or known BMFS.

Results

17 patients were included in the present analysis, which will be updated with an estimated number of 6 more patients prior to the meeting. Regarding conventional cytogenetics, the required 20 metaphases for CBA were only obtained in one of the 17 patients. One patient with no metaphases after culture showed a gain in chromosome 1q by FISH in 7% of the cells, which was confirmed by OGM. FISH results were negative in the rest. In contrast, OGM was able to obtain results in all cases fulfilling the required quality control metrics, and detected SVs in 13 of them. Furthermore, we found several cryptic submicroscopic alterations by OGM that were not described by CBA and FISH.

Conclusions

OGM emerges as a powerful tool with the ability to reduce multiple techniques into a single assay with much higher resolution than standard cytogenetics. In the case of cytopenic BMFs, we were able to obtain results in all cases, which suggests that OGM can overcome the limitations of conventional techniques. Furthermore, in addition to confirming the abnormalities detected by conventional techniques, OGM found new alterations beyond their detection limits.

OC 26

A COUNTRYWIDE STUDY OF GATA2 DEFICIENCY IN ITALY REVEALS NOVEL SYMPTOMS AND GENOTYPE-PHENOTYPE CORRELATION

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Key words: GATA2 deficiency, Inborn errors of immunity, Cytopenia, Sensorineural deafness, Lymphedema

Background and aims

GATA2 deficiency is a rare disorder encompassing a broadly variable and continuously evolving phenotype. First described in 2011, up to 500 patients have been reported. Here, we describe 31 Italian patients (26 families) aiming to describe novel symptoms and investigating genotype-phenotype associations.

Methods

All the Italian Association of Pediatric Hematology and Oncology (AIEOP) centers were

contacted. Patients with pathogenic, likely pathogenic, or variants of unknown significance (VUS) in the GATA2 gene were enrolled in this study.

Results

Patients' median age at onset of symptoms was 12.5 years. Infections (39%), hematological malignancies (23%) and undefined cytopenia (16%) were frequently reported at the onset. Their prevalence increased during the follow-up and further clinical manifestations, i.e hepatosplenomegaly, haemorrhagic diathesis, thrombosis, bone marrow failure (BMF), lymphedema, pulmonary alveolar proteinosis and sensorineural deafness, occurred. Clinical penetrance was highly variable, with severely affected pediatric patients coexisting with asymptomatic adults in the same pedigree. Once established the diagnosis, congenital deafness became part of the clinical picture of GATA2 deficiency in two individuals. The majority of patients (55%) underwent hematopoietic stem cell transplantation, performed in case of myeloid neoplasia (13/17) and BMF (3/17), but also in one patient with immunodeficiency. The event free survival and overall survival were 23% and 90% at the age of 20 and 16% and 78% at the age of 40, respectively. The majority of GATA2 variants were located at zinc-finger 2 (ZF2) domain of the GATA2 protein. Null (52%) and missense (42%) mutations were the most frequent variants. Intronic variants were found in 6% of the cohort. Four missense mutations out of the 7 cases of lymphedema were described. Family screening was essential to identify asymptomatic patients (6%).

Conclusions

Our study highlights new (pilonidal cyst/sacrococcygeal fistula, cholangiocarcinoma and gastric adenocarcinoma) phenotypes and shows that lymphedema may be associated with missense mutations in patients with GATA2 deficiency.

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CHEK2 GERMLINE VARIANTS AND ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT OUTCOMES

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Background and aims

Germline alterations in genes functioning in DNA damage repair are increasingly recognized as factors in hematologic malignancy predisposition and treatment related toxicity. CHEK2 variants regulating CHEK2 protein synthesis are frequently identified in individuals tested for hereditary cancer predisposition. Their effect on success of high intensity therapies is not known. Our aim was to assess the correlation between germline CHEK2 variants and allogeneic hematopoietic stem cell transplantation (HSCT) outcomes.

Methods

This is a retrospective study of a Finnish national cohort of allogeneic HSCT patients. We analyzed the whole exome sequencing and clinical data of 432 patients transplanted between 1999 - 2020. The patient series comprised of Helsinki University Hospital cohort and the Finnish Bone Marrow Transplantation Registry cohort (adult patients) and a national pediatric cohort. We focused on recipients carrying pathogenic (P) or likely pathogenic (LP) variants. We compared HSCT recipients without variant to recipients with a CHEK2 variant, recipients with a variant causing hereditary hematological malignancies (HHM), and recipients with a variant associated with predisposition to solid tumors (others than CHEK2). The patients carrying multiple gene variants (N=8) were excluded.

Results

We identified a germline CHEK2 P/LP alteration in 10.6% of the HSCT recipients. We found no correlation in the overall survival (P=.339), non-relapse mortality (P=.185) or risk of relapse (P=.723) between HSCT recipients with CHEK2 variants vs no variant. No difference was

observed in frequency of graft failure, acute or chronic graft versus host disease (GVHD) (0%, 42.2% and 44.4% vs 2.7%, 50.3% and 44.4%, respectively). Recipients with HHM variants showed higher frequency of acute liver GVHD (23.1% vs 5.5%, P=<.001), tendency to higher frequency of graft failure (9.5% vs 2.7%, P= 0.097) and early non-relapse mortality (19.2% vs 11.3%, P=0.158).

Conclusions

Germline variants in CHEK2 do not affect HSCT outcome supporting standard HSCT care for recipients with a germline CHEK2 variant.

OC 28

PEDIATRIC MDS IN GATA2-DEFICIENCY: ENHANCED HISTONE TRIMETHYLATION AND DEREGULATED APOPTOSIS AS DRIVER?

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Key words: Myelodysplastic syndrome, MDS, GATA2, Pediatric, GATA2 Transformation, Venetoclax, Azacitidine, MAC - Score

Background and aims

GATA2-deficiency is a heterogeneous multi-system disorder characterized by a high risk of developing myelodysplastic syndrome (MDS) and increased risk of transformation in acute myeloid leukemia (AML). We aimed to understand the pathogenesis in pediatric GATA2-deficiency and to identify drivers and potential biomarkers for individual risk of progression. Therefore, we systematically analysed bone marrow biopsies focusing on the hematopoiesis and the hematopoietic niche including the composition of its microenvironment in pediatric MDS.

Methods

We studied a cohort of children (n=57) diagnosed with MDS with excess blasts (MDS-EB) or refractory cytopenia of childhood (RCC) with or without GATA2-deficiency. Fluorescence multiplex immunohistochemistry combined with multispectral imaging, gene expression profiling, and RNA-in situ hybridization were used to identify prognostically and therapeutically relevant markers or pathways. We also evaluated the potential of venetoclax/azacitidine combinatorial therapy using the "Mediators of Apoptosis Combinatorial Score" (MAC-Score (Waclawiczek et al., Cancer Discovery, 2023)).

Results

Patients with $GATA2^{mut}$ MDS-EB (GATA2-EB, n=6) showed a high expression of the anti-apoptotic protein BCL2, which could not be observed in $GATA2^{mut}$ RCC (GATA2-RCC, n=24) or other pediatric MDS subgroups $GATA2^{WT}$ RCC (RCC, n=17) and $GATA2^{WT}$ MDS-EB (MDS-EB, n=10) without GATA2-deficiency. GATA2-EB was further characterized by a

robust expression of the methyltransferase EZH2 associated with increased levels of histone trimethylation indicated by overexpression of H3K27me3. Multiplex fluorescence imaging of additional BCL2 family members revealed a significantly increased MAC-Score in GATA2-EB patients with potential therapeutic implications.

Conclusions

Bone marrow biopsies of children with GATA2-EB are characterized by high expression of BCL2 and EZH2 associated with enhanced histone trimethylation, compared to patients with GATA2-RCC or without GATA2 mutation. These genes are potential targets for venetoclax and azacitidine therapy, suggesting that GATA2-EB patients may benefit from regimens that include these agents. Importantly, the increased MAC-Scores observed in children with GATA2-EB might indicate a good response to venetoclax/azacitidine alone or in combination.

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VENETOCLAX-BASED THERAPIES IN PEDIATRIC ADVANCED MDS AND RELAPSED/ REFRACTORY AML: A MULTICENTER RETROSPECTIVE ANALYSIS

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Key words: venetoclax, pediatric, MDS, AML, HSCT

Background and aims

Venetoclax combination therapies are safe and promising in adult AML. Data in pediatric myeloid neoplasms are still limited.

Methods

We report a multicenter retrospective analysis of pediatric patients with refractory/relapsed (r/r) AML, post-cytotoxic therapy MDS/AML (tMDS/tAML), or MDS with excess blasts (MDS-EB) who received venetoclax-containing therapies. Data about patient and disease characteristics, venetoclax administration, toxicity, and response were collected. Response was classified as complete (CR), partial (PR), and non-response (NR) if bone marrow blasts after therapy were ≤ 5%, 5-20%, and ≥20%, respectively.

Results

Thirty-one patients were included. Median age was 10.2 (1.3-17.4) years. Diagnoses were MDS-EB (4), r/rAML (18), and tMDS/tAML (9). Patients received a median of 2 venetoclax cycles (1-15) at a median dose of 350 mg/m^2/daily (125-500) for a median time of 28 days (9-28). In combination, 19 patients received hypomethylating agents, nine cytotoxic agents, and three both. One patient discontinued venetoclax due to severe pancytopenia. No other serious adverse effects were reported. Best response achieved was CR and PR in 16 (51,6%) and 6 (19,4%) patients. Eight (25.8%) NR were reported. In venetoclax-cytotoxic agents group, CR and PR were reported in 6 (66.7%) and 1 (11.1%), while in venetoclax-hypomethylating agents group in 7 (36.8%) and 5 (26.3%). Three out of four MDS-EB achieved a CR while one did not respond. Twenty patients were bridged to HSCT, after a median of 3.3 months (0.6-31.1) from the start of therapy. Median follow-up was 7.7 months (1.1-32.3). Estimated OS at 30 months after the start of treatment was 29.9%±14.4 for the whole cohort and 74.4%±13.8 for

patients undergoing HSCT. Causes of death included disease progression before HSCT (8), transplant-related mortality (3) and relapse after HSCT (2).

Conclusions

Our data confirmed the efficacy and safety of venetoclax combinations in pediatric advanced myeloid disorders, resulting in a promising approach as bridge to HSCT.

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JUVENILE MYELOMONOCYTIC LEUKEMIA (JMML) CELLS ESCAPE IMMUNE SURVEILLANCE BY MULTIPLE MECHANISMS

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Key words: Juvenile myelomonocytic leukemia, CD47, CD39, CD73, immune escape

Background and aims

Immune escape mechanisms contribute to the growth and relapse of multiple tumors but were not yet described for JMML. Here, we characterized different mechanisms by which JMML cells modulate the immune system.

Methods

We focused on the most aggressive PTPN11-mutated JMML subtype. Primary human JMML cells and murine leukemic $Ptpn11^{D61Y/+}$ cells were analyzed by mass cytometry, conventional flow cytometry and RNAseq for known immune escape molecules. In addition, functional assays for phagocytosis and T cell activation were performed in vitro.

Results

Mass cytometric analysis revealed strong expression of different immunoregulatory molecules including CD39, CD47, Siglec-7, NOX2 and PD-L1 on human JMML cells. On splenic myeloid cells of Mx-Cre;$Ptpn11^{D61Y/+}$ mice, high expression levels of CD47, PD-1, PD-L1, PD-L2, CD73 and VISTA were found. We selected the purinergic signaling regulators CD39 and the “don’t eat me” signal CD47 for further analysis. On a functional level, immune escape was demonstrated in co-culture experiments, where murine leukemic cells inhibited the proliferation and activation of wildtype murine T cells. Treatment with POM1 (CD39 inhibitor) resulted in the full restoration of T cell proliferation and activation. Blocking CD47 increased phagocytosis of human and murine leukemic cells by macrophages, both wildtype and Ptpn11 mutated ones. The use of anti-CD47 antibody magrolimab in PDX model resulted in a strong reduction of leukemic cells in spleen but not bone marrow. No additive effects were observed when azacitidine and magrolimab were combined.

Conclusions

JMML cells escape T cell response by ATP hydrolysis mediated by CD39 and CD73. We will analyze in vivo, whether inhibition of this pathway increases the immune response to JMML and prevents relapse. In addition, CD47 might represent a good target to reduce relapse risk.

OC31

PATHWAY-DIRECTED THERAPY APPROACH IN PEDIATRIC MDS AND AML

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Key words: AML, MDS, targeted treatment

Background and aims

Acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) are rare disorders in childhood. Whereas AML is usually treated with intensive conventional chemotherapy and a stem cell transplantation (HSCT) in at least 25% of patients, MDS patients with excess of blasts (MDS-EB) usually proceed to HSCT without previous chemotherapy. Despite an intensive frontline treatment, more than 30% of patients with AML will experience a relapse. Moreover, the survival of children transplanted for MDS-EB is only 60% due to relapse and transplant-related toxicity. Hence, there is a considerable need for alternative treatment options. In the previous years, multi-omic studies have led to a better understanding of AML and MDS biology, and fueled identification of numerous genetic alterations, both in adult and pediatric myeloid disease. Targeted therapies, emerging from these discoveries, are now being investigated or even incorporated into current treatment strategies. In this presentation, the genetic landscape of pediatric AML and MDS will be addressed with specific attention for pathways with the potential to be used as a therapeutic target. The focus will be on the currently available and promising targeted agents, either or not yet under investigation, such as FLT3, BCL2, IDH, MEK, DOT1L and menin inhibitors. Despite several challenges which have already become apparent, such as the potential toxicity of some compounds and the heterogeneous biology of AML and MDS-EB, targeted drugs are likely to change the therapeutic landscape of these disorders and will hopefully improve the patients outcome and quality of life.

OC 32

DEVELOPMENT OF NEW GENE EDITING APPROACHES FOR BONE MARROW FAILURE AND MDS PREDISPOSITION SYNDROMES

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Key words: myelodysplastic syndromes, bone marrow failure, gene and cell therapy

Background and aims

Germline predisposition to bone marrow failure (BMF) and myelodysplastic syndromes (MDS) cause substantial economic and psychosocial burden and early death in affected individuals. Due to the limits and risks of hematopoietic stem cell transplantation, there is an urgent need for novel therapies. Conventional lentiviral-based gene therapy is not feasible for most genes (i.e., TERT, SAMD9/L or GATA2) because uncontrolled overexpression is detrimental. However, gene therapy using new techniques called base and prime editing, holds great therapeutic promise.

Methods

PARADIGM (partnership to advance development of individualized genomic medicines) is a St. Jude investigator-initiated project to develop a preclinical proof-of-concept platform for therapeutic gene editing in BMF/MDS patients. In pursuit of this objective, we are utilizing existing technology and concurrently refine adenine base editors (ABEs) and cytosine base editors (CBEs) for ultra-precise allele correction.

Results

From our BMF/MDS biobank, comprehensive genomic analyses uncovered 65 patients with unique pathogenic mutations in 33 genes. After systematic variant type/specificity analysis, we identified single nucleotide variants (SNVs) in 78.5% (51/65) patients, of which 64.7% (33/51) are candidates for base editing, either using CBE (7/33) or ABE (26/33). Additionally, 38.5% (25/65) of mutations (18 SNVs and 7 indels), which are not correctable by ABE/CBE, were predicted to be prime editable. To model selected candidate mutations in vitro, we successfully created 19 induced pluripotent stem cell lines (iPSC) with base/prime-editable mutations in GATA2, SAMD9, SAMD9L, DKC1, TINF2, RPS19, RPS26, ANKRD26, ETV6, ERCC6L2, SRP54, and CSF3R genes. We currently modify ABEs/CBEs sequence to enhance activity and specificity. In parallel we develop genetic and cellular readouts in primary cells and cell lines.

Conclusions

In summery, 89.2% (58/65) of patient mutations are amenable to either base or prime editing, providing a first milestone towards gene editing in patient cells which is underway.

OC 33

ILLUSTRATION OF CLONAL ARCHITECTURE IN JUVENILE MYELOMONOCYTIC LEUKEMIA BY TARGETED SINGLE-CELL DNA SEQUENCING

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Key words: JMML, clonal architecture, single cell sequencing

Background and aims

The significance of subclonal secondary mutations in JMML is increasingly appreciated. We hypothesize that the early presence of cell subpopulations that give rise to therapy-resistant cell clones is a key factor determining the risk of progression or relapse. We created genetic maps at the single-cell level in well-annotated clinical samples and associated these with hematologic and clinical information.

Methods

A microfluidic single-cell targeted DNA sequencing approach was used interrogating an in-house customized panel of genes with specific relevance to JMML. The design consisted of 490 amplicons and represented 24 genes. In a pilot experiment, JMML cells from bone marrow or spleen of 4 patients at time of diagnosis and bone marrow cells from one healthy donor were analyzed.

Results

The median number of individually sequenced cells per sample was 3,068. 3 cases showed linear clonal evolution whereas 1 case exhibited branching clonal evolution. Patient 1 represented an example of an NRAS-driven case of JMML without clonal diversity. Patient 2 exhibited a minor subclone with an ASXL1 variant on top of the PTPN11 driver mutation. Whereas patient 3 was likely driven by PTPN11, cells carrying only this mutation were not detectable, suggesting early evolution with two additional variants in ASXL1 and SETBP1. An interesting tertiary feature was a subclonal NF1 alteration which later underwent loss of heterozygosity. Patient 4 had the same NRAS founder mutation as Patient 1, but a large clone with a secondary SETBP1 variant was also present which evolved into two independent subclones with either a second NRAS mutation or a PTPN11 mutation.

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Conclusions

The data provide novel and fascinating insight. The branching architecture of case #4 would not have been recognized by bulk sequencing. Single-cell DNA analysis allows the identification of individual cell populations with specific mutational make-up and therefore facilitates understanding their significance in the context of the disease.

OC 34

ONCO-FETAL REPROGRAMMING DRIVES HIGH-RISK JUVENILE MYELOMONOCYTIC LEUKEMIA, WHICH CAN BE TARGETED BY ANTI-CD52 TREATMENT

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Key words: JMML, Multi-OMICS, targeted treatment, biomarkers

Background and aims

Juvenile myelomonocytic leukemia (JMML) is caused by genetic activation of RAS signaling and has a heterogeneous clinical course. JMML epitypes resolve this heterogeneity but high-risk patients lack efficient curative treatment options. To date, the mechanisms driving disease heterogeneity remain unclear. This study aimed to decipher the underlying molecular programs in order to identify disease-specific aberrations for diagnostic and therapeutic purposes.

Methods

We employed a multi-omics approach to dissect the epitype-specific molecular programs in primary JMML patient samples. Our findings were validated using an inducible Ptpn11-E76K knock-in mouse and a patient-derived xenotransplantation (PDX) model.

Results

Multi-modal analysis demonstrated conservation of epigenetic subgroups in hematopoietic stem cells (HSCs) of JMML patients. Epigenomic dysregulation affected binding motifs of developmental transcription factors and correlated with ectopic expression of fetal HSC signatures in high-risk patients, including HMGA2 and fetal hemoglobin. Mapping JMML HSC methylomes onto the normaldevelopmental trajectory from fetal to adult HSCs, generally revealed a post-natal HSC state. However, high-risk JMML HSCs were epigenetically more immature and presented fetal-like methylation patterns. Employing a JMML mouse model with postnatal induction of the Ptpn11-E76K mutation resulted in reactivation of fetal-like expression programs in HSCs akin to those observed in high-risk JMML, suggesting that high-risk JMML HSCs hijack fetal programs. In line with this, integrative analysis identified several subgroup-specific molecular markers which might serve as prognostic biomarkers for high-risk JMML. One of those markers, CD52, was both differentially methylated and highly expressed in high-risk JMML HSCs. Targeting CD52 with alemtuzumab in a JMML PDX mouse model demonstrated reduced human engraftment in treated recipients and increased survival of 2° recipients.

Conclusions

In summary, we identified onco-fetal reprogramming as a hallmark of high-risk JMML. We determined unique molecular programs which can be used to develop new treatment strategies for high-risk JMML and provide pre-clinical evidence for anti-leukemic activity of alemtuzumab.

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P1

UNRAVELING THE MOLECULAR BASIS OF PEDIATRIC MYELODYSPLASTIC SYNDROME: INSIGHTS FROM CRISPR/CAS9-EDITED IPSCS BEARING THE SAMD9 P.I1567M MUTATION.

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Key words: SAMD9, hiPSCs, CRISPR/Cas9, Familial MDS, Germline variants, Hematopoiesis

Background and aims

Pediatric Myelodysplastic Syndromes (pMDS) are a rare group of hematological disorders, characterized by ineffective hematopoiesis with a high risk to progress to acute myeloid leukemia (AML). While most of the adult MDS/AML cases are associated with random somatic mutations, an increasing number of pediatric cases are associated to germline variants. Nowadays, we know more than 20 predisposing MDS genes, with SAMD9 as one of the most common mutated. However, understanding the molecular basis that leads to pMDS development by SAMD9 mutations remains unexplored. To understand the effect of germline SAMD9 mutations on hematopoiesis, CRISPR/Cas9 system was used to introduced heterozygous and homozygous p.I1567M mutation in a healthy human induced pluripotent stem cell (iPSC) line.

Methods

To assess whether SAMD9 mutation affects hematopoiesis, both iPSC-SAMD9mut lines were differentiated toward blood progenitors. FACS analysis revealed that SAMD9 mutation blocks the maturation of early hematopoietic progenitors (CD34+CD43+CD45+).

Results

The increased hematopoietic output of hiPSC-SAMD9mut lines could be related to the higher proliferation/survival of early HPCs. However, cell-cycle analysis of CD34+CD43+ cells revealed no significant differences among hiPSC-SAMD9mut and control. These data suggest a specific effect of SAMD9 mutation on blood specification rather than proliferation. In line with this finding, differentiation of HSPCs to myeloid terminal lineages leads to a significant decrease in monocytes (CD14+CD45+) formation when compared to their isogenic control. These data were also validated by CFU assay. Finally, given the role of SAMD9 in inflammation, we stressed the system culturing hiPSC-SAMD9-mut in the presence of proinflammatory cytokines (IFNγ and TNFα).

Conclusions

Interestingly preliminary data of FACS analysis suggests that SAMD9mut in a stressed hematopoiesis leads to a significant decrease of HSPCs differentiation. Overall, our study provides new insights into the role of SAMD9 p.I1567M mutation in familial MDS and highlights the potential of iPSC-based models to unravel the pathogenesis of inherited hematological disorders.

P2

VARIANT PROFILING IN PEDIATRIC CHRONIC MYELOID LEUKEMIA

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Key words: pediatric chronic myeloproliferative disorders, pediatric chronic myeloid leukemia

Background and aims

Chronic myeloid leukemia (CML) is very rarely diagnosed in childhood and adolescence. Consequently, there is limited knowledge on the pathogenesis of CML in these age groups. Whereas in the adult population, the mutational landscape of the disease and its impact on treatment response and outcome have been extensively studied in the chronic phase (CP) as well as in the blast phase (BP), data on the variant profile of this disease in pediatric patients still hardly exist.

Methods

Based on targeted next-generation sequencing covering 142 leukemia-associated genes, we present the first large and systematic variant profiling in pediatric CML including 90 children and adolescents diagnosed with CML in CP. In addition, we performed whole genome sequencing (WGS) in a unique subcohort of 18 individuals with de novo or secondary CML BP. All patients had been enrolled in the German national CML-PAED registry.

Results

In the first cohort of pediatric patient with CML in CP, fourteen individuals (16%) harbored at least one pathogenic somatic variant. The ASXL1 gene was recurrently mutated in six cases. A pathogenic germline variant was detected in three patients (3%). In 36 patients (40%), no variant was found. No significant correlation between mutation status and initial hematologic parameters, response characteristics or survival was found in this cohort. Individuals with CML BP showed a more complex variant profile and apart from mutations in the ABL1 gene recurrent alterations in ASXL1 were again most prominent.

Conclusions

In summary, we observed parallels of the somatic variant profile between pediatric and adult patients with CML. However, we could not establish prognostic markers in this analysis. Therefore, larger studies in children and adolescents with CML are required, which can be enabled only through joint international efforts.

P3

SPECTRUM OF CLINICAL PHENOTYPES AND SOMATIC VARIANTS IN RUNX1-ASSOCIATED FAMILIAL PLATELET DISORDER WITH PREDISPOSITION TO HEMATOLOGIC MALIGNANCIES

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Background and aims

Pathogenic germline variants in RUNX1 cause familial platelet disorder with predisposition to hematologic malignancies (RUNX1- FPD), characterized by incomplete penetrance and a broad phenotypic spectrum even among affected family members. Causal germline variants are presumed to promote an inflammatory microenvironment in which somatic alterations accumulate and can finally lead to malignant transformation and frank leukemia. The interplay of RUNX1 germline variants and acquired variants remain to be elucidated.

Methods

We included 33 individuals with likely pathogenic or pathogenic (lp/p) RUNX1 germline variants from 12 independent families. Of these, 47 bone marrow or peripheral blood samples were sequenced using a customized NGS panel encompassing 148 genes (i.e., entire coding regions or hot spot exons) associated with hematologic malignancies. To our knowledge, this is the largest cohort of RUNX1-FPD individuals screened for somatic variants to date.

Results

In 47 samples, a median of three acquired variants (i.e., classified as variant of unknown significance (VUS) or lp/p) were detected per sample (range 0-12). In samples from asymptomatic individuals (i.e., no known hematologic malignancy, cytopenia and/or platelet dysfunction) or with thrombocytopenia, a median of two acquired variants (range 0-7, n=20) with 1.5 VUS and no lp/p variant were found. Frequently altered genes were ANKRD26, BCOR, and CSF3R. In contrast, MDS, AML, CMML samples harbored more variants (median 4, range 1-12, n=27) per sample. Here, a median of three VUS and one lp/p variant were found. Frequently altered genes were CSF3R, SRSF2, and ZRSR2.

Our analysis supports the theory of stepwise malignant transformation in RUNX1-FPD. Further data analysis including phenotype- genotype correlation and inclusion of additional patients is ongoing. It will eventually support elucidation of malignant transformation being the prerequisite for future risk-adapted surveillance, potential chemoprevention, and indication for hematopoietic stem cell transplantation prior to overt leukemia. Funding: BMBF MyPred (01GM1911B).

P4

PUMA-INDUCED APOPTOSIS DRIVES BONE MARROW FAILURE UPON TELOMERE SHORTENING AND LEUKEMIA IN A MOUSE MODEL OF DYSKERATOSIS CONGENITA

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Key words: MDS, DC, bone marrow failure, TERC, telomere, mouse model

Background and aims

Dyskeratosis congenita (DC) is a telomeropathy characterized by severe hematological complications including risk of secondary MDS and AML. Mechanistically, critically short telomeres cause a DNA damage response with p53-mediated cell cycle arrest, senescence and/or apoptosis; the latter primarily mediated via activation of PUMA. Inactivating p53 signaling could possibly mitigate the hematopoietic phenotype but would increase risk of genomic instability and leukemia. Based on our earlier mouse model of secondary leukemia (Genes Dev, 24(15):1602-7), we hypothesized that exclusive inhibition of p53-mediated apoptosis could delay hematopoietic failure and prevent transformation.

Methods

We created a DC model by serially transplanting HSPCs from generation 3 mTerc$^{-/-}$(G3 mTerc$^{-/-}$) mice with dysfunctional telomerase, into lethally irradiated wildtype recipients.

Results

After secondary transplantation, 45% of the recipients died of bone marrow failure. Surviving recipients displayed severely reduced HSPC viability and a DC phenotype. With the aim to inhibit apoptosis, we deleted Puma in G3 mTerc$^{-/-}$ mice. Puma deficiency significantly improved bone marrow cellularity, HSPC viability (72% vs. 50% viable cells, $p<0.01$), and blood formation. Most importantly, only 21% of secondary recipients died in the absence of Puma. Telomere length in G3 mTerc$^{-/-}$Puma$^{-/-}$ HSPCs but not fibroblastes was significantly longer compared to their Puma proficient counterparts indicating reduced cell turn-over. Importantly, no signs of genetic instability, karyotypic abnormalities, and spontaneous or irradiation-induced leukemogenesis were observed in G3 mTerc$^{-/-}$Puma$^{-/-}$ donor or recipient mice.

Conclusions

Our data indicate that inhibition of the intrinsic apoptosis pathway is sufficient to prevent loss of cells with critically short telomeres. The resulting proliferation pressure on HSPCs can be reduced and stem cell exhaustion prevented. We conclude that PUMA inhibition represents an adaptive event improving blood cell formation while maintaining genomic stability. Whether therapeutic application of PUMA inhibitors is sufficient to prevent the acquisition of mal-adaptive, oncogenic events such as TP53 mutations has to be further investigated.

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P5

ASSOCIATION AND INTERACTIONS OF THE RIO KINASES IN THE CONTEXT OF DIAMOND-BLACKFAN ANEMIA

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Key words: Diamond-Blackfan Anemia, Ribosomopathie, RIOK

Background and aims
Diamond-Blackfan anemia (DBA) is a rare inherited bone marrow-failure syndrome with erythroblastopenia and other variable, non- hematological phenotypes. DBA is considered as a ribosomopathy since most causative mutations involve genes responsible for composition and maturation of ribosomes. In a patient presenting with DBA but without any known mutations, we identified a micro- duplication of chromosome 6 including the RIOK1 gene locus. RIOK (right open frame kinase) proteins (i.e. RIOK1-3) play a plethora of roles, especially in the maturation of the 40s subunit of ribosomes. Interestingly, RIOK2 was recently described to play a major role in hematopoiesis by affecting transcription factors like GATA1 and GATA2, SPI1, RUNX3 and KLF1. We are investigating whether also RIOK1 is involved in hematopoiesis and responsible for the DBA phenotype in our patient. In addition, we are investigating whether and how RIOK1 and RIOK2 influence each other.

Methods
We use lentiviral knockdown and overexpression of RIOK 1 and RIOK 2 in human cord blood-derived hematopoietic stem and progenitor cells, followed by erythroid differentiation. In addition, we analyzed RIOK1 haploinsufficent mice.

Results
While reduced levels of RIOK 1 in murine and human hematopoietic cells did not result in any major hematological phenotype, reduced levels of RIOK 2 showed an incline towards granulocyte-macrophage progenitors. RIOK 1 overexpression resulted in a rapid loss of hematopoietic cells cultured in conditions promoting erythroid differentiation and in colony forming assays. Surviving RIOK 1 overexpressing cells showed a maturation defect towards the erythroid lineage. Electron microscopy of patient derived EBV cells revealed an altered morphology, including enlargement of the nucleolus and accumulation of autophagosomes.

Conclusions
We observe an effect of RIOK 1 reminiscent of our patient, but further analysis on ribosomal maturation and transcriptome are ongoing to elucidate the pathogenesis of RIOK1-mediated anemia.

P6

NPM1 MUTATIONS IN CHILDREN WITH MYELODYSPLASTIC SYNDROME WITH EXCESS BLASTS

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Key words: NPM1, MDS-EB

Background and aims
Nucleophosmin 1 (NPM1) mutations are found in 30% of adult AML associating with a favorable outcome, while they are less frequent in pediatric AML (5-8%). There is limited data on the prevalence and clinical significance of NPM1 mutations in children with myelodysplastic syndrome with excess blasts (MDS-EB)

Methods
Between 07/1998 and 06/2021, 322 patients (<18 years) with MDS-EB were registered in the EWOG-MDS-98/2006 studies. Targeted next generation sequencing for somatic aberrations as well as molecular analysis for germline GATA2, SAMD9/9L and RUNX1 mutations were performed.

Results
NPM1 mutation was identified in 14 of 235 patients with material for genetic testing available (6%, M/F=7/7, median age 12.7 years). Hematological features were characterized by a high incidence of patients with normoblasts in peripheral blood, increased bone marrow cellularity and presence of Auer rods (64%, 62% and 43% of patients, respectively). All but one patient (45, X,-Y) had a normal karyotype. One patient had a germline SAMD9 mutation. All patients had 1-3 additional somatic mutations including PTPN11 (36%), NRAS (43%) and WT1 (21%), but no UBTF-TD or FLT3-ITD. Allogeneic hematopoietic stem cell transplantation (HSCT) was performed in all patients; prior to HSCT, four patients received intensive chemotherapy followed by complete remission (1), blast persistence (2) or aplasia without blasts (1), and one patient thioguanine. The 5-year-event-free survival following HSCT was 70% (95%CI 45-95%, alive in remission n=10, relapse n=1, transplant related mortality n=3).

Conclusions
NPM1 mutations were found in 6% of children with MDS-EB. Like in NPM1-mutated AML, they were associated with a normal karyotype and additional somatic mutations. The small number of NPM1 mutated children in this cohort does not allow definite conclusions on outcomes in MDS-EB. Considering ongoing clinical trials exploring targeted therapy for NPM1-mutated disease, the spectrum of this clinically heterogeneous group of myeloid neoplasia needs to be re-evaluated.

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P7
IDENTIFICATION OF HIGH-RISK JMML BY BMP4 BISULFITE NEXT-GENERATION SEQUENCING

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Key words: JMML, BMP4, Methylation, NGS, Biomarker

Background and aims
Juvenile myelomonocytic leukemia (JMML) is epigenetically classifiable based on genome-wide DNA methylation profiling. The present study explored the diagnostic value of locus-specific DNA methylation in the bone morphogenetic protein 4 (BMP4) gene as single predictor. The data were contrasted with CpG methylation data from previous genome-wide microarray studies.

Methods
Clinical samples were collected from 116 children diagnosed with JMML. Leukemic bone marrow or peripheral blood samples were separated into mononuclear cells (MNCs) and granulocytes by density gradient centrifugation. Cell samples from 9 children without malignant bone marrow infiltration were included as normal controls. Genomic DNA was converted with sodium bisulfite. PCR was performed and unique DNA barcode sequences for targeted NGS were attached to each sample and sequenced. The reads encompassed 9 CpG sites located at -274 to -139 bp upstream the BMP4 transcriptional start site.

Results
The composition of the patient cohort reflected the typical clinical, genetic and epigenetic landscape of JMML. Methylation levels of BMP4, assessed by targeted bisulfite next-generation sequencing, were heterogeneous within the JMML cohort and associated with the known risk factors age > 2 years, genetic subtype, and HbF > 15%. BMP4 methylation was highly comparable with microarray- based genome-wide methylation classification, especially for specific genetic and hematologic subtypes such as PTPN11, CBL, or HbF>15%. Quartile-based classification and subsequent survival analysis revealed lower 5-year disease-free survival (DFS) for the BMP4- high methylation class as compared to patients with normal BMP4 methylation. When considering rank-ordered absolute BMP4 methylation values (highest versus lowest 20%), the 5-year DFS was 0.38 for high versus 0.66 for low (p=0.03) and the 5-year cumulative incidence of relapse was 0.54 versus 0.23 (p=0.03).

Conclusions

The results of this study suggest that focal BMP4 methylation may be a useful biomarker and an efficient alternative to genome-wide methylation profiling for the clinical management of higher-risk JMML cases.

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P8

RIC VS MAC IN ALLO-HSCT FOR CHILDHOOD MYELODYSPLASTIC SYNDROME

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Key words: Childhood myelodysplastic syndrome, treatment, allo-HSCT.

Background and aims

MDS in children is uncommon, with an annual incidence of 1–4 cases per million, and may occur in the context of genetic predisposition. Allo-HSCT is the only curative treatment but the outcome varies according to the type of childhood MDS.To study long-term efficacy of allo-HSCT in different types of children MDS according to conditioning regimens.

Methods

We performed a retrospective analysis of 61 patients (pts) (28 (46%) females and 33 (54%) males) with MDS). All children received allo - HSCT between 1992 and 2022. Median age was 9 years (range 1.0-18). The diagnosis was established in accordance with
international criteria EWOG-MDS: RCC – 14 pts (23%), MDS-EB – 47 pts (77%). Following cytogenetic findings were registered: normal karyotype- 18 pts (29,5%); monosomy 7 – 24 (39%), complex karyotype - 4 pts (6,5%), other cytogenetic changes, in 15 (25%). The median time from diagnosis to allo-HSCT was 11 months (2 -86). As a stem cell source BM was used in 43 pts (70,5%), peripheral blood stem cells (PBSC), in 18 cases (29,5 %). MSD, MUD and haploidentical donors were used in 7 pts (11%),42 (69%) and 12 (20%), respectively. MAC was used in 27 pts (44%) and RIC, in 34 pts (56%). GVHD prophylaxis was Cy-base -22 pts (36%), ATG- 34 pts (56%),
other – 5 (8%).

Results

OS was 57%, DFS was 49 %. OS according to MDS type: RCC -77%; MDS-EB – 50% (p=0,104). DFS according to MDS type: RCC -77%; MDS-EB – 43% (p=0,05). DFS after RIC -57%, MAC -49% (p=0,205). Incidence of primary graft failure was 13.5% and secondary graft failure – 14.8%. CIR was 16,9% and NRM was 16,4 %. CI of acute GvHD II-IV was 43%, chronic GVHD - 17%.

Conclusions

Allo-HSCT is curative option for childhood MDS but requires individual approach for different types of MDS.

P9

OUTCOMES OF HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PEDIATRIC PATIENTS WITH MYELODYSPLASTIC SYNDROME AND ACUTE MYELOID LEUKEMIA SECONDARY TO INHERITED BONE MARROW FAILURE SYNDROMES

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Key words: MDS, AML, HSCT, IBMFS

Background and aims
Inherited bone marrow failure syndromes (IBMFS) may predispose to the development of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) with poor prognosis.

Methods
Retrospective study of 12 pediatric patients with a diagnosis of MDS/AML secondary to IBMFS who underwent hematopoietic stem cell transplantation (HSCT) between November 2009 and February 2023 at a tertiary centre.

Results
Diagnoses of IBMFS consisted of: severe congenital neutropenia (n=3), Shwachman-Diamond syndrome (n=3), Fanconi anemia (n=2), GATA2 deficiency (n=2) and MIRAGE syndrome (n=2). Nine had MDS at the time of HSCT and 3 had AML. Cytogenetic alterations present at the time of HSCT were: monosomy 7/del7q (n=8), del5q (n=2) and complex karyotype (n=1). Median ages at disease presentation, time of MDS/AML and time of HSCT were 3.6 months (range 1 month-14 years), 7.3 years (range 0.8-17.2 years) and 7.6 years (range 1.2-17.3 years), respectively. Two patients with AML and one with advanced MDS underwent chemotherapy prior to HSCT. The type of donor and source of hematopoietic progenitors were: bone marrow from identical sibling (n=2), bone marrow from HLA 9-10/10 unrelated donor (UD) (n=3), peripheral blood from haploidentical relative (n=2) and umbilical cord blood from UD (n=5). Conditioning regimens were myeloablative in ten patients and reduced-intensity in two. There were 3 graft failures (2 of them were rescued with successive transplants). 58% of patients had acute graft versus host disease (GVHD) grades II-IV, and 17% grades III-IV. No patients had chronic GVHD. The 5-year overall survival and event-free survival were 78,6% (64,7-92,5%) and 47,6% (25,9- 69,3%), respectively. Median follow-up was 4.7 years (range: 0.1-13.4 years). Two patients died, one from AML progression, and the other from invasive fungal infection.

Conclusions
Despite being a limited series, it shows that HSCT in recent years offers a curative option in most pediatric patients with MDS/AML secondary to IBMFS.

P10

BIOLOGY OF BONE MARROW DISORDER CHARACTERIZE DISTINCT SUBTYPES OF REFRACTORY CYTOPENIA OF CHILDHOOD (RCC)

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Key words: Refractory cytopenia of childhood (RCC), bone marrow failure, SAA-like, genetic aberrations

Background and aims
Refractory cytopenia of childhood (RCC), defined by persistent cytopenia and bone marrow dysplasia is a specific entity of pediatric MDS. Hypoplastic bone marrow and specific cytogenetic abnormalities (monosomy 7 and/or germline predisposition) are major criteria. Hypoplastic RCC lacking genetic aberrations (80%) is challenging to discrminate from aplastic anemia (AA) in a spectrum of bone marrow failure (BMF).

Methods
To describe RCC-heterogeneity, we performed retrospective analysis of clinical, hematological, immune and genetic parameters at diagnosis in a series of Czech patients diagnosed between 2006 - 2022 (n=64). SAA-diagnostic criteria, karyotyping and BMF-WES- panel were applied to cluster in subcategories; depth of cytopenia, interval to aplasia and clonal evolution to characterize biology of BMF. All parameters and clinical outcome in distinct subcategories were compared each-to-other and to a group of AA (n=62) diagnosed in the same period.

Results
BMF in „SAA-like-RCC"(n=24) is characterized by deep cytopenia (53%), short interval to aplasia (4,3 weeks) and young age (median 8 yrs), analogue to that of AA.„Non-SAA-like-RCC"(n=41) comprise all cases with genetic aberrations (n=17) and cases with indolent course of BMF (n=24). BM-disorder in „RCC-with-genetic-aberrations" presented later (median age 13,9 yrs), with longer time to aplasia (51 weeks) and specific immune profile, reflecting germline predisposition. Risk of clonal evolution in this group was 41% compared to 4% in both „SAA-like" and „non-SAA-like". Totally 29 RCC patients meeting SAA-criteria were scheduled for immunosuppressive therapy (IST). Long-term outcome in „SAA-like-RCC" was similar to that of aplastic anemia OS: 96%/92,1% (p=0,49), EFS: 43%/49,2% (p=0,68).

Conclusions

RCC is a heterogeneous disorder with variable natural course and need for risk-adapted treatment strategies. In our observations, SAA-criteria and molecular pattern characterize biological subtypes of RCC better than morphology. „SAA-like-RCC" seems analogous to aplastic anemia. RCC-with-genetic-aberrations is a real clonal disease profitting from early HSCT. More genetic studies are warranted to describe heterogeneous group of „non-SAA-like-RCC". Supported by CZ - NU23-05-00353 and NU20J-007-00028

P11

PREVALENCE OF EXPOSURE TO SOCIO-ENVIRONMENTAL FACTORS IN MYELODYSPLASTIC SYNDROMES - A STUDY OF THE BRAZILIAN PEDIATRIC MYELODYSPLASTIC SYNDROME GROUP (GCB-SMD-PED)

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Key words: Myelodysplastic Syndromes, Pediatric, Socio-environmental Factors

Background and aims

Myelodysplastic Syndromes (MDS) are rare hematologic disorders in children, most commonly in the age group 6-10 years. Adult- onset MDS has been associated with environmental exposures. The etiology of MDS in children and the impact of environmental exposures are currently entirely unknown. The aim of this project is to investigate the association between socio-environmental risk factors and the prevalence of pediatric MDS.

Methods

Cross-sectional study of children with cytopenias and under investigation for MDS, between 0 to 18 years old, who were evaluated by the GCB-SMD-PED since 1997 to 2022. Their parents were interviewed and after diagnosis two groups were defined: children with de novo MDS, and children with cytopenias of benign origen (pitfalls- such as infections and primary immunodeficiencies). The prevalence ratio between de novo MDS exposed and non-exposed to socio-environmental factors was generated in SPSS.

Results

400 children were eligible, 188 (47%) in the de novo MDS group and 212 (53%) in the no MDS group, and the mean age at diagnosis was 8 and 7 years, respectively. Children of low-educated fathers and smoker mothers were associated with a 31% (PR:1.31, CI95%1.03-1.66) and 45% (PR:1.45, CI95%1.10-1.91) higher prevalence, respectively, of de novo MDS compared to those with a medium-high level education fathers and nonsmoker mothers. Children with abnormalities (PR:1.27, CI95%0.99-1.63), exposed to toxic substances (PR:1.15, CI95% 0.91-1.46), children of a smoker father (PR:1.29, CI95%0.98-1.69) and low-educated mothers (PR:1.23, CI95%0.97-1.55) showed higher prevalence of developing de novo MDS compared with those not exposed, however they were not statistically significant. Sex, color, family history of cancer, infections, or living in rural area were not associated with occurrence of de novo MDS.

Conclusions

There is an association between low paternal education and maternal smoking with a higher prevalence of de novo MDS. Future analyses will be needed to investigate the causal relationship of these associations.

P12

A CASE OF KRAS-MUTATED JMML WITH HIGH METHYLATION PROFILE AND PERPLEXED CLINICAL COURSE.

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Key words: JMML, KRAS, HSCT, high methylation profile

Background and aims

JMML is a rare clonal hematopoietic malignancy of childhood. The deregulation of the RAS/MAPK signaling pathway caused in more than 90% of the cases by somatic or germline mutations in PTPN11, NRAS, KRAS, NF1, or CBL contributes to the pathogenesis of the disease. Clinical and molecular risk factors are used to stratify patients in risk groups and the treatment varies from watch-and-wait strategy to allogeneic bone marrow transplantation (HSCT), accordingly. The aim is to present a case with KRAS-mutated JMML, harboring unfavorable risk factors, determining his complicated course.

Methods

A 3.5 year-old boy was diagnosed with JMML. Initial work-up revealed low platelets, elevated HbF(82%), normal karyotype and a KRAS mutation c.35G>C;[p.G12A], VAF 41%.

Results

The patient received 4 courses of azacitidine with no response (KRAS-VAF 33%) and he was transplanted from MSD(sister) with TreoFluTT regimen. First relapse with abnormal karyotype 49,XY(+4,+11,+2) occurred 12 months after 1stHSCT (KRAS-VAF 41%). Treatment with 3 cycles of azacitidine-venetoclax resulted initially to molecular response (KRAS-VAF<1%) that was sustained <3 months (KRAS-VAF28%). The patient underwent 2nd MSD-HSCT (brother) with BuMel regimen, 4 doses of prophylactic DLI and 4 cycles of venetoclax post-HSCT (KRAS-VAF<1%). Sixteen months after the 2ndHSCT, a 2nd relapse occurred with abnormal karyotype 46,XY,add(18), KRAS-VAF 31%. Therapy with FLA was administered to achieve molecular remission and a 3rd HSCT (MUD 10/10) was performed with BuFluTT regimen. The patient achieved complete remission and he is on maintenance with azacytidine (7thcycle) and ruxolitinib for GvHD. However, an unresolved severe pneumonia has recently occurred requiring mechanical ventilation.

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Conclusions

Somatic KRAS mutations account for 10-15% of JMML cases, usually with low/intermediate DNA methylation profile. However, in our case KRAS mutation was combined with exceptionally elevated HbF and high methylation profile, highlighting the crucial role of epigenetics also in KRAS-mutated cases as a determinant of the eventual disease phenotype and outcome.

P13

THE CLINICAL SPECTRUM AND BIOLOGICAL FINDINGS IN RAS-ASSOCIATED JUVENILE MYELOMONOCYTIC LEUKEMIA AND RAS-ASSOCIATED AUTOIMMUNE LEUKOPROLIFERATIVE DISORDER

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Key words: JMML, RALD

Background and aims

Identical mutations in KRAS and NRAS can be observed both in children with juvenile myelomonocytic leukemia (JMML) and RAS- associated autoimmune leukoproliferative disorder (RALD). JMML is a severe hematopoietic malignancy of early childhood, whereas RALD is considered a benign condition associated with lymphoproliferation and autoimmune phenomena. The objective of this study was to establish clinical and biological hallmarks that could differentiate JMML from RALD in patients with somatic KRAS and NRAS mutations.

Methods

We conducted a systematic review including case reports and cohort studies describing patients diagnosed with KRAS and NRAS mutated JMML or RALD. To assess the differences between both diseases the Chi-Square test and Fisher's Exact test were used to compare qualitative variables and the independent two-sample t-tests and Mann-Whitney U tests for quantitative variables. All statistical analysis were performed using SPSS Statistics 28.

Results

A systematic literature search resulted in the inclusion of 56 studies. From these articles, clinical and genetic characteristics from 127 patients with JMML and 35 with RALD could be extracted. JMML patients were younger, and more frequently males. Splenomegaly is more frequent in JMML patients (p=0.05). Autoimmune phenomena are described in half of the RALD patients vs in one JMML patient. White blood cell counts, monocyte counts, lymphocyte counts, and percentage of circulating blasts are higher in JMML. An abnormal karyotype was exclusively found in JMML patients. Interestingly, pooling all studies, only male gender and presence of monocytosis favor a diagnosis of JMML, whereas autoimmune phenomena are suggestive for RALD.

Conclusions

The conducted systematic review found similar clinical and laboratory characteristics between JMML and RALD, although some significant differences (monocytosis, abnormal karyotype in JMML and autoimmune phenomena in RALD) could be observed. Given the overlap, we believe JMML and RALD are likely to be two extremities of the same disease spectrum rather than two distinct entities.

P14

THIRTY YEARS OF EXPERIENCE IN THE TREATMENT OF CHILDHOOD APLASTIC ANEMIA IN LITHUANIA

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Key words: Children, Aplastic anemia, HSCT

Background and aims

Aplastic anemia (AA) is a rare heterogeneous disorder of hematopoietic stem cells causing pancytopenia and marrow hypoplasia. We aimed to review the epidemiology, diagnostics and treatment results of childhood AA (acquired) in Lithuania.

Methods

We performed a retrospective analysis of pediatric AA cases diagnosed and treated at the Center for Pediatric Oncology and Hematology, VULSK, in Lithuania from 1992 to 2021. Patients with AA are centralized at our institution. Data was obtained from medical records. The study period was split in two groups: 1992-2001 and 2002-2021. The reason for this distribution was the initiation of pediatric hematopoietic stem cell transplantation (HSCT) in 2002 in Lithuania.

Results

From 1992 to 2021 49 children were diagnosed and treated for acquired AA (idiopathic n = 40, following viral hepatitis n = 8 and toxic n = 1). 21 cases (43.0%) in the first period (1992-2001), and 28 cases (57.0%) in the second period (2002-2021) were included into the study. The median age at diagnosis was 9.02 years (1.9-17.8 years). 71.4% of patients died within the first period (1992-2001). They were treated with prednisolone/cyclosporin/ATG/granulocyte-colony stimulating factor/androgen. There was no opportunity to perform HSCT. The results of the second period: 71.4% of patients survived in this period. HSCT was performed on 19 patients (67.9%): 10 patients from HLA identical sibling donors; for 9 patients from HLA unrelated donors, but 4 of whom died after HSTC (2 due to GVHD, 1 – CMV infection, 1 – Zygomycosis). HSCT was performed after median 2.17 months (1-5 months) after AA diagnosis.

Conclusions

Patients' survival before treatment with HSCT era was very low – 28,6% compared with treatment with HSCT (71,4%), p=0.02. Our results show that allogeneic HSCT is the first-line treatment for children with AA. Aplastic anemia requires bone marrow transplantation as soon as possible

P15

HOSPITAL ADMISSIONS OF APLASTIC ANAEMIA: REAL WORLD EVIDENCE FROM UK CHILDREN ADMITTED FROM 2017 – 2022

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Key words: Administrative database, Electronic health records, Epidemiology, Stem cell transplant, Constitutional aplastic anaemia

Background and aims

Aplastic anaemia is a rare, heterogenous, life-threatening haematological disorder. There is limited contemporary data on the burden of the disease within the paediatric population in the UK. Though there is no national AA registry there are datasets that capture isolated parts of the patient journey i.e. hospital admissions. We wanted to determine the hospital activity of children with aplastic anaemia.

Methods

We performed a retrospective health service evaluation to determine the demographics and number of new patients admitted to hospital each year with a diagnosis of aplastic anaemia and their treatment. The primary source for this analysis was the Hospital Episode Statistics (HES) database and patients were identified using ICD-10 coding.

Results

For the period 1st April 2017-31st March 2022, there were 125 new patients under the age of 18 years of age diagnosed with aplastic anaemia, an average of 25 new cases per year. Most patients received a diagnosis of 'AA, unspecified' – 56%. 48% of patients were female. 60% of patients were white. The ethnicity with the highest patients per 100,000 population was 'Any other Asian background' followed by 'Pakistani (Asian or Asian British)'. Twenty-five patients (20% of the whole cohort) subsequently underwent a stem cell transplant during the analysis period. 80% of transplants were allogenic peripheral blood stem cell transplant and 20% were bone marrow transplants. The mean time from diagnosis to transplant was 183 days.

Conclusions

To our knowledge, this is the first epidemiological data on AA in UK children. There are geographic variations in the way data is classified making the analysis difficult and supporting the idea that a national registry to determine incidence, prevalence and survival of children with aplastic anaemia in the UK is needed as is seen in other countries.

P16

REFRACTORY CYTOPENIA OF CHILDHOOD: CLINICAL FEATURES, PATHOLOGIC FINDINGS AND EXPERIENCE OF BRAZILIAN COOPERATIVE GROUP OF PEDIATRIC MYELODYSPLASTIC SYNDROME

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Key words: Myelodysplastic syndromes, Child, Immunophenotyping, Pancytopenia, Flow cytometry

Background and aims

Myelodysplastic Syndromes (MDS) estimated incidence is 1 to 4 cases per million, with Refractory Cytopenia of Childhood (RCC) being the most common subtype of pediatric MDS. However, it can be a challenging diagnosis. In these cases, cytogenetic alterations as well as immunophenotyping by flow cytometry contribute to diagnostic workup. Knowing the profile of these children can improve clinical reasoning, suspicion, and identification of patients. OBJECTIVE: To describe the clinical and pathological profile of patients referred to the BCG-MDS-PED with refractory cytopenia of childhood diagnosis.

Methods

Descriptive cross-sectional study with retrospective data collection from 91 children (0 to 18 years old) referred to the group from 1997 to May 2022 who were diagnosed with MDS - RCC. Statistical analyzes were performed using SPSS Software for Windows® version 21.

Results

We had 56% of female cases and the Caucasian ethnicity was the most prevalent (65.9%). Age ranged from 1 to 17 years, with a median of 8.9 years. Regarding clinical profile, pallor was the most common alteration, affecting 64.8% of patients and skin/mucosal bleeding was observed in 39.6%. In laboratory profile, 57.1% of patients had anemia at diagnosis; 70.3% neutropenia and 80.2% thrombocytopenia. Pancytopenia was present in 39.6%. In morphologic evaluation, most patients had hypocellular bone marrow (82.5%). Cell decrease was more intense in the granulocyte population. All evaluable patients (N=59) had at least 1 immunophenotypic abnormality and 6.3% had aberrant expression of CD7 in myeloid precursors. Clonal alterations were observed in 16 patients (20.7%) with 27% of these related to chromosome 7 (monosomy and long arm deletion).

Conclusions

The present work contributes with national data for this rare pathology, facilitating further studies on pediatric MDS and increasing understanding of the disease.

P17

RECURRENCE OF UNDERLYING CONGENITAL NEUTROPENIA WITHOUT MDS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION IN A PATIENT WITH ELANE-MUTATED CONGENITAL NEUTROPENIA AND SECONDARY MDS

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Key words: congenital neutropenia, secondary MDS, ELANE

Background and aims

Severe congenital neutropenia (SCN) represents a disorder of haematopoiesis with leukemic predisposition. Mutations in CSF3R and
RUNX1 are usually involved in multistep progression to secondary MDS/leukemia.

Methods

We report on an infant with an ELANE-mutated SCN and secondary MDS, who demonstrated a unique post-transplant course.

Results

The patient was diagnosed as ELANE mutated SCN shortly after birth due to agranulocytosis and the affected mother. The patient showed inadequate response to G-CSF therapy, leading to high doses (30µg/kg/day) by the age of 10 months. At that time point, she also developed pancytopenia, needing transfusions for erythrocytes and platelets, and hepatosplenomegaly. The bone marrow (BM) examination demonstrated hypocellularity with hyperplastic and dysplastic erythropoiesis, megakaryopoietic hypoplasia and severe maturation deficiency of granulopoiesis without blast excess, which led to the diagnosis of secondary MDS. The karyotype was normal. No mutations were found in CSF3R and RUNX1 genes, while a KRAS mutation (c.183A>C) was detected with a low VAF (4%). The patient underwent hematopoietic stem cell transplantation (HSCT) from an HLA-compatible unrelated donor after conditioning with thiotepa, fludarabine, treosulfan at the age of 16 months. Unfortunately, mixed chimerism was detected soon after HSCT, leading to nearly complete autologous reconstitution 1.5 years after HSCT without reappearance of the KRAS mutation. Due to declining granulocyte count, G-CSF (5µg/kg/day) was given intermittently again with an adequate response. The patient is in a good condition without cytopenia 22 months after HSCT.

Conclusions

The current patient demonstrated a transformation to secondary MDS with severe pancytopenia, inadequate G-CSF response, ineffective hematopoiesis and a small KRAS mutated clone. The unique post-HSCT course was characterized by recurrence of SCN and autologous reconstitution without relapse of MDS. The significance of the KRAS mutation in pathogenesis of secondary MDS of this patient remains unclear.

P18

IMMUNOPHENOTYPE IN SAMD9/9L SYNDROME: CORRELATIONS WITH MORPHOLOGY

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Key words: immunophenotype, SAMD9, SAMD9L

Background and aims

Immune dysfunction is a frequent feature in SAMD9/9L syndromes with scarce reports on lymphocytic subsets in patients with MDS and germline SAMD9 and SAMD9L mutations (SAMD9/9L^{mut}) and no correlations with morphology.

Methods

Retrospective, central analysis of peripheral blood (PB) and/or bone marrow (BM) immunophenotype in consecutive patients with pediatric MDS and germline SAMD9/9L^{mut}, diagnosed in Greece and enrolled in the EWOG-MDS-SAA study. Age-adapted references for lymphocytic subsets were used.

Results

Six patients were included, SAMD9^{mut} N=4, SAMD9L^{mut} N=2, 4 boys/2 girls of median age 6.8 years (range 1.2-15.5 years). RCC and MDS-EB was diagnosed in 5 and 1 patients, respectively. Cellularity was severely reduced, moderately reduced and increased in 2, 3 and 1 patients, respectively. Monosomy 7, inv(17p) and normal karyotype was found in 4, 1 and 1 patients, respectively. Somatic mutations included SAMD9 and RUNX1 in one patient each. An additional germline PTPN11^{mut} was found in one patient with RCC and increased cellularity. Immunophenotype was performed on PB, BM and PB+BM in 3, 2 and 1 patients, respectively. Median PB percentage and absolute count of B-cells were 3.05% (range 0.88-16.2%) and 92/mm^3 (range 17-319/mm^3), respectively. Median percentage and absolute count of NK-cells in PB were 3.5% (range 0.3-6.1%) and 62/mm^3 (range 9-335/mm^3), respectively. Median

percentage of hematogones in BM was 0.046% (range 0.03-1.2%). Overall, B-cells and NK-cells were reduced in PB in all patients but the patient with additional germline PTPN11^{mut}. Hematogones were reduced in all three patients with BM immunophenotype. The T- cell compartment was normal in both PB and BM in all patients.

Conclusions

B and NK lymphopenia were observed in children with MDS with germline SAMD9/9L^{mut}. Immunophenotype alterations might correlate with genetic causes, especially in hypocellular cases, pointing out that immunophenotype on PB/BM is an integral part of diagnostic workup in pediatric MDS.

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TELOMERE LENGHT AND CYTOPENIAS: ANALYSIS OF CHILDREN REFERRED TO BRAZILIAN COOPERATIVE GROUP OF CHILDHOOD MYELODYSPLASTIC SYNDROME

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Key words: myelodysplastic syndrome, telomere biology disorders, juvenile myelomonocytic leukemia

Background and aims

Telomeres are nucleoprotein structures whose function is to maintain genomic integrity. In humans, telomere dysfunction translates into the development of diseases involving different organs (telomeropathies), and cytopenias can be the initial finding.
Myelodysplastic syndromes (MDS) are rare in the pediatric population. Refractory Citopenia of Childhood (RCC), the most common subtype, clinically manifests with persistent cytopenias, low blasts and hypocellular bone marrow in about 80% of cases. In this context, the differential diagnosis of RCC must be made with aplastic anemia, telomere biology disorders and other bone marrow failure syndromes. The aims of this study were to determine the telomere length of cytopenic patients and to perform next generation sequencing (NGS) in those with shortened telomeres.

Methods

Patients referred to Brazilian Cooperative Group of Pediatric MDS without a diagnosis, and with at least one lineage cytopenia were included. Peripheral blood samples were collected and telomere length were evaluated by flow-fish. Those samples with telomere shortening were then sent to NGS analysis of 54 genes involved in telomere biology disorders.

Results

From December 2021 to May 2023, 54 patients were included. Results are available for 50 patients and 4 patients (7,4%) had short telomeres and are now under NGS analysis (data not available). None of these patients had any clinical signs of telomere biology disorders. None had final diagnosis of MDS. Patients 1 and 3 presented with pancytopenia (diagnosis were autoimmune lymphoproliferative syndrome with FAS mutation and bone marrow failure syndrome), patient 2 had isolated neutropenia (preliminary diagnosis of benign ethnic neutropenia) and patient 4 had anemia, thrombocytopenia and monocytosis (diagnosis of Juvenile Myelomonocytic Leukemia with mutations in KRAS and WT1 genes).

Conclusions

In patients with cytopenias and suspected MDS, it is important to exclude telomere biology disorders, as well as other bone marrow failure syndromes, as they may present without any recognizable sign.

P20

CHILDHOOD MYELODYSPLASTIC NEOPLASM: GENETIC VARIANTS AND THEIR IMPACT ON DIAGNOSIS AND PROGNOSIS

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Key words: Childhood MDS, cytogenetic alterations, genetic variants

Background and aims

Childhood myelodysplastic neoplasm (cMDS) has a risk of evolution to acute myeloid leukemia. Allogeneic hematopoietic stem cell transplantation (HSCT) is the only treatment with a cure potential for cMDS. The normal karyotype is observed in approximately 50% in these patients. So, it is fundamental the identification of molecular alterations, which could help to predict prognosis. This study aimed to identify genetic variants in cMDS and their associations with clinical outcomes.

Methods

A customized Next-Generation-Sequencing (NGS) panel was performed with Ion Torrent Personal Genome Machine for genes: GATA2, RUNX1, CEBPA, ANKRD26, ETV72, SAMD9, SAMD9L, PTPN11, NRAS, SETBP1, DDX41, TP53, FLT3, SRP72, JAK3. Analyses were performed with NextGENe Software, genetic variants were classified using dbSNP, 1000 genomes, COSMIC, and Varsome databases.

Results

We analyzed bone marrow (BM) samples from eight cMDS patients, five with childhood refractory cytopenia and three with MDS with excess of blasts. The disease evolution was observed in five patients. Abnormal karyotypes were identified in six patients: three with monosomy 7, one with complex karyotype, one with biclonal chromosomal alteration, and one with t(3;8)(p26;q21) constitutional. NGS identified pathogenic variants in NRAS, GATA2; like pathogenic in ETV6, DDX41, ANKRD26, FLT3; variant with uncertain significance (VUS) in JAK3, SRP72, ANKRD26, and SAMD9L. During the donor selection, germline variants were observed in two cMDS families. A patient with normal karyotype shared the same genetic variants with her mother and siblings, being like pathogenic variants (DDX41, ANKRD26), and VUS (SAMD9L). As well as the constitutional t(3;8)(p26;q21) patient who had VUS (SRP72, ANKRD26) of maternal origin.

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Conclusions

Although the number of patients studied was small, this study showed the importance of using NGS to aid in the diagnosis, prognosis mainly for patients with normal karyotype, identifying genetic predisposition for genetic counseling, therapeutic considerations, donor selection for HSCT, and a better understanding of cMDS pathogenesis. Acknowledgment: FAPERJ/E-26/201.218/2022.

P21

PITFALLS IN THE DIAGNOSTICS OF SAA/RCC: CAN WHOLE EXOME SEQUENCING LEAD US THE WAY?

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Key words: whole exome sequencing, congenital amegakaryocytic thrombocytopenia, refractory cytopenia of childhood

Background and aims

The initial diagnostic work-up of severe aplastic anemia (SAA) and hypocellular refractory cytopenia of childhood (RCC) focuses on differentiating between immune-mediated, idiopathic and constitutional diseases such as Fanconi Anemia or GATA2 deficiency.
Guidelines recommend prompt initiation of immunosuppressive therapy (IST) for supposedly non-constitutional SAA/RCC patients without a matched sibling donor - with only narrow prior genetic diagnostics. However, over 50% of patients fail to respond to first- line IST, and treatment delay negatively affects outcome. We hypothesize that whole exome sequencing (WES) can support the identification of patients not suitable for IST.

Methods

We performed WES in addition to bone marrow examination and flow cytometry in a girl born to consanguineous parents who presented with severe pancytopenia at the age of 6 (hemoglobin 6.5 g/dL, MCV 106 fl, thrombocytes 5 G/L, ANC 0.85 G/L).

Results

Bone marrow examination suggested the diagnosis of RCC with severe hypocellularity, aplasia of megakaryopoiesis, and signs of erythropoietic dysplasia. Flow cytometry revealed a severely reduced number of CD34+ progenitors with a skewed maturation profile depleted of MPPs, EMPs and CMPs, but with residual LMMPs and GMPs. WES identified a novel homozygous germline missense variant (c.941T>Gp.Phe314Cys; CADD: 26.1) in exon 6 of the MPL gene, affecting a highly conserved phenylalanine in the extracellular domain of the thrombopoietin receptor. A previously reported patient with a homozygous missense variant in exon 6 had a similar phenotype with first signs of multi- lineage cytopenia after the age of 5 (Gemeshausen et al., Haematologica 2021).

Conclusions

WES led to the diagnosis of congenital amegakaryocytic thrombocytopenia (CAMT-MPL) with hematopoietic stem cell transplantation as the only available curative treatment option. This case illustrates the challenge of promptly initiating IST and exemplifies the value of WES in therapeutic decision making in RCC, suggesting that systematic genetic investigations could contribute to an improved identification of non-immune-mediated SAA/RCC patients.

P22

IDENTIFICATION OF FUNCTIONAL DEFECTS LEADING TO BONE MARROW FAILURE IN GATA2 DEFICIENCY

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Key words: GATA2 Deficiency, Bone Marrow Failure

Background and aims

Hematopoiesis is the process in which multipotent hematopoietic stem cells differentiate through progenitor cells into different blood cell lineages. One transcription factor governing this process is GATA2, playing a role in hematopoietic stem and progenitor cell (HSPC) development and differentiation. Germline monoallelic GATA2 mutations lead to variable phenotypes like immunodeficiency, cytopenia, lymphedema and development of myelodysplastic syndromes. To decipher the pathophysiological characteristics of GATA2 deficiency, our group established a mouse model showing spontaneous leukemogenesis in the context of GATA2 heterozygosity. This model showed that transplantation of $Gata2^{+/-}$ HSPCs into lethally irradiated mice can induce bone marrow failure and secondary leukemia. Moreover, $Gata2^{+/-}$ HSPCs showed poor engraftment within the first weeks after transplantation probably due to an increased apoptotic susceptibility. The aim of this project is to characterize the apoptosis signaling in $Gata2^{+/-}$ HSPCs in detail and focus on various stress signals including kinase inhibitors.

Methods

We have used several in vivo (transplantation of limited numbers of LSK cells) and in vitro methods (proliferation/differentiation, apoptosis using different kinase inhibitors and DNA damage techniques) to understand the functional defects of $Gata2^{+/-}$ cells.

Results

Analysis at different time points during the first two weeks after transplanting $Gata2^{+/-}$ HSPCs into lethally irradiated mice revealed less LSK cell numbers in the BM compared to WT recipients. Differentiation experiments performed in Methocults using transplanted LSK cells showed a lower number of colonies when exposed to the multi-kinase inhibitor Staurosporine. Furthermore, this drug showed an increased apoptosis rate in $Gata2^{+/-}$ HSPCs compared to WT in vitro. Proliferation experiments showed that aged $Gata2^{+/-}$ cells have a decreased proliferation rate compared to WT cells.

Conclusions

The results obtained from this study revealed a tendency towards higher apoptosis rate in $Gata2^{+/-}$ LSK cells as well as less proliferation under kinase inhibitor treatment.

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THERAPY-RELATED MYELODYSPLASTIC SYNDROMES (t-MDS) IN CHILDHOOD: THE EXPERIENCE OF THE HELLENIC STUDY GROUP FOR MDS/JMML/SAA, MEMBER OF THE EWOG-MDS/SAA WORKING GROUP

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Key words: therapy-related-MDS

Background and aims

Therapy-related MDS (t-MDS) are a distinct group of pediatric-MDS occurring after exposure to chemotherapy and/or radiationtherapy for treatment of a previous unrelated malignancy. The correlation between t-MDS and genetic predisposition is being studied. We present four (4) t-MDS patients as the 7.5-year experience (2015-2022) of the Hellenic study group for MDS/JMML/SAA, as a member of the EWOG MDS/SAA.

Methods

Four t-MDS patients registered to the Hellenic MDS/JMLL/SAA group are presented, being diagnosed after appropriate detailed clinical, laboratory and cytogenetic investigations (reviewed by national reference centers and EWOG-MDS/SAA).

Results

The 4 t-MDS patients constitute 10% of the hellenic MDS cohort (period 10/2015-4/2023). Sex: 3-female/1-male. Median/Mean age: 6.4/6.5-years. Underlying Malignancy: Neuroblastoma Stage-IV 2/4, Meduloblastoma Grade-IV 1/4 and Nephroblastoma Stage-I-HR 1/4. The patients were treated for primary disease according to current treatment protocols [chemotherapy 4/4, radiotherapy 2/4, autologous hematopoietic stem cell transplantation (SCT) 3/4 and 3 received SCT >2 times]. At diagnosis of t-MDS in CR1/CR2/CR3 were 2/1/1 patients respectively. Median/mean time since diagnosis of primary disease: 1,144/1,160-days (median/mean time since end of treatment: 121/249-days). All had pancytopenia (CBC) and dysplasia (BM) in two (2/4) or three (2/4) hematopoietic series. None was presented with excess blasts (t-MDS-EB). Cytogenetic testing: normal karyotype 2/4, structural cytogenetic lesion 1/4, and complex karyotype 1/4 (resolved to normal karyotype 17 months post t-MDS diagnosis). Further screening (NGS/WES) to highlight genetic predisposition lesions for myeloid neoplasms revealed PTPN11 somatic-mutation in the medulloblastoma patient. None with TP53 mutation. All patients followed watchful-waiting strategy and showed partial hematological recovery (median/mean follow-up time from t-MDS diagnosis: 24.3/31.5 months respectively). Three are alive and one died due to primary disease (advanced neuroblastoma).

Conclusions

Molecular-genetic lesions' detection is important for better diagnosis and follow-up of patients with t-MDS. Close clinical and hematological monitoring is important for identifying transition to RAEB/AML and timely initiation of SCT.

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NATIONAL EXPERIENCE WITH IMMUNOSUPPRESSIVE THERAPY AND ELTROMBOPAG IN CHILDREN WITH APLASTIC ANEMIA- REAL WORLD DATA

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Key words: aplastic anemia, eltrombopag, immunosuppressive therapy

Background and aims

Immunosuppressive treatment (IST) with the use of antilymphocyte or antitymocyte globulin (ATG) is the standard in severe aplastic anemia (SAA) in children, who lack sibling donor. However, treatment failures after IST are common. Eltrombopag (EPAG), an oral thrombopoietin receptor agonist, showed improved hematologic response in a combination with first-line standard IST in adults with SAA.We present retrospective results of IST with EPAG in newly diagnosed AA in children from 6 Polish pediatric oncology and hematology centers. Patients were diagnosed and evaluated according to the EWOG-SAA guidelines.

Methods

In years 2020-2023, 15 patients (6 girls, 9 boys) aged from 1 year and 10 months to 16 years with AA (14 SAA , 1 MMA) were qualified for upfront IST (rATG or hATG). EPAG was started in 4 patients from the beginning of IST, and in 11 between 2 - 4 weeks from IST. The starting dose was 25-50mg /day depending on age and increased to a maximum of 150mg / day depending on hematological response. EPAG treatment was planned for 6 months, but lasted from 1 month to 2 years

Results

Seven patients (46.6%) responded to IST-EPAG combination. Complete remission (CR) was achieved in 3 children, and partial remission (PR) was in 4 patients. After EPAG discontinuation, 2 patients remain in CR, and 2 relapsed and were referred for HSCT. Three patients continue EPAG. Of 8 patients who did not respond to treatment, 7 underwent HSCT from an unrelated donor and are alive and well, and 1 child died before transplantation. EPAG was very well tolerated, and prolonged therapy was uneventful. No evidence of clonal disease was found during therapy.

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Conclusions

The reported group is heterogenous and EPAG did not significantly improve the efficacy of IST treatment, but good tolerance can endorse prospective studies in children and suggests possibility of prolonged thromobomimetics administration.

P25

The rare cases of co-occurrences of Down Syndrome Disease and Juvenile Myelomonocytic Leukemia

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Key words: JMML, Down Syndrome

Background and aims

Juvenile myelomonocytic leukemia is a rare clonal disease of early childhood characterized by excessive proliferation of granulocytes and monocytes cells. Although 30% of Down syndrome (DS) patients present a transient myeloproliferative disease (TMD) in the first few months after birth with potential subsequent development of an acute myeloid leukemia, the association with JMML has yet to be reported. Here we present the exceptional situation of two infants with 47,XX+21c karyotype and early onset of a JMML disease.

Methods

Whole Exome Sequencing and custom panel followed by Illumina sequencing were used to identify somatic mutations and for the screening of PTPN11 Variant Allele Frequency (VAF). Methylation was carried out by EPIC array.

Results

Both the patients were referred to our Center for the appearance of hyperleukocytosis and thrombocytopenia. We initially performed a peripheral blood smear showing a large amount of immature myelomonocytic cells associated with less than 5% of
undifferentiated blasts, confirmed also by flow-cytometry immunophenotype. Subsequently, a bone marrow (BM) aspirate was performed, confirming the prevalence of myelomonocytic precursor, hyperplasia and low blast count at cytomorphology and immunophenotyping. These findings, similar in both peripheral blood and BM samples, were not consistent with the typical presentation of TMD of DS, considering the high amount of myelomonocytic cells, but raised the suspicion of a different myeloproliferative disease. Both samples were positive for a somatic mutation in PTPN11 gene. Of note, we did not identify any hot spot mutations in GATA1 gene, known to be associated with TMD. Both the patients received eight cycles of Azacytidine, followed by HSCT for one patient. VAF in PTPN11 gene was also screened to follow clinical response to treatment.

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Conclusions

In conclusion, this is the first demonstration of JMML in DS. Our findings are critical for the proper diagnosis of myeloproliferative conditions in DS infants and consequently therapy management.

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INVESTIGATING AND MODULATING LEUKEMIA INITIATING CELLS TO REDUCE RISK OF POST-TRANSPLANT RELAPSE IN JMML

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Key words: HSPCs, PTPN11 mutated LICs,KRAS mutated LICs, relapse risk

Background and aims

JMML is a myeloproliferative neoplasm of early childhood driven by constitutive active RAS signaling and epigenetic mechanisms. Allo-HSCT is the only curative treatment for most JMML patients. Surprisingly, relapse risk after allo-HSCT is different in the genetic JMML subtypes. In particular, PTPN11 mutated and DNA-hypermethylated JMML relapse in up to 50% cases, while relapse in C JMML is rare. One conceivable explanation is that PTPN11 mutated leukemia initiating cells (LICs) might have stronger competitive fitness than healthy hematopoietic stem and progenitor cells (HSPCs)while KRAS mutated LICs might be outcompeted by donor cells. We are studying LIC fitness on a functional and transcriptomic level to modulate them to reduce relapse risk.

Methods

We characterize HSPCs isolated from bone marrow and spleens of mice expressing the $Ptpn11^{D61Y/+}$ and $Kras^{G12D+/}$mutants and compare them to wildtype HSPCs with regard toproliferation, differentiation, apoptosis and self-renewal.

Results

Our preliminary data show that Ptpn11 mutated HSPCs accumulate in the spleen. They proliferate more and faster when compared to WT HSPCs. In addition, leukemic stem cells have short-term survival advantages and differentiate more. However, their self-renewal ability is reduced.

Conclusions

Ptpn11 knock-in HSPCs are fitter than WT cells with regard to proliferation and differentiation, but they lose their self-renewal ability prematurely. We will now characterize Kras knock-in HSPCs in vitro and perform functional in vivo assays.

P27

MYELODYSPLASIA-RELATED CYTOGENETIC ABNORMALITES IN CHILDHOOD ACUTE MYELOID LEUKEMIA – A 20-YEAR I-BFM- AML COLLABORATIVE DATABASE STUDY

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Key words: monosomy 7, complex karyotype, MDS, AML

Background and aims

The leukemogenesis of childhood acute myeloid leukemia (AML) with myelodysplasia-related cytogenetic abnormalities is largely unknown. Childhood AML harbor cytogenetic aberrations related to myelodysplastic syndrome (MDS) such as monosomy 7 and complex karyotype (CK). Monosomy 7 occurs in 25-40% childhood MDS and <5% of childhood AML. CK occurs in 15-20% of childhood MDS and 15% of childhood AML. Childhood AML may have developed from antecedent MDS, irrespective of blast count at diagnosis. Our aim is to describe the myelodysplasia-related cytogenetic abnormalities in childhood AML at diagnosis.

Methods

Approximately, 1.000 children within the I-BFM-AML diagnosed with AML with myelodysplasi-related cytogenetic abnormalities between 2000-2021 are eligible for inclusion. Characteristics including leukemic blasts in peripheral blood (PB) and bone marrow (BM), white blood cell count (WBC), and FAB classification at diagnosis are reported. Karyotypes are grouped by numerical and structural variants. CK are defined as at least three unrelated cytogenetic abnormalities. Extended genetic profiling will be performed in the subset of children with available biological material.

Results

Thirty-six children have been included by May 2023. Preliminary results show a median age at diagnosis of 3 years (range: 0-18), evenly distributed between sexes (male, n=22, 61%). Three children had a history of prolonged cytopenia prior to diagnosis. At diagnosis, median blasts in PB and BM were 32% (range: 0-100%) and 62% (range: 13-100%), respectively and median WBC was 14 x 10^9/L (range: 0.9 – 308 × 10^9/L). FAB M7 (n=12) and FAB M5 (n=6) were the most frequent morphological subtypes. Trisomy 8 was the most frequent numerical cytogenetic abnormality (n=9). Monosomy 7 was present in 3 children. CK was present in 21 children.

Conclusions

Our study is expected to provide insight into whether AML with myelodysplasia-related cytogenetic abnormalities constitutes a distinct subgroup of AML or represents MDS evolving to a higher blast count.

P28

RELAPSED UBTF-TD MDS TREATED WITH VENETOCLAX AND AZACITIDINE

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Key words: MDS UBTF-TD therapy

Background and aims
UBTF-TD associated MDS was recently described as a subgroup constituting 25% of MDS-EB in children. The outcome of UBTF-TD MDS-EB is poor with very sparse reports of successful therapy.

Methods
We report our experience on venetoclax and azacitidine therapy in a patient with relapsed UBTF-TD MDS following two hematopoietic stem cell transplantations (HSCT).

Results
A previously healthy 13-year-old girl presented with pancytopenia and was diagnosed as MDS-EB with 15% blasts and 47,XX,+8. One month later the blast count has increased to 20-25%. One course of FLA reduced the blast count to below 5%. HSCT with unrelated donor and busulfan/cyclophosphamide/melphalan conditioning was performed with grade I aGvHD and early tapering of immune therapy. Relapse with similar phenotype but with normal karyotype and WT1 mutation occurred two years from HSCT. Haplo-HSCT was performed using alfa-beta depleted PB stem cells and treosulfan based conditioning. Grade III aGvHD (gut and skin) was treated with prednisolone, ECP, and ruxolitinib. Second relapse occurred one year from haplo-HSCT. Cytogenetics had evolved to 46,X,t(X;12)(p11;q11) while WT1 mutation persisted. UBTF-TD-TD was detected in retrospect at diagnosis and both relapses

Four courses of azacitidin (125 mg sc for five days) and venetoclax 100 mg/day for 28 days (during concomitant azole therapy) were given. The therapy was well tolerated but the courses were complicated by signs of fungal and viral infections and persistent cytopenia necessitating pauses of the venetoclax therapy. MRD monitoring with UBTF-TD and WT1 expression showed a dramatic response to therapy with UBTF- TD <0.5% and normal WT1 expression. The patient remains in good clinical condition with morphologic, cytogenetic, and molecular remission although still cytopenic seven months from second relapse.

Conclusions
The optimal therapy of UBTF-TD MDS is not known. Our patient showed marked response to venetoclax and azacitidine calling for more studies of this combination in children with UBTF-TD associated MDS-EB.

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DYSLIPIDEMIA AFTER INTENSIVE IMMUNOSUPPRESSIVE THERAPY VERSUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN APLASTIC ANEMIA: A SINGLE-CENTER EXPERIENCE

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Background and aims
Intensive immunosuppressive treatment (IST) and allogeneic hematopoietic stem cell transplantation (HSCT) have significantly improved survival of aplastic anemia (AA) patients, but also induce treatment-related complications. Dyslipidemia and associated cardiovascular events are known to occur more frequently after HSCT, but the influence of IST alone on lipid metabolism is not known. This study analyzed lipid profiles of all patients who underwent either first-line IST or HSCT for AA at our center.

Methods
This retrospective, single-centre cohort study included 109 adult AA patients treated with IST (n= 63) consisting of horse antithymocyte globulin, cyclosporine +/- eltrombopag or HSCT (n= 46), in which lipid values at 6, 12 and 24 months were available for comparison.

Results
Median total cholesterol and LDL after 6, 12 and 24 months were 4.56, 4.72 and 4.48 mmol/l and 2.29, 2.65 and 2.53 mmol/l, respectively after HSCT, compared to 5.52, 5.42 and 5.24 mmol/l and 3.31, 2.91 and 2.96, respectively after IST. There was no significant association between therapy modality and total cholesterol, LDL or triglyceride levels. After HSCT and IST therapy hypercholesterolemia (total cholesterol ^{3} 5 mmol/l) was significantly more prevalent (pre- versus posttherapy: 2 % vs 15 % and 12 % vs 26 %, respectively, both $p < 0.01$).

Conclusions
Dyslipidemia occurs in an equal higher rate after HSCT and IST in AA. There is a trend for persisting higher lipid values in patients treated with IST alone, emphasizing the need for stringent long-term screening and management of dyslipidemia during AA follow up to potentially improve cardiovascular outcome in these patients.

P30

CLINICAL AND HEMATOLOGICAL PROFILE OF CHILDREN WITH CYTOPENIAS REFERRED TO THE BRAZILIAN COOPERATIVE GROUP OF PEDIATRIC MYELODYSPLASTIC SYNDROME

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Key words: Myelodysplastic syndromes, Thrombocytopenia, Anemia, Neutropenia, Child, Pancytopenia

Background and aims

Refractory cytopenia of childhood (RCC) is the most common subtype of pediatric myelodysplastic syndrome (MDS). Cytopenia and cellular dysplasia can also occur due to other different etiologies, such as infections and chronic diseases. Differential diagnosis can be very complex, and there is an evident need for a better understanding of clinical and hematological profile of these patients with different causes of cytopenia. OBJECTIVE:To describe the clinical and hematological profile of patients referred to the Brazilian Cooperative Group of Pediatric Myelodysplastic Syndrome (BCG-PED-MDS) with or without a diagnosis of myelodysplasia.

Methods

Descriptive cross-sectional study with retrospective data collection of children referred from January 1997 to March 2021. Patients aged 0-18 years registered in the BCG-PED-MDS with diagnosis of MDS - RCC and patients with other diagnoses unrelated to MDS were included, totalizing 303 cases. Those without a defined diagnosis and complete blood count data were excluded. Patients with Down Syndrome, myeloproliferative diseases, Juvenile Myelomonocytic Leukemia, and high-grade MDS were also excluded. Statistical analyzes were performed using SPSS Software for Windows® version 21.

Results

Of 303 cases, 79 were diagnosed as RCC and 224 with other diagnosis. There was statistical association between female sex and RCC (p = 0.018). Median age was 7.8 years. Pallor was frequently found in both groups and its association with RCC had a p value of 0.031. There was also correlation between skin/mucosal bleeding (p = 0.000), anemia (p = 0.04) thrombocytopenia < 50,000/mm3 (p = 0.000) and elevated fetal hemoglobin (p = 0.004) with RCC. Among patients with isolated anemia, none had the diagnosis of RCC. There was no statistical association regarding neutrophil count or hemoglobin values between groups.

Conclusions

Despite a small number of patients with RCC, this study points out that we can find correlation between hematological and clinical criteria that differentiates patients with RCC from other pathologies.

P31

INFANT WITH SAMDL9 MUTATION, MONOSOMY 7 AND ACUTE LYMPHOBLASTIC LEUKEMIA

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Key words: SAMDL9 mutation, monosomy 7, acute lymphoblastic leukemia, bone marrow transplant

Background and aims

Our patient is male infant who presented with pancytopenia and CMV infection at age of 3 months. He was previously healthy, and he was not dysmorphic. Bone marrow aspiration was consistent with bone marrow failure / aplasia, and on flow cytometry we noticed absence of CD 34 + cells, but also complete absence of B cells.The child required regular transfusions of RBCs and platelets. CMV infection was treated by gancyclovir. We also performed aditional immunologic assessment. The child had normal for age imunoglobulin levels, normal lymphocyte proliferation test, normal isohemaglutinnin levels, and appropriate anti HBs levels.

Methods

At University Clinical Center in Freburg we did targeted NGS -custom pediatric MDS Panel from peripheral blood and identified a missense variant of uncertain significace in SAMD9L c.3842>C which can lead to MDS. We continue to follow his blood count and in the age of 10 months his blood cound improved and he was transfusion free. At age of 13 months we noticed elevated MCV with another episode of pancytopenia. In bone marrow we found 8% B precursor lymphoblasts (by flow cytometry) and monosomy 7. Molecular finding showed positive clonality for TCRgamma and IgH genes. As he was the only child in family we preformed genetic analysis in mother and it was negative for SAMD9L mutation.

Results

After contacting few centers in Europe we decided to give induction therapy for ALL according to BFM IC ALL 2009 protocol. After achieving complete remission we performed bone marrow transplant from unrelated donor. Bu Cy Mel conditioning regimen was used.

Conclusions

The resulting haploinsufficiency of genes located on chromosome 7 can lead to MDS or AML, especially when additional cooperating mutations are somatically acquired. This is the first case of SAMD9L mutation that lead to ALL evolution and was succesfully treated wit BM

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INFANT EXTRAMEDULLARY KMT2A REARRANGED T/MYELOID ACUTE LEUKEMIA MIMICKING LANGERHANS CELL HISTIOCYTOSIS

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Key words: infant, low blast count leukemia

Background and aims

A previously healthy 3-month-old girl was admitted to local hospital for progressive dyspnea. Chest X-ray revealed pneumomediastinum and patient was transferred to specialized hospital where the respiratory insufficiency progressed with necessity of intubation; low-dose corticosteroids were administered. CT scan showed massive bilateral bullous lung changes. The patient was transferred to our resuscitation unit in critical condition. On admission, WBC was 49,000/ul with 1.5% of blasts. Biochemical results showed metabolic acidosis and tumor lysis syndrome. On the following day, WBC spontaneously decreased with no further detectable blasts.

Results

Bone marrow aspiration was performed without evidence of leukemic cells by morphology. Flow cytometry revealed 6% of atypical cells of T/myeloid immunophenotype (CD7+CD33+CD4+HLA-DR+CD117dim(i)CD3dim). Bronchoalveolar lavage was performed with PCR low-positivity of Pneumocystis jirovecii and rhinovirus. Flow cytometry detected 15% of atypical cells (CD117+CD33+CD4dimCD1a+CD7+). BRAF mutation was negative from both blood and bronchoalveolar lavage. The patient's condition worsened, with progressive multiorgan failure. For suspicion of Langerhans cell histiocytosis, corticoids (60mg/m2) and trametinib (0.032mg/kg) were started. Two days later the patient died of multiorgan failure. Post mortem, FISH from bone marrow revealed translocation (9:11) (KMT2A::MLLT3) in 6% of the cells. The autopsy determined the massive infiltration by monocytic blasts predominantly in lymph nodes, bone marrow, liver, spleen, kidneys, colon and lungs, where bilateral cystic remodeling of tissue was also found. High positivity of KMT2A::MLLT3 fusion gene was found in various tissues. Next generation sequencing for clonal Ig/TCR rearrangements was negative. Whole exome sequencing for genetic disorders of surfactant dysfunction and primary immunodeficiencies was performed with negative results.

Conclusions

In conclusion, we present an unusual case of extramedullary KMT2A rearranged T/myeloid acute leukemia with low blast count in the bone marrow and massive organ infiltration. We hypothesize that bilateral pseudocystic remodeling of the lung tissue was the consequence of leukemic infiltration in prenatal or early postnatal period.

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HSCORE CALCULATION AT DIAGNOSIS AND DURING PATIENT FOLLOW-UP IN TWO CHILDREN WITH MALIGNANCY-ASSOCIATED HEMOPHAGOCYTIC SYNDROME

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Key words: HScore, children, leukemia, lymphoma, hemophagocytosis

Background and aims
The HScore was recently created to identify reactive hemophagocytic syndrome (HPS).We present two children with malignancy-associated HPS, for whom the HScore was calculated for diagnosis and therapy response.

Methods
Hemophagocytic syndrome was diagnosed using at least 5 of the 8 criteria listed in the hemophagocytic lymphohistiocytosis (HLH)- 2004 protocol as follows: Cytopenia, hypertriglyceridemia/hypofibrinogenemia, hyperferritinemia, prolonged fever, splenomegaly, soluble elevated sCD25 activity, decreased NK cell activity and bone marrow HPS. The HScore is calculated at https://saintantoine.aphp.fr/score/. HScore was also measured at diagnosis and during the patient's follow-up. The best HScore cut- off value for predicting HPS was 169. A bone marrow sample, flow cytometry, cytogenetics, and PET scan were used to diagnose malignancy.

Results
A 17-year-old boy was diagnosed with precursor-B acute lymphoblastic leukemia (ALL). At diagnosis, his HS score was 215. The ALL- BFM 2000 protocol was initiated. After induction chemotherapy, he did not achieve complete remission. During his follow-up, his HS score was usually more than 169. After each chemotherapy recovery, his HScore with the excess blast was 243, 276, and 291, respectively. He died from sepsis and refractory leukemia. Anaplastic large cell lymphoma (ALCL) was identified in a 12-year-old boy. He had HPS for at least 6 months prior to the ALCL diagnosis. His HS score was 258 at the time of HPS diagnosis. The HLH-2004 protocol was started. Complete remission was achieved in the first four weeks of HLH therapy. ALCL developed at the 8 weeks of the HLH-2004 treatment. His PET scan showed positive three times after each chemotherapy recovery. Simultaneously, his HScores were 286, 306, and 322, respectively. He died from a hemorrhage due to persistent lymphoma.

Conclusions
Our findings show that HScore calculation may assist with the diagnosis and treatment response of children with refractory leukemia-lymphoma.

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CAN A THIRD HAEMATOPOIETIC STEM CELL TRANSPLANTATION BE A VIABLE OPTION FOR JMML RELAPSE? A CASE REPORT

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Key words: Juvenile myelomonocytic leukemia, HSCT

Background and aims
Allogeneic haematopoietic stem cell transplantation (HSCT) is the only cure for most JMML patients. One third of patients relapse after transplantation and second HSCT is a potentially effective option in a proportion of these patients.

Methods
We describe the case of a boy diagnosed with JMML who relapsed after two allogeneic HSCTs and received a third haploidentical transplantation.

Results
In May 2019, a 9-month-old boy was diagnosed with JMML, PTPN11 (c.227A>G) somatic mutation, intermediate methylation class, HbF 65%. In July 2019 allogeneic HSCT was performed from HLA 10/10 identical brother (source BM). Conditioning regimen: busulfan, cyclophosphamide, melphalan. Moderate VOD was treated with defibrotide. Ciclosporine (CsA) was discontinued in October 2019. In November 2020, haploidentical HSCT was performed from the father (source BM) because of disease relapse. Conditioning regimen: treosulfan, thiotepa, fludarabine. GvHD prophylaxis: PTCY, CsA, MMF. Due to primary graft failure (BM chimerism 95% first donor), unmanipulated BM infusion from the brother was performed. Prophylactic azacitidine was administered for 8 cycles (last on January 2022). In March 2022, the boy experienced a second disease relapse. Haploidentical HSCT was performed from the mother (source BM) in June 2022. Conditioning regimen: treosulfan, thiotepa, fludarabine. GvHD prophylaxis: PTCY, CsA, MMF. Stable engraftment without organ toxicities occurred. CsA was halted on day+40 and prophylactic azacitidine was started on day+45. The boy developed steroid- dependent skin acute GvHD treated with ruxolitinib and, after the first prophylactic DLI infusion, gut GvHD (grade II), successfully treated with steroids. Eleven months after HSCT, the boy is well and in disease remission (full donor chimerism, VAF PTPN11 0% on BM, HbS 0%). He is still on azacitidine (8 cycles administered so far).

Conclusions
This report suggests that a third HSCT can be considered in selected JMML patients as a safe and viable option in case of disease relapse.

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HEPATITIS-ASSOCIATED MYELODYSPLASTIC SYNDROME IN CHILDREN: REPORT OF 2 CASES

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Key words: hepatitis, RCC, children, MDS

Background and aims

A well-known association is described between aplastic anemia and hepatitis, but less is reported on pediatric myelodysplasia.

Methods

Data from children with hepatitis-associated refractory cytopenia of childhood (RCC) were collected.

Results

A 4-year-old boy presenting with abdominal pain, fatigue, dark urine, and diarrhea was diagnosed with acute hepatitis (ALT 1,119 U/mL). He partially responded to a six-week course of steroid treatment (ALT 568 U/mL), but two weeks later he presented a marked increase in ALT values (ALT 1,018 U/mL) associated with bilinear cytopenia (leukocytes 1,730/uL, neutrophils 910/uL, platelets 14,000/uL). The bone marrow biopsy evaluation was consistent with RCC, according to the WHO classification. He received several immunosuppressive treatments but did not achieve a response at liver level, and finally received hematopoietic stem-cell transplantation (HSCT) from a sibling donor. No major complications occurred, and liver abnormalities normalized during conditioning. At last follow-up, four years after HSCT, he was in good clinical condition with normal blood counts and liver tests. A 10-year-old girl presenting with abdominal pain, jaundice, fatigue, and dark urine was diagnosed with acute hepatitis (ALT 2,252 U/mL) and received immunosuppressive treatment with gradual normalization of liver abnormalities. Three months later, she developed trilinear cytopenia (hemoglobin 8.3 g/dL, leukocytes 2,120/uL, neutrophils 587/uL, platelets 53,000/uL) requiring weekly red blood cell and platelet transfusions. The bone marrow biopsy showed a hypocellular and dysplastic pattern consistent with RCC. She therefore received HSCT from a matched unrelated donor. At HSC reinfusion, she developed a posterior reversible encephalopathy syndrome, and the hematopoietic reconstitution was delayed. At last follow-up, three years after HSCT, she was in good clinical condition with normal blood counts and liver tests.

Conclusions

As for aplastic anemia, also pediatric MDS can be associated with acute hepatitis.

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CASE REPORT: AN UNUSUAL CASE OF REFRACTORY CYTOPENIA OF CHILDHOOD IN A PATIENT WITH DOWN SYNDROME?

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Key words: Myelodysplastic Syndrome, RCC, Down Syndrome

Background and aims

Down Syndrome patients have an unique bone marrow microsystem. We present a case report of a patient with trisomy 21 with persistent pancytopenia and pathologic bone marrow.

Methods

Descriptive report based on medical records and literature review.

Results

A 10-years old male boy with Down Syndrome, admitted to hospital in April-2022, with an acute history of fever, pallor, respiratory tract infection and pancytopenia in blood tests (Hemoglobin 5g/dl; Leucocytes: 1100/mm³; Neutrophils: 429/mm³; Platelets: 19.000/mmm³). He received transfusion support and antibiotics with fast resolution of respiratory tract infection and underwent investigation of pancytopenia. His past medical history included beyond the Down Syndrome, a severe Autism and a cardiac surgery in newborn period. Physical examination showed the usual features of Down Syndrome, marked pallor and bruising in arms and legs, with no organomegaly or lymphadenopathy. Initial tests for differential diagnosis of pancytopenia were performed, without alteration. Bone marrow aspiration resulted deeply hypocellular, without blasts, with mild dysplasia. Biopsy showed a hypocellular bone marrow (cellularity range 1-20%, with a very irregular distribution of hematopoiesis). Megakaryocytes were rare, isolated, small and hypolobated. A new evaluation was performed after six months, with similar pattern. Deb test and Thelomerophaties investigation resulted negative. Karyotype was 47, XY,+21. Next-generation sequencing (NGS) identified a DNMT3A mutation at a variant allelic frequency (VAF) of 25%. The patient is now in outpatient follow-up, with no major complications and sporadic transfusion need, although he maintains pancytopenia.

Conclusions

Although we are probably facing an unusual case of Myelodysplastic Syndrome (Refractory Cytopenia of Childhood), these findings in a patient with trisomy 21, constitute a challenge in choosing the best treatment approach. Therefore, more studies are needed to understand the biological behavior of myelodysplastic findings in Down Syndrome, out of context of Myeloid Neoplasms GATA-1 associated.

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JMML WITH NRAS MUTATION IN ELANE ASSOCIATED SEVERE CONGENITAL NEUTROPENIA

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Key words: ELANE mutation, NRAS, JMML

Background and aims
ELANE mutations can be associated with severe congenital neutropenia (SCN), cyclic neutropenia and autoimmunity.

Methods
We describe a child with ELANE-SCN, who developed a somatic NRAS mutation and juvenile myelomonocytic leukaemia (JMML).

Results
The child was diagnosed two months old with an ELANE mutation (LRG_57t1:c.[136T>C];[126=].LRG_57p1:p.(Ser46Pro);(Ser46=)) following two septic episodes with severe neutropenia. Blood counts were normal except for the neutropenia and slightly increased monocytes. Granulocyte-Colony Stimulating Factor (G-CSF) was initiated at the age of 3 months old due to repeating infections. During the first months of G-CSF-therapy, leucocytes increased to maximum of 30×10^9/L, dominated by monocytes, eosinophiles and lymphocytes. Thrombocytes decreased to 50-100×10^9/L. He developed mild microcytic anaemia with normal reticulocytes. Bone marrow examination (BM) one year after initiation of G-CSF showed hypercellularity dominated by monocytes and eosinophils. Granulopoiesis was arrested at the promyelocytic/myelocytic stage. Dysplasia was not detected, but blast cell level was slightly increased (4%). EWOG reference pathologists interpreted the findings in blood and bone marrow as probable JMML. NRAS mutation (NM_002524.4(NRAS):c.34G>A (50%)*) was detected in 48% of reads. There were no signs of RUNX1 or G-CSF receptor mutations. Re- analyses of blood samples from time of diagnosis of the ELANE mutation found no NRAS mutation, nor was it detected in DNA from cultured fibroblasts form a skin biopsy. The NRAS mutation was thus interpreted as somatic. The findings were confirmed in repeated BM examinations. The boy received an haematopoietic stem cell transplantation (HSCT) with a 10/10 HLA-identical unrelated donor at 2 years and 9 months. He was conditioned with busulfan (AUC 90), cyclophosphamide, melphalan and Thymoglobulin, with MTX and CyA as GVHD prophylaxis. BM at 40 days and 1 year post-HSCT was without JMML/NRAS or ELANE mutations.

Conclusions
This is the first description of a patient with ELANE-SCN who developed a somatic oncogenic NRAS mutation and JMML following G- CSF therapy.

P38

INTENSIVE CHEMOTHERAPY AS BRIDGING TO STEM CELL TRANSPLANTATION IN 5 YEAR OLD GIRL WITH JUVENILE MYELOMONOCYTIC LEUKEMIA– CASE REPORT

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Key words: JMML, Juvenile myelomonocytic leukemia, FLA, PTPN11, low dose cytarbine, azacytydine

Background and aims
Juvenile myelomonocytic leukemia (JMML) is a Ph-negative, clonal myeloproliferative disease of the monocytic system, in which the percentage of blasts and promonocytes in the bone marrow does not exceed 20%, and in 90% of cases activating mutations in the RAS pathway are found.

Results
A 5-year-old girl was diagnosed in October 2021 in Ukraine with JMML. Initial treatment consisted of 6-mercaptopurine, cytarabine and cis-retinoic acid and the child was referred abroad for allogeneic stem cell transplantation (HSCT), but had to come to Poland due to war outbreak. On admission to our Clinic, the girl was in poor general condition with dyspnoea and presented features of extreme cachexia. Physical examination and imaging studies revealed lymphadenopathy and massive hepatosplenomegaly with ascites. Leukocyte count was 20 x 10^3/ul with 10% of blast cells, Hb 10.2 g/dl PLT 11 × 10^3/ul. Bone marrow biopsy and trephine biopsy were performed. The myelogram showed 6.2% of young mononuclear cells. The cytogenetic examination excluded the BCR/ABL fusion, monosmy 5 and 7. The PTPN11 mutation was confirmed. The HbF concentration was 24%. Due to poor general condition and ineligibility for HSCT, treatment with azacytidine was initiated. After two cycles, azacytidine was stopped due to patient's deterioration and lack of efficacy, and LD cytarabine was started, resulting in a decrease in leukocytosis and reduction of organomegaly. One week later a "rebound" with pleural effusion requiring drainage was observed. After two cycles of LD cytarabine, two cycles of FLA chemotherapy were administered, after which the patient's condition stabilized. The patient underwent megatherapy Bu-Cy-Mel and PB - HSCT from an unrelated donor. Currently, the patient is a year after alloHSCT in full JMML remission and off immunosuppressive treatment.

Conclusions
Patients with JMML even with aggressive disease can benefit from HSCT. The standard mild to intensive chemotherapy can be used as a bridging to HSCT.

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NATURAL HISTORY OF RALD: A 20 YEAR FOLLOW-UP OF A NRAS MUTATED PATIENT EXCLUDING A MALIGNANT PROGRESSION

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Key words: Ras-associated autoimmune leukoproliferative disorder, NRAS gain of function, inborn error of immunity, juvenile myelomonocytic leukaemia

Background and aims

Ras-associated autoimmune leukoproliferative disorder (RALD) is a rare condition resulting from a somatic gain-of-function mutation in NRAS/KRAS genes that impairs leukocyte apoptosis and homeostasis. Overlapping inborn error of immunity (IEIs), autoimmune diseases and haematological malignancies, RALD is often difficult to define. It has been reported that certain RALD patients progress to juvenile myelomonocytic leukaemia (JMML) or acute myeloid leukaemia. We report the clinical history of a 22-year old male with RALD. Our purpose is to identify predictive markers indicative of disease progression.

Methods

We performed a 20-year longitudinal evaluation of the case through serious investigations. The in-depth immunophenotype was repeated at different time points. Targeted deep-sequencing was studied to exclude other somatic variants suggestive of clonal progression. Risk to progression in JMML was further evaluated through periodic in vitro cultures of the patient's peripheral blood mononuclear cells (PBMC).

Results

The patient presented with splenomegaly, absolute mono-lymphocytosis and severe thrombocytopenia during the first month of life, non-responding to high-dose intravenous immunoglobulins and steroids. He maintained good clinical conditions, with persistent splenomegaly, moderate thrombocytopenia and monocytosis. The in-depth immunophenotype demonstrated low naïve in both CD4+/CD8+ T cells, low recent thymic emigrant and Treg. At the age of 16, a diagnostic Haloplex panel for IEIs related genes had been performed on DNA extracted from PBMCs, revealing a heterozygous mutation p.G12D, in NRAS gene. Its absence in buccal swab DNA confirmed the somatic nature. Clonogenic assay showed spontaneous growth of granulocytic-macrophage progenitors although in limited colonies, contrary to what typically observed in JMML. Targeted deep sequencing did not show any other somatic event.

Conclusions

Our report of RALD case stresses the importance of an integrated approach in which clinical parameters, along with functional assays and the longitudinal assessment for additional myeloid co-mutations, may allow to better predict the real risk of malignant progression in this rare condition.

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EPIDEMIOLOGICAL ANALYSIS OF CONSTITUTIONAL AND ACQUIRED APLASTIC ANEMIA IN LATVIA: 1998-2022

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Key words: aplastic anemia; epidemiology

Background and aims

Aplastic anemia (AA) represents a rare and potentially fatal manifestation of bone marrow failure. The primary objective of this study was to comprehensively examine the epidemiology encompassing diverse subtypes of AA within the geographical context of Latvia, spanning the extensive duration from 1998 to 2022.

Methods

The retrospective study included all constitutional and acquired AA cases at Children's Clinical University hospital in Riga. Parameters such as incidence, etiology, therapy type, survival, and mortality rates were analyzed. The data were analyzed by IBM SPSS.

Results

During the study period, 32 cases of AA were diagnosed: 18 with acquired idiopathic aplastic anemia (IAA), 5 associated with acute hepatitis, and 9 with congenital Fanconi anemia. The incidence of acquired AA (aAA) ranged from 1.75 to 8.36 per million inhabitants under 18 years per year, while for Fanconi anemia it ranged from 0.46 to 1.5 live births. The median age of patients with aAA was 129 months, predominantly boys (78%). The distribution of moderate, severe, and very severe aAA cases was 56%, 35%, and 9%, respectively. Among the 23 aAA patients, 16 received immunosuppressive therapy (IST), and 12 underwent hematopoietic stem cell transplantation (HSCT). Adult specialists were involved in the care of 70% of aAA patients, 6 cases resulted in fatalities. The mean survival time of surviving aAA patients have been 63.3 months (std.12.004), while fatal cases have a mean survival of 32.8 months (std.5.722). Fanconi anemia patients had a median age of 92 months, with 67% being girls. HSCT has been performed in 5 out of 9 cases, and only 1 patient died in the early post-transplantation stage.

Conclusions

Aplastic anemia is a rare and severe disease. Treatment has evolved, with IST and androgens previously used for acquired AA and Fanconi anemia, respectively. However, HSCT is now the leading treatment for both types.

P41

IMMUNOSUPPRESSIVE THERAPY IN A PATIENT WITH SAA-like RCC AND GATA2 DEFICIENCY

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Background and aims

GATA2 deficiency is a rare autosomal dominant disorder that predisposes to a range of hematologic and immunologic manifestations, including refractory cytopenia in childhood (RCC) and myelodysplastic syndrome (MDS). Recent data suggest that RCC/MDS caused by a pathogenic GATA2 germline mutation should be treated according to the EWOG MDS treatment algorithm (Bortnick et al., Bone Marrow Transplant. 2021) where hematopoietic stem cell transplantation (HSCT), rather than IST, is recommended in case of severe pancytopenia and karyotypic aberrations. Conversely, borderline diagnoses of SAA-like RCC have been described. Hence, clinicians face significant decision-making pressure, as delaying immune suppressive treatment (IST) for SAA can affect patient outcomes.

Methods

We present the case of a 7-year-old Ukrainian refugee who was transferred to our hospital with severe pancytopenia known to prevail for several weeks. On admission the patient had transfusion-dependent anemia and thrombocytopenia, with an absolute neutrophil count (ANC) < 0.2 G/L. Histopathology indicated SAA without signs of dysplasia or cytogenetic abnormalities. As a matched sibling donor was not available, we swiftly initiated IST according to EWOG-SAA 2010 guidance.

Results

The patient responded well, achieving transfusion independence and an ANC > 1.0 G/L after 30 days of treatment. However, during this time, results from initial examinations revealed a pathogenic GATA2 splice-site variant (c.871+2T>C). On day 120, the patient exhibited continued improvement (according to criteria poor partial response [PPR] with ANC at 1.0 G/L without G-CSF), with no karyotypic aberrations or infection-related complications (major concern of IST).

Conclusions

This case highlights the diagnostic and treatment challenges faced in merely clinical diagnosis of SAA/RCC and raises the question of management (swift commencement of IST, wait for molecular results). In addition, it raises the question of further management of patients with GATA2 mutation on treatment with IST because of "SAA-like RCC".

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GATA2 DEFICIENCY SYNDROME: MDS / ACUTE MYELOID LEUKEMIA IN AN ADOLESCENT PATIENT

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Key words: GATA2, leukemia, monosomy 7, myelodysplastic syndrome

Background and aims

GATA2 deficiency resulting from heterozygous germline mutations in the gene encoding the zinc-finger transcription factor GATA2 has been identified as the most common hereditary cause of myelodysplastic syndrome (MDS) in adolescents with monosomy 7. In this abstract we wanted to present the progress of an adolescent girl with monosomy 7 and GATA2 mutation to emphasize the critical role of screening for inherited variants in relatives during hematopoetic stem cell transplantation (HSCT) donor selection in order to avoid donor derived myeloid malignancies.

Methods

In 2018, a 12-year-old girl with features of bone marrow failure was diagnosed as monosomy 7 and hypocellular MDS. Allogeneic HSCT from her HLA-matched mother was performed since her mother was the only suitable HSCT donor and her clinical condition was urgent. Six months later, the results of genetic analysis were available; GATA2 mutation was positive in both our patient and her mother. The patient was followed up closely for progression to MDS/AML.

Results

In 2023, the patient presented with fever for two days. On physical examination, she was well, lymphadenopathy and hepatosplenomegaly were not present. Her Hb level was 10.8 g/dL, leukocyte count was 800/mm3, absolute neutrophil count was 100/mm3, and platelet count was 20,000/mm3. Her bone marrow aspiration smear showed hypocellularity and 70% granular myeloblasts. Flow cytometric analysis of the bone marrow aspiration sample demonstrated CD13, CD33, CD117, CD2, and CD7 positivity. Monosomy 7 and monosomy 5 were positive in genetic analysis. She had MDS which was transformed to AML. Infectious causes were all excluded. Azacitidine was started to reduce the number of myeloblasts in the bone marrow. Allogeneic HSCT is planned as soon as possible.

Conclusions

Germline mutations should be screened especially in adolescent patients with MDS and monosomy 7 and screening for inherited variants in relatives is very important to avoid development of myeloid malignancies.

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PERIPHERAL AND BONE MARROW G-CSF-RELATED MYELOBLASTS RATE IN A PATIENT WITH SEVERE CONGENITAL NEUTROPENIA

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Key words: severe congenital neutropenia, G-CSF, blastosis

Background and aims

GCS-F has improved the quality of life of patients with severe congenital neutropenia (SCN); however, they have an intrinsic risk of developing MDS/AML, which increases with the degree of G-CSF exposure.

Results

C.M. is a 20-year-old girl who received a diagnosis of ELANE-mutated SCN at the age of 3 years. Since diagnosis, G-CSF treatment was introduced, reaching a maintenance dose of 1 µg/kg/die. She had a good quality of life, except for osteoporosis, and she performed a "surveillance" bone marrow aspirate annually. On March 2019, she developed oral stomatitis with severe neutropenia; after excluding the presence of MDS/AML, we increased G-CSF dose to 4.45 mcg/kg/die, achieving a temporary improvement of neutrophil count. On September 2019, response to G-CSF was gradually lost. This time morphological evaluation of bone marrow showed a complete maturational block at promyelo/myelocyte level and no evidence of MDS/AML. Cytogenetics assays showed a pseudodiploid clone 46, XX with 7q partial deletion in 9 metaphases, of whom 2 had a balanced translocation t(4p;21q) involving the RUNX1 gene, confirmed at FISH. The CSFR3-mutated clone (c.2215 C>T) increased up to 15,8 % and HSTC was absolutely recommended. One month later, complete blood count showed trilinear cytopenia and 6% myeloblasts. Bone marrow aspirate confirmed the progression to myeloproliferative acute disease evidencing 50% myeloperoxidase-positive blasts. G-CSF was immediately discontinued. Ten days after treatment withdrawal, bone marrow myeloblasts remained stable while peripheric blasts gradually decreased until they disappeared. In November 2019 she had haplo-identical HSCT from her brother. Two years later she's on good clinical condition with normal blood count and full donor chimerism.

Conclusions

In our patient continuative G-CSF-treatment led to genomic instability and progression to myeloproliferative disease. Different to previous reports, G-CSF discontinuation correlated to reduction of peripheral blastosis but we did not obtain morphologic nor cytogenetic remission on bone marrow.

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ALLOGENEIC STEM CELL TRANSPLANTATION FOR MYELODYSPLASTIC SYNDROME COMPLICATED BY SYNCHRONOUS ACUTE GRAFT-VS-HOST DISEASE AND CYTOMEGALOVIRUS INFECTION

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Key words: Myelodysplastic Syndrome, Graft-vs-Host Disease, Allogeneic hematopoietic stem cell transplantation

Background and aims

Myelodysplastic Syndrome (MDS) is a clonal disease of bone marrow stem cells accounting for 3–7% of hematologic malignancies in children.

Clinical manifestations are usually attributable to pancytopenia. The main diagnostic feature is the presence of dysplastic anomalies in bone marrow cells.
The only curable treatment for MDS is allogeneic hematopoietic stem cell transplantation (HSCT), a procedure with potential serious complications such as Graft-vs-Host Disease (GvHD) and infections.

Methods

Case report.

Results

A previously healthy 7-year-old male presented with 2 weeks of abdominal pain, vomiting and anorexia. On physical examination was pale and with petechiae. The peripheral blood revealed hemoglobin 6.4g/dL, leucocytes 2.410/uL with 3% blasts, platelets 200.000/uL. Bone marrow assessment showed erythroid dysplasia, 8.7% of myeloid blasts and normal karyotype. The diagnosis of MDS with excess of blasts was made and the patient enrolled in HSCT program. After engraftment, he developed cutaneous Acute GvHD (aGvHD) with good response after 1 week of corticosteroids. During corticosteroids slow taper, he developed Cytomegalovirus Reactivation and Enterocolitis. Therapy with Ganciclovir and Immunoglobulin was started. After no clinical and no viremia response, he was switched to Foscarnet. After colon biopsy, a diagnosis of Intestinal aGvHD was made and the patient started on Infliximab. After 4-weekly administrations, no response was observed and he was admitted in the intensive care unit with lower gastrointestinal tract hemorrhage and respiratory and hepatic failure. After corticosteroids and Infliximab refractoriness, he was treated with Ruxolitinib with no intestinal improvement. He died due to hemorrhagic shock secondary to intestinal aGvHD.

Conclusions

HSCT is the only curative option for pediatric MDS patients. Even with GvHD prophylaxis and improvement of supportive measures, HSCT still imposes significant toxicity. Refractory aGvHD and viral infections are major factors for the morbidity and mortality risk of these procedure, especially when they occur simultaneously.

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DIAGNOSIS OF JUVENILE MYELOMONOCYTIC LEUKEMIA, IN CHILDREN WITH NEUROFIBROMATOSIS TYPE 1 – CASE REPORT

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Key words: Juvenile Myelomonocytic Leukemia, JMML, Neurofibromatosis type 1

Background and aims

Juvenile Myelomonocytic Leukemia (JMML) is a rare and aggressive disease of early childhood. This disorder represents less than 3% of all pediatric hematologic malignancies. Hematopoietic stem cell transplantation (HSCT) is the best option treatment.

Methods

Case report.

Results

2-year-old boy with Neurofibromatosis type 1 (NF1) was referred to our consultation after being observed in an oncological consultation for his neurofibromatosis. He had a blood count with leucocytosis (57.000/uL) with neutrophilia (21.250/uL), monocytosis (12.060/uL), eosinophilia (2.300/uL) and basophilia (500/uL), without blasts, during an acute otitis media. Physical examination showed multiple café au lait spots, inguinal lymphadenopathy, without splenomegaly and no other clinical features. After the infection, his blood count showed only monocytosis. Significant familial patterns were observed: his mother and grandmother died from tumors and had NF1, so, this child had been oriented to an oncological consultation. Besides this, his father and 2 paternal uncles had had Wilms tumor in childhood. The bone marrow smear showed hypercellular bone marrow with myeloid hyperplasia, without excess blasts, with augmented monocytes (20.3%). Genetic molecular study confirmed mutation in the NF1 gene. HSCT was proposed and HLA study showed an international donor, since his sibling wasn´t compatible. As the father was working abroad, we transferred to another center in France for transplantation.

Conclusions

JMLL diagnosis is difficult, due to its unspecific characteristics and is rarity. Genetics are an important risk factor for developing JMML. The absence of splenomegaly in the initial presentation is not common, described only in 7% of the cases, but this patient was oriented early because he was followed in an oncological consultation, due to his neurofibromatosis and cancer family history. Despite of the unfavorable prognosis, HSCT is the only potential curative with survival rates near 50%.

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JUVENILE MYELOMONOCYTIC LEUKEMIA WITH TELOMERE SHORTENING: CASE REPORT

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Key words: juvenile myelomonocytic leukemia, telomere lenght disorders

Background and aims

Juvenile Myelomonocytic Leukemia (JMML) is a rare neoplasm of early childhood characterized by mutations in RAS-pathway genes (KRAS, NRAS, CBL, NF1, PTPN11) and myelodysplastic/myeloproliferative features. Telomere biology disorders (TBD) are diseases associated with germline mutations in genes related to telomere maintenance and can present with bone marrow failure without other symptoms. There is no clear relation between JMML and TBD

Methods

Case report

Results

10 year-old boy with a previous diagnosis of autism spectrum disorder and a history of fatigue and pallor for 7 months. At the beginning, physical examination was normal and complete blood count (CBC) showed anemia (hemoglobin 9.5 g/dl), normal leukocytes (3.4 k/µl) and monocytes, and thrombocytopenia (143 k/µl). Fetal hemoglobin level of 12.1%. Bone marrow was hypercellular, with mild dysplasia and increase in monocytes. Karyotype was normal (46, XY) and telomeres were short. Family history was negative for TBD and hematological diseases. After 4 months the patient developed splenomegaly, hepatomegaly and CBC showed a worsening in anemia (hemoglobin 6.8), thrombocytopenia (46 k/µl) and leukocytosis (leukocytes 32.7 k/µl) with monocytosis (12.1 k/µl). A new bone marrow evaluation, showed hypercellularity with 6% of blasts. Karyotype was normal. BCR-ABL1 was negative. Next generation sequencing (NGS) showed mutations in KRAS exon 2 (c.38G>A, p.Gly13Asp) with variant allele frequency (VAF) of 19% and WT1 exon 7 (c.548dup, p.Tyr183Ter) with VAF of 47%. NGS for genes related to TBD is under analysis. Final diagnosis was JMML, despite of the unusual age of presentation. He is now receiving treatment with Azacytidine and preparing to bone marrow transplantation. To date, we found only 1 case report of JMML and TBD with TERT mutation

Conclusions

TBD can be present in a variety of hematological neoplasms, such as myeloproliferative neoplasms and myelodysplastic syndrome, but is a rare occurrence in patients with JMML.

GENERAL INFORMATION

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Symposium videos will be available on the online platform for 2 months.

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Registration Fees include:

- Access to the Scientific Sessions, Symposium Material, Opening Ceremony

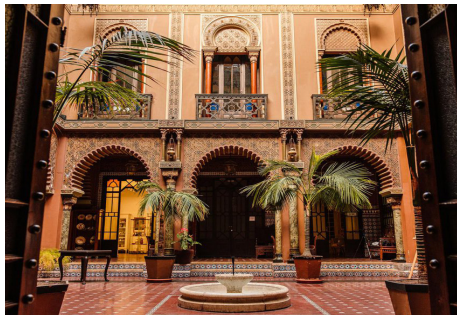
Social Activies

- Welcome Reception: September 28, 2023 at the Venue

  Included in the Registration Fee (registration required)

- Symposium Dinner: September 29, 2023.

Bus Pickup from Museu da Electricidade, walking tour around old town followed by dinner at Casa do Alentejo



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