Bundesamt
für Strahlenschutz

Ressortforschungsberichte zum Strahlenschutz

7. Internationaler Workshop zur Ursachenforschung von Leukämie im Kindesalter

Vorhaben 3622I02478

Zum Goldenen Hirschen Berlin GmbH

Das Vorhaben wurde mit Mitteln des Bundesministeriums für Umwelt, Naturschutz, nukleare Sicherheit und Verbraucherschutz (BMUV) und im Auftrag des Bundesamtes für Strahlenschutz (BfS) durchgeführt.

Impressum

Bundesamt für Strahlenschutz
Postfach 10 01 49
38201 Salzgitter

Tel.: +49 30 18333-0
Fax: +49 30 18333-1885
E-Mail: ePost@bfs.de
De-Mail: epost@bfs.de-mail.de

www.bfs.de

BfS-RESFOR-214/23

Bitte beziehen Sie sich beim Zitieren dieses Dokumentes immer auf folgende URN:
urn:nbn:de:0221-2023071238468

Salzgitter, Juli 2023

Inhalt

1. Aufgabenstellung

Beobachtungen aus epidemiologischen Studien weisen auf einen möglichen Zusammenhang zwischen niederfrequenten Magnetfeldern und einem erhöhten Risiko für Leukämie im Kindesalter hin. Auch gibt es Beobachtungen, dass niedrige Dosen ionisierender Strahlung mit einem erhöhten Auftreten von Leukämie in Kindern einhergehen. Für beide Beobachtungen sind bisher keine zufriedenstellenden, wissenschaftlich fundierten Erklärungen gefunden worden.

Dies veranlasste das Bundesamt für Strahlenschutz (BfS), die Forschung zu Leukämie im Kindesalter zu intensivieren und den möglichen Ursachen und Zusammenhängen in internationalen Workshops nachzugehen.

Im Rahmen des 7. Internationalen Workshops wurde der aktuelle Forschungsstand mit internationalen Experten in einer dreitägigen Veranstaltung umfassend beleuchtet, um sich der Thematik aus unterschiedlichen wissenschaftlichen Perspektiven zu nähern, Wissenslücken zu identifizieren und, wo nötig, neue Forschung zu initiieren.

1.1 Zielsetzung

Unmittelbares Ziel der Veranstaltung war es, einen Überblick über den aktuellen Forschungsstand aus der Perspektive verschiedener wissenschaftlicher Disziplinen zu erhalten und offene Fragen zu identifizieren, auf deren Grundlage die Forschung fortgeführt werden sollte. Hierfür wurden internationale Referentinnen und Referenten, die als Experten auf ihrem jeweiligen Fachgebiet gelten, nach München eingeladen. Zusätzlich bestand die Möglichkeit für Wissenschaftler*innen Abstracts zu ihrer Forschung einzureichen, die als Kurzvorträge in das Programm aufgenommen wurden. Erweitert wurde der Teilnehmerkreis durch weitere Expertinnen und Experten, die den Workshop als Teilnehmende mit ihrem Fachwissen ergänzten.

Die inhaltlichen Details und Vorbereitungen des Workshops wurden vom Bundesamt für Strahlenschutz und dem wissenschaftlichen Komitee erarbeitet. Die organisatorische Umsetzung erfolgte mit Unterstützung eines externen Dienstleisters (Zum goldenen Hirschen Berlin GmbH).

2. Planung und Ablauf des Workshops

Die Aufgaben für die Organisation des Workshops wurden in drei Arbeitspakete eingeteilt.

AP 1: Planung des Workshops:
- Auswahl eines Veranstaltungsortes
- Einrichtung der Microsite
- Teilnehmermanagement (Einladungen, Erinnerungen, Save the Date, Kontakt für organisatorische Anfragen)
- Organisation des Caterings und der Mittagstische
- Einrichtung der notwendigen technischen Infrastruktur
- Ablaufmanagement und Koordination der Tagung

AP 2: Durchführung des Workshops
- Koordination der Dienstleister vor Ort
- Betreuung der Gäste vor Ort (Registrierung, Beantwortung von organisatorischen Rückfragen)
- Einrichtung eines Empfangs und Informationsstandes

AP 3: Nachbereitung
- Abschlussbericht

2.1 Veranstaltungsort

Der öffentliche Workshop fand vom 28. bis 30. November im Kolpinghaus München-Zentral als Hybridveranstaltung statt. Der Workshop richtete sich an interessierte Wissenschaftler*innen wie Biologen, Humanmediziner, Epidemiologen und Wissenschaftler*innen anderer Fachgebiete. Insgesamt nahmen vor Ort und online 64 Gäste aus 10 Ländern an dem Workshop teil.

2.2 Beteiligte Akteure

2.2.1 Programm-Komitee

Janine Schmidt | German Federal Office for Radiation Protection, Neuherberg, Germany
Gunde Ziegelberger | German Federal Office for Radiation Protection, Neuherberg, Germany
Sabine Hornhardt | German Federal Office for Radiation Protection, Neuherberg, Germany
Arndt Borkhardt | Heinrich-Heine-University, Düsseldorf, Germany
Joachim Schüz | International Agency for Research on Cancer (IARC/WHO), Lyon, France

2.2.2 Eingeladene Redner*innen

Arndt Borkhardt | Heinrich-Heine-University, Düsseldorf, Germany
Joachim Schüz | International Agency for Research on Cancer (IARC/WHO), Lyon, France
Dan Baaken | University Medical Center Mainz, Germany
Julia Hauer | Technical University of Munich, Germany
Joseph Wiemels | University of Southern California, Los Angeles, USA
Mihai Netea | Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands
Shai Izraeli | Schneider Children's Medical Center & Tel Aviv University Israel, Israel
Gianni Cazzaniga | University of Milano-Bicocca, Monza, Italy
Pablo Menendez | University of Barcelona, Spain
Kim Nichols | St. Jude Children's Research Hospital, Memphis, USA
Sameer Bakhshi | Dr B. R. A. Institute Rotary Cancer Hospital, New Delhi, India
Logan Spector | University of Minnesota, Minneapolis, USA

Jacqueline Clavel | Institut National de la Santé et de la Recherche Médicale (INSERM), France
Friederike Erdmann | University Medical Center of the Johannes Gutenberg University Mainz, Germany
Anssi Auvinen | Tampere University, Finland & Radiation and Nuclear Safety Authority, Vantaa, Finland
Ben Spycher | University of Bern, Switzerland
Michael Hauptmann | Brandenburg Medical School Theodor Fontane, Neuruppin, Germany
Aleksandra Pandyra | Heinrich-Heine-University, Düsseldorf, Germany
Helene Cavé | Université Paris Cité & Hôpital Robert Debré, France
Salvatore Nicola Bertuccio | University of Bologna, Italy
Thomas Mercher | Université Paris-Saclay, France

2.2.3 Eingereichte Abstracts für Kurzvorträge

Felix Onyije | International Agency for Research on Cancer (IARC/WHO), Lyon, France
Maike Wellbrock | University Medical Center of the Johannes Gutenberg University Mainz, Germany
Gregor Reid | University of British Columbia, Vancouver, Canada
Phung Tran | Electric Power Research Institute (EPRI), Palo Alto, California, USA
Katharina Gößling | University Hospital Duesseldorf, Germany
Christina-Evmorfia Kampitsi | Institute of Environmental Medicine, Karolinska Institutet, Sweden
Jill McKay | Northumbria University, UK
Erin Marcotte | University of Minnesota, USA
Julia Vogt | Technical University of Munich, Germany
Jessica Saville | Northumbria University, UK
Franziska Auer | Technical University of Munich, Germany
Ali Farrokhi | BC Children's Hospital Research Institute, Vancouver, Canada
Denis Acunzo | University of Milano-Bicocca, Monza, Italy
Mayla Bertagna | University of Milano-Bicocca, Monza, Italy

2.3 Programm

Der dreitägige Workshop wurde in sechs inhaltlich aufeinander abgestimmte Sessions eingeteilt und durch zwei zusätzliche Sessions, in denen die eingereichten Abstracts vorgestellt wurden, ergänzt.

In 24 Vorträgen von eingeladenen Experten sowie 13 eingereichten Kurzvorträgen wurde über folgende Themengebiete referiert:

- Internationale Perspektiven auf Leukämie im Kindesalter
- Infektionen und Immunität
- Umweltrisikofaktoren
- Entzündungen und die Knochenmarks-Mikroumgebung
- Genetik und Epigenetik

Zwischen den Programmpunkten konnte das Publikum Fragen zu den Präsentationen stellen und Probleme oder Streitpunkte diskutieren. Teilnehmende des Workshops tauschten sich hier über das Gehörte aus und erörterten gemeinsam Ansatzpunkte für die Weiterentwicklung der Forschung. Auf Basis des erhaltenen Feedbacks wird nun die weitere Vorgehensweise erarbeitet.

In den Programmpausen sowie bei einem gemeinsamen Conference Dinner am Abend des zweiten Veranstaltungstages konnten die Gäste den Austausch sowie das Networking im lockeren Rahmen fortsetzen.

3. Ergebnisse

Für einen Überblick über den während des Workshops präsentierten Forschungsstand sind im Folgenden das Programm sowie die Abstracts mit den Kurzzusammenfassungen jedes Vortrags aufgelistet.

3.1 Finales Programm

Montag, 28. November

From 11:00	Registration | Welcome coffee	
Session 1 | International Perspectives on Childhood Leukemia Chair: Gunde Ziegelberger, Co-Chair: Janine Schmidt		
12:30	Opening and summary of past workshops	S. Hornhardt, BfS Germany
12:45	Social inequalities in the incidence of childhood cancer: key points from the WHO European Report on Inequalities in Childhood Cancer	F. Erdmann, Germany
13:05	Four Decades of Learning and Progress About Acute Lymphoblastic Leukemia in India	S. Bakhshi, India
13:30	PEDIAC: the French national program on the origins and causes of pediatric cancers	T. Mercher, France
13:45-14:15	*Coffee break*	
Session 2 | Infections and Immunity Chair: Arndt Borkhardt, Co-Chair: Omid Azimzadeh		
14:15	Trained immunity: a memory for innate host defense	M. Netea, Netherlands
14:40	Training Immunity to prevent ALL – challenges and prospects	J. Hauer, Germany
15:05	Early infections and immune development in the etiology of childhood acute lymphocytic leukemia	J. Wiemels, USA
15:30	Modeling infection and trained immunity in acute lymphoblastic leukemia	A. Pandyra, Germany
15:55	Incidence, time of diagnosis and delivery of healthcare among children with leukaemia during the COVID-19 pandemic: evidence from the German Childhood Cancer Registry	F. Erdmann, Germany
16:20-16:30	Short break	
Proffered papers I | 16:30-17:00 Chair: Janine Schmidt, Co-Chair: Gunde Ziegelberger		
16:30	Oncogene induced senescence in ETV6::RUNX1 pre-leukemia phase of childhood acute lymphoblastic leukemia	D. Acunzo, Italy
16:45	IL-12p40 homodimer/monomer dependent depletion of B cell precursor ALL cells following early-life infection in mice	A. Farrokhi, Canada
From 19:00	*Informal get-together at "Bohne & Malz" | Sonnenstraße 11, Munich*	

Dienstag, 29. November

Session 3 | Environmental Risk Factors		
Chair: Joachim Schüz, Co-Chair: Peter Scholz-Kreise		
09:00	Low dose ionizing radiation from medical imaging procedures	M. Hauptmann, Germany
09:25	Natural background radiation & air pollution	B. Spycher, Switzerland
09:50	Residential radon as a risk factor for childhood leukemia – What is the evidence?	A. Auvinen, Finland
10:15-10:45	Coffee break	
10:45	Extremely low-frequency magnetic fields and childhood leukaemia: an overview	J. Schüz, France
11:10	The Geocap study	J. Clavel, France
11:35	Childhood Leukemia Environmental Risk Factors (CLERF) Project	D. Baaken, Germany
12:00-13:00	**Breakout Session**	
CLERF pilot study		
13:00-14:00	*Lunch*	
Proffered papers II | 14:00-15:00		
Chair: Janine Schmidt, Co-Chair: Gunde Ziegelberger		
14:00	Parental occupational exposure to combustion products, metals, silica and asbestos and risk of childhood leukaemia	F. Onyije, France
14:15	Overview of EPRI Research on Powerlines, Plant Nurseries, and Childhood Leukemia	P. Tran, USA
14:30	Mode of delivery and childhood leukemia risk— a Swedish population-based cohort study	C.-E. Kampitsi, Sweden
14:45	28-year incidence and time trends of childhood leukaemia in former East Germany compared to West Germany after German reunification: A study from the German Childhood Cancer Registry	M. Wellbrock, Germany
15:00-15:30	*Coffee break*	
Session 4 | Inflammation and the Bone Marrow Microenvironment		
Chair: Omid Azimzadeh, Co-Chair: Sabine Hornhardt		
15:30	Role of signaling through the TSLP/IL7R in initiation and propagation of acute lymphoblastic leukemia	S. Izraeli, Israel
15:55	Early hematopoietic stages of pediatric acute megakaryoblastic leukemia	N. Bertuccio, Italy
16:20	ETV6::RUNX1 pre-leukemic niche: role of infections and bone marrow microenvironment in leukemic onset	M. Bertagna, Italy
From 19:00	*Conference Dinner at „Schneider Bräuhaus" | Tal 7, Munich*	

Mittwoch, 30. November

Proffered papers III | 08:45-10:30		
Chair: Sabine Hornhardt; Co-Chair: Gunde Ziegelberger		
08:45	Exploring a potential mechanistic role of CpG-specific methylation in the relationship between environmental exposures and childhood acute lymphoblastic leukaemia	J. McKay, UK
09:00	Exploring the potential role of environmentally-associated DNA methylation to contribute to risk of different subtypes of childhood acute lymphoblastic leukaemia	J. Saville, UK
09:15	Breakpoint-agnostic screening for childhood leukemia	E. Marcotte, USA
09:30	Clinical criteria for genetic testing in pediatric oncology show a low specificity and miss every 4th child carrying a cancer predisposition	F. Auer, Germany
09:45	Characterization of a novel JAK3 variant in a family with a history of leukemia and lymphoma	J. Vogt, Germany
10:00	Virus-specific functional hyperresponsiveness in children with ETV6::RUNX1 positive acute lymphoblastic leukemia (ALL)	K.L. Gößling, Germany
10:15	Pathogen sensor engagement and host immune responsiveness dictate the outcome of TLR ligand exposure on precursor B-ALL cell populations	G. Reid, Canada
10:30-11:00	*Coffee break*	
Session 5 | Genetics and Epigenetics		
Chair: Janine Schmidt, Co-Chair: Sabine Hornhardt		
11:00	Identification, functional characterization and management of genetic variants associated to predisposition to childhood Acute Lymphoblastic Leukemia	G. Cazzaniga, Italy
11:25	Lessons from a low incidence population: The Admixture and Risk of Acute Leukemia (ADMIRAL) study	L. Spector, USA
11:50	Role of ontogeny and transcriptional alterations in pediatric acute megakaryoblastic leukemia	T. Mercher, France
12:15	Childhood ALL in the context of RASopathies – What does it tell us about leukaemogenesis?	H. Cavé, France
12:45-13:45	Lunch	
13:45	ETV6 Regulates Hematopoietic Stem Cell Function and Represses TNF During Stress Hematopoiesis	K. Nichols, USA
14:10	Biological insights into the pathogenesis of aneuploidies in childhood B-ALL	P. Menendez, Spain
14:35	Hyperdiploidy: the longest known, most prevalent but still most enigmatic form of acute lymphoblastic leukemia in children	A. Borkhardt, Germany
Session 6 | Discussion		
15:00	Looking around and forward – what to do next?	BfS-Team, BfS Germany
15:45-16:15	*Coffee and Farewell*	

3.2 Abstracts

3.2.1 Session 1: International Perspectives in Childhood Leukemia

Friederike Erdmann

Social inequalities in the incidence of childhood cancer: key points from the WHO European Report on Inequalities in Childhood Cancer

Division of Childhood Cancer Epidemiology, Institute of Medical Biostatistics, Epidemiology and Informatics (IMBEI), University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany

Department of Prevention and Evaluation, Leibniz Institute for Prevention Research and Epidemiology – BIPS, Bremen, Germany

Inequalities in health according to social conditions are regarded as unnecessary and unjust. Socioeconomic influences on health matter at all ages including childhood, for which childhood cancer is the leading cause of disease related death in high-income countries (HIC).

Substantial differences in the reported incidence of childhood cancers have been observed globally by socioeconomic development of a population. This is reflected in the higher incidence rates reported for countries with higher position in the Human Development Index (HDI), particularly for acute lymphoblastic leukaemia, and for cancer in infants (below 1 year of age), compared to countries with lower HDI scores. It is however assumed that most (if not all) of the variations in incidence rates are due to underdiagnoses and underreporting of cases. Considerable inequalities across countries and position according to the HDI are also noted for childhood cancer survival, with substantially lower survival rates seen in most low- and middle-income countries compared to high-income countries.

Within individual countries, socioeconomic and gender-based inequalities can be seen in terms of incidence, detection and outcome. The relationship between socioeconomic status (SES) and childhood cancer risk has been internationally most exhaustively studied for leukaemia and specifically acute lymphoblastic leukaemia. Findings are overall inconsistent across studies and limited to studies from HICs. On the contrary, observations on social inequalities in survival within countries are accumulating and indicate that survival inequalities do not only concern resource-poor countries but also high-income populations including European countries. In turn, a childhood cancer diagnosis in itself may have implications on the parents´ socioeconomic situation as well as on the later socioeconomic life after having survived the disease. Childhood cancer survivors are at risk of various adverse socioeconomic consequences with respect to school performance, attained educational level, employment, income level and uptake of social security benefits.

Every year over 34,000 children in the WHO European Region (WHO/Europe), are diagnosed with cancer, 20% of them die from their illness. On International Childhood Cancer Day, 15 February 2022, the WHO Regional Office for Europe launched the first WHO Report on Inequalities in Childhood Cancer. The report brings together evidence and information from a wide range of sources to present the nature and magnitude of inequality in childhood cancer in the European region and calls for action to begin closing these gaps.

My talk aims to give an overview about the most recent evidence about social inequalities in the incidence of childhood cancer- across countries and within countries, point to important research gaps and summarise the key points from the WHO European Report on Inequalities in Childhood Cancer.

Sameer Bakhshi

Four Decades of Learning and Progress About Acute Lymphoblastic Leukemia in India

Dept of Medical Oncology, Dr B. R. A. Institute Rotary Cancer Hospital,
All India Institute of Medical Sciences, New Delhi, India

Acute lymphoblastic leukemia (ALL) prior to 1984 had a very low survival rate in India, and even the exact data was also not known. The first major change in the treatment of ALL was initiated after a collaboration was set up between the National Cancer Institute, Bethesda and Cancer Institute, Chennai. A uniform protocol for treatment of ALL was initiated under the name Multicentric Protocol 841 (MCP-841); this protocol was initiated at Chennai, All India Institute of Medical Sciences, New Delhi and Tata Medical Hospital, Mumbai. The protocol was based on contemporary US and Europeon protocols but modified for the Indian context (such as avoiding high doses of methotrexate administration); the protocol was reasonably intense keeping in mind the advanced stage of presentation in the Indian set up at that time. The key findings of this intervention study which enrolled little more than 1000 patients aged 1-25 years from 1990-1997 was that T-ALL is relatively higher in Chennai (as high as 43% of all cases); toxic death rates varied from 11-23% and overall EFS was 41-60% at the three centers. However, no factor emerged significant to for predicting survival.

Subsequently, a new protocol was initiated by the same three centers in collaboration with international Cancer Treatment Network and Research (INCTR), and this was INCTR 02-04. This protocol was having the backbone of the MCP-841 protocol but was relatively more intense so as reduce relapses, and CNS radiation was reduced. However, the interest in this protocol dwindled and except AIIMS, the other two centers had incomplete recruitment and stopped midway. Of the 397 patients enrolled from 2004-2009, the proportion of T ALL reduced to 35% in Chennai, the toxic death rates were between 17-23% and EFS varied from 38-66%.

Thereafter, at the end of the third decade, the pediatric oncology community got together to form a group named Indian Pediatric Oncology Group (INPOG), and simultaneously, a working group for treating ALL with a new approach was created between the major centers in Delhi, Mumbai, Chandigarh and Kolkatta; this group for ALL was named ICICLE and this then became a flagship collaborative group under INPOG. Since the previous two protocols, namely, MCP-841 and INCTR-0204 had achieved reasonable progress but had then become static with no identification of risk groups, the group decided nevertheless to go with a risk adapted approach. This required agreement on a common protocol for risk adaptation, and for this the major effort was to standardize MRD assessment at all centres. A pretrial cohort was done from 2013-2016, and thereafter the trial was initiated with two randomized questions: use of 2 weeks vs 4 weeks

of steroids in induction therapy, and the second question was randomization between doxorubicin vs mitoxantrone in the delayed intensification phase. The preliminary results from the pre-trial cohort suggest that the CR rates are more than 96% and that relapses are 25%. The data of the trial cohort are still not mature.

Not only has the group standardized risk adapted therapy, standardized MRD assessment, but has done various lab-based sub-studies. Recently there has been a growth of indigenous CAR T cell development in India with several private enterprises trying to develop the same. Overall the last decade has seen a stupendous improvement in survival of ALL in India.

References:

1. Magrath I, Shanta V, Advani S, et al. Treatment of acute lymphoblastic leukaemia in countries with limited resources; lessons from use of a single protocol in India over a twenty year period. Eur J Cancer. 2005 Jul;41(11):1570-83.
2. Arya LS, Kotikanyadanam SP, Bhargava M, et al. Pattern of relapse in childhood ALL: challenges and lessons from a uniform treatment protocol. J Pediatr Hematol Oncol. 2010 Jul;32(5):370-5.
3. Arora RS, Bakhshi S. INPOG- Collaborative research in India comes of age. Ped Hematol Oncol Journal 2016; 1: 13-17.
4. Das N, Banavali S, Bakhshi S, et al. Protocol for ICiCLe-ALL-14 (InPOG-ALL-15-01): a prospective, risk stratified, randomised, multicentre, open label, controlled therapeutic trial for newly diagnosed childhood acute lymphoblastic leukaemia in India. Trials. 2022 Jan 31;23(1):102.
5. Das N, Gupta R, Gupta SK, et al. Critical evaluation of the utility of pre- and post-therapy immunophenotypes in assessment of measurable residual disease in B-ALL. Ann Hematol. 2021 Oct;100(10):2487-2500.
6. Gupta SK, Bakhshi S, Kamal VK, et al. Proposal and clinical application of molecular genetic risk scoring system, „MRplus“, for BCR-ABL1 negative pediatric B-cell acute lymphoblastic leukemia-report from a single centre. Leuk Res. 2021 Dec;111:106683.
7. Gupta SK, Bakhshi S, Kumar L, et al. Gene copy number alteration profile and its clinical correlation in B-cell acute lymphoblastic leukemia. Leuk Lymphoma. 2017 Feb;58(2):333-342.
8. Gupta SK, Bakhshi S, Chopra A, et al.Molecular genetic profile in BCR-ABL1 negative pediatric B-cell acute lymphoblastic leukemia can further refine outcome prediction in addition to that by end-induction minimal residual disease detection. Leuk Lymphoma. 2018 Aug;59(8):1899-1904.
9. Gupta SK, Singh M, Chandrashekar PH, et al. Clinical and Prognostic Impact of Copy Number Alterations and Associated Risk Profiles in a Cohort of Pediatric B-cell Precursor Acute Lymphoblastic Leukemia Cases Treated Under ICiCLe Protocol. Hemasphere. 2022 Sep 30;6(10):e782.

Thomas Mercher

PEDIAC: the French national program on the origins and causes of pediatric cancers

Institut Gustave Roussy, INSERM U1170, Université Paris-Saclay, 94800 Villejuif, France

The PEDIAC project brings together eleven teams of basic research scientists from distinct major french cancer centers with expertise spanning a wide research area, including epidemiology, immunology, physiological modeling, genetic analyses and molecular biology of pediatric tumor cells. This consortium stems from an original french national funding scheme and will develop a 4-year pluri-disciplinary research program on different pediatric cancer entities (https://programme-pediac.com/en/home/).

PEDIAC's main research objective is to understand the causes and origins of the development of aggressive cancer subtypes at specific ages during childhood. The project is divided into three workpackages (WP). WP1 aims at identifying risk factors in childhood cancers. This part will include the identification of genetic predispositions by relying on existing cohorts of patients considered more likely to carry germline mutations, the identification of environmental, potentially preventable, risk factors, the estimation of the potential impact of modifiable and non-modifiable risk factors in France and the characterization of epigenetic signatures of environmental exposures.

WP2 aims at identifying ontogenic determinants of pediatric oncogenes specificities. This part will work on the characterization of pediatric cancer entities through available and novel data to decipher cellular and molecular differences within intra and extra-tumoral compartments using single cell approaches. Importantly, we will also use appropriate experimental models to characterize the cellular and molecular impact of the changes observed during different stages of ontogeny on tumor formation.

WP3 will represent the functional module of the project where integrated molecular bases of pediatric cancers will be investigated and modeled. This part will work on the identification of altered intrinsic functional nodes, including epigenetic regulators, the characterization of essential ligand-receptors interactions contributing to pediatric tumor emergence or maintenance and the development of improved approaches to tumor ecosystem reconstruction aiming at a better modeling of relevant interactions between the intrinsic factors and the micro or macro-environment in pediatric cancers.

Together, PEDIAC aims at identifying potentially preventable risk factors and novel markers of cancer predisposition as well as at modeling more faithfully the situation found in patients to identify molecular mechanisms specific to pediatric tumor cells.

3.2.2 Session 2: Infections and Immunity

Mihai Netea

Trained immunity: a memory for innate host defense

Department of Medicine, Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands

The inability of innate immunity to build an immunological memory, considered one of the main characteristics differentiating it from adaptive immunity, has been recently challenged by studies in plants, invertebrates, and mammals. Long-term reprogramming of innate immunity, that induces adaptive traits and has been termed trained immunity characterizes prototypical innate immune cells such as natural killer cells and monocytes, and provides protection against reinfection in a T/B-cell-independent manner. In contrast, trained immunity has been shown to be able to induce protection against reinfection in a lymphocyte-independent manner. Non-specific protective effects dependent on trained immunity have also been shown to be induced after BCG vaccination in humans. Specific signaling mechanisms including the dectin-1/Raf1 and NOD2-mediated pathways induce trained immunity, through induction of histone modifications (methylation, acetylation) and epigenetic reprogramming of monocyte function. Complex immunological and metabolic circuits link cell stimulation to long-term epigenetic reprogramming of the function of myeloid cells and their bone marrow progenitors. The concept of trained immunity represents a paradigm change in immunity and its putative role in infection and inflammation may represent the next step in the design of future vaccines and immunotherapeutic approaches.

Julia Hauer

Training Immunity to prevent ALL – challenges and prospects

Children's Hospital, Technical University of Munich, Munich, Germany

B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is the most common form of childhood cancer. Chemotherapy is associated with life-long health sequelae and fails in ~20% of cases. Thus, prevention of leukemia would be preferable to treatment. Childhood leukemia frequently starts before birth, during fetal hematopoiesis. A first genetic hit such as translocations (ETV6-RUNX1) leads to the expansion of preleukemic B-cell clones, which are detectable in healthy newborn cord blood (up to 5%) or germline genetic predisposition cause hematopoietic stem cell /precursor vulnerability.

We and others provide experimental evidence that suggests that a major driver of conversion from the preleukemic to the leukemic state is exposure to immune challenges. The Pax5+/- model serves as an ideal model to study the interplay of genetic predisposition and immune challenges in childhood ALL as it nicely mimics children with PAX5 G183S germline predisposition.

Furthermore, novel insights have shed light on immune host responses and how they shape the complex interplay between (1) inherited or acquired genetic predispositions, (2) exposure to infection, and (3) host immune response.

Joseph L. Wiemels

Early infections and immune development in the etiology of childhood acute lymphocytic leukemia

Center for Genetic Epidemiology, University of Southern California, Los Angeles

Epidemiologists have long investigated patterns of infectious exposure and response in early life as risk-modulating factors for acute lymphoblastic leukemia (ALL). Two non-exclusive hypotheses describe these patterns in relation to risk: (i) delayed exposure to common infections and microbial colonization in infancy may lead to aberrantly vigorous and damaging immune responses later in childhood, enhancing ALL risk (the "delayed infection hypothesis" from Greaves 1), and (ii) a specific leukemia-causing virus may infect children who are immunologically naïve to such a virus in situations where enhanced population mixing occurs at community-wide scales 2. No specific infectious agent was discovered until recently when cytomegalovirus (CMV) was found within childhood ALL blasts at diagnosis, as the only virus that distinguished ALL blasts from acute myeloid leukemia blasts 3. The mere presence of CMV at diagnosis may not be the key feature of its involvement since evaluation of neonatal blood spot-derived DNA in California subjects revealed the presence of CMV at birth at a 3-5 fold higher frequency in children who contracted ALL later in childhood when compared to children that remained cancer-free.3 The CMV-ALL association therefore does not conveniently fit into either of the two prevailing hypotheses concerning infection and ALL; certainly neonatal infection cannot be portrayed as "delayed" thereby ruling out CMV as a causative agent for the postnatal, delayed second hit in leukemia in Greaves' hypothesis. As to the second hypothesis from Kinlen, CMV has never been proven to be a cancer virus, and its ubiquity in our population (>80% seropositivity worldwide, in both developed and developing countries) argues that CMV itself should not be the causative agent given the rarity of ALL in children.

The incidence of congenital infection of CMV has not concurrently changed over the time period that ALL incidence has increased in developed countries, this ALL increase being about 2-fold over the past 70 years.4,5 Due to this single fact, neonatal CMV infection seems not to be singularly causative for development of ALL. Indeed, CMV does not fulfil even one of 'Koch's postulates' regarding evidence of a causal relationship between microorganisms and disease. Instead, CMV's role in etiology of ALL may pertain to timing of CMV infection as the key feature, and requires collaboration with postnatal events. CMV exposure at any age leads to lifelong infection and altered immune function; exposure in the neonatal period (rather than later in child or adulthood) may lead to the development of immune function that promotes or permits oncogenic stimuli or could stimulate outgrowth of hematopoietic stem cell precursors thereby enhancing the opportunity for further mutational damage leading to ALL.

This hypothesis is made more emphatic with the observation that a single subtype of ALL is linked to CMV in leukemic blasts – that is, high hyperdiploid ALL, the most common subtype.6 Early CMV exposure therefore may alter the hematopoietic status upon which other infectious stimuli play a more direct, causative role, promoting specific subtypes of ALL in conjunction with other causative factors. Neonatal CMV infection is therefore likely an adjuvant to the "delayed infection" hypothesis put forward by Greaves 1 rather than a representing an entirely new causal pathway for ALL.

Additional supportive clinical evidence for the CMV-ALL association is provided from Swedish registries where CMV infection diagnosed during pregnancy in the mother of children who contracted hematologic malignancies, or at birth in the newborn, was observed at a more than 10-fold higher frequency when compared to the remainder of the age-matched population.7 The evidence therefore in sum supports an etiologic association between CMV and ALL that is tantalizing but more research is needed before the virus itself could be considered a potential target for prevention or clinical management of ALL.

CMV infection at birth has long been associated with sensorineural hearing loss and other birth defects, but not previously recognized as a risk factor for ALL in case-control epidemiology studies. The extremely low levels of CMV found at birth in the California study (where 3% of healthy controls were positive 3 compared to less than 1% in most neonatal CMV studies) suggest that exposure to CMV early rather than fulminant infection may be the key epidemiologic feature. Again, such a scenario would support a oncomodulatory role for CMV rather than direct transformative role – and potentially responsible for the altered cytokine profile at birth associated with ALL.8 Whether CMV exposure happens during pregnancy or during the delivery itself is a possibility which would have implications in the possible prevention of neonatal CMV.

Profound questions remain in the elucidation of etiologic or pathogenic connections between neonatal CMV infection and ALL. While a viral-associated APOBEC-mediated mutational signature is a key feature of some virally-induced cancers including ALL, this mutational signature is conspicuously absent in high hyperdiploid ALL,9 which has the strongest clinical association with CMV.6 This mutational signature being a broad feature of other virally-induced cancers suggests the potential for other viral involvement (apart from CMV) in the etiology of ALL. This relationship reveals another key feature needing clarification in the relationship between CMV and the many features of neonatal immune function and postnatal infection relationships in the pathway towards ALL and ALL prevention.

References:

1. Greaves M. A causal mechanism for childhood acute lymphoblastic leukaemia. Nat Rev Cancer. 2018;18(8):471-484.
2. Kinlen LJ. Epidemiological evidence for an infective basis in childhood leukaemia [editorial]. Br J Cancer. 1995;71(1):1-5.
3. Francis SS, Wallace AD, Wendt GA, et al. In utero cytomegalovirus infection and development of childhood acute lymphoblastic leukemia. Blood. 2017;129(12):1680-1684.
4. Barrington-Trimis JL, Cockburn M, Metayer C, Gauderman WJ, Wiemels J, McKean-Cowdin R. Trends in childhood leukemia incidence over two decades from 1992 to 2013. Int J Cancer. 2017;140(5):1000-1008.
5. Gurney JG, Davis S, Severson RK, Fang JY, Ross JA, Robison LL. Trends in cancer incidence among children in the U.S. Cancer. 1996;78(3):532-541.
6. Gallant RE, Arroyo K, Bracci PM, et al. Clinical characteristics of cytomegalovirus-positive pediatric acute lymphoblastic leukemia at diagnosis. Am J Hematol. 2022;97(6):E198-E201.
7. Wiemels JL, Talback M, Francis S, Feychting M. Early Infection with Cytomegalovirus and Risk of Childhood Hematologic Malignancies. Cancer Epidemiol Biomarkers Prev. 2019;28(6):1024-1027.
8. Chang JS, Zhou M, Buffler PA, Chokkalingam AP, Metayer C, Wiemels JL. Profound deficit of IL10 at birth in children who develop childhood acute lymphoblastic leukemia. Cancer Epidemiol Biomarkers Prev. 2011;20(8):1736-1740.
9. Brady SW, Roberts KG, Gu Z, et al. The genomic landscape of pediatric acute lymphoblastic leukemia. Nat Genet. 2022;54(9):1376-1389.

Alexandra A. Pandyra

Modeling infection and trained immunity in acute lymphoblastic leukemia

Department of Pediatric Oncology, Hematology and Clinical Immunology, Medical Faculty, Heinrich- Heine- University, Düsseldorf, Germany

German Cancer Consortium (DKTK), partner site Essen/Düsseldorf, Düsseldorf, Germany

Pax5 heterozygosity (Pax5+/-) in mice mimics germline or somatic Pax5 dysregulation (resulting in reduced Pax5 levels) observed in the clinical setting. In previous studies, we provided the first evidence for infection-driven leukemogenesis in Pax5+/- mice. While a link between general, non-specific infectious exposure and B-cell precursor acute lymphoblastic leukemia (BCP-ALL) in the Pax5+/- model was demonstrated, the effects of a specifically tractable infections are poorly understood. In the current studies, we therefore aimed to characterize the long-term effects of viral infections and immune training in vivo. Pax5+/- mice were infected with the chronic Lymphocytic choriomeningitis virus (LCMV) Docile strain or the Vesicular stomatitis virus (VSV). LCMV is a non-cytopathic virus and has been extensively utilized to investigate virus-induced immunopathology, effector responses and immune tolerance. VSV by contrast, is used to model acutely cytopathic viruses. Innate and early adaptive responses were not impaired in Pax5+/- mice following infection with both LCMV and VSV. However, Pax5+/- mice were susceptible to infection with LCMV resulting in shorter long-term survival compared to infected WT mice. However, at day 15 and 90 post-infection, LCMV-specific T cells were present within the bone marrow micro-environment and tetramer specific CD8+ T cells raised against the LCMV nucleocapsid protein (np 396) were increased in the BM of Pax5-/+ infected mice. Although most mice could clear the virus from all organs by day 120, mice that eventually succumbed to infection were characterized by detectable viral titres in organs. Preliminary data indicates that pre-treatment with the immune trainer β-Glucan improved the survival of WT but not Pax5+/- mice. Trained immunity describes the ability of innate immune cells such as monocytes to retain a memory-like phenotype driven by epigenetic programming following initial immune or metabolic stimulation culminating in amplified responses upon secondary stimulation.

Monocytes are generally pro-tumorigenic but as with many immune infiltrates, this population is characterized by an inherent plasticity whose immunosuppressive effects can manipulated through immune training. To complement our murine models of BCP-ALL predisposition, we are in parallel generating cellular compartment specific knockouts of the CREB binding protein (CBP). CBP is a transcriptional co-activator and functions as a histone acetyltransferase. Furthermore, somatic oncogenic CBP mutations are associated with B cell neoplasias including relapsed BCP-ALL in children. Specifically, we have generated mice where CBP

is knocked out in the early B cell lineage and myeloid compartment. Preliminary data indicates that deletion of CBP results in hyperproduction of the pro-inflammatory cytokine IL-6 in response to TLR-specific innate immune stimuli. Taken together, this study demonstrates that pre-leukemic Pax5-/+ mice are differently affected by chronic infection and immune training compared to WT mice supporting the observations that immune dysregulation contributes to the emergence of the leukemic clone. Future investigations will delve into further deciphering immune trained responses and combining our BCP-ALL genetically pre-disposed models with the newly generated CBP systems to better understand how specific immune subsets contributing to leukemia development.

Friederike Erdmann

Incidence, time of diagnosis and delivery of healthcare among children with leukaemia during the COVID-19 pandemic: evidence from the German Childhood Cancer Registry

Division of Childhood Cancer Epidemiology, Institute of Medical Biostatistics, Epidemiology and Informatics (IMBEI), University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany

Department of Prevention and Evaluation, Leibniz Institute for Prevention Research and Epidemiology – BIPS, Bremen, Germany

Background: More than two years have passed since the outbreak of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic and its first COVID-19 lockdowns with extensive social distancing policies, stay-at-home orders and other societal restrictions imposed across Germany and worldwide. The unprecedented detrimental consequences of the COVID-19 pandemic on cancer care and timely diagnosis are of increasing concern. At the same time, the pandemic with its societal restrictions enables a unique opportunity to gain a better understanding about the hypothesised role of the immune system in the pathogenesis of childhood lymphoblastic leukaemia (ALL). Assessing the direct or indirect impact of the COVID-19 pandemic on the incidence of childhood ALL on a population level is therefore very informative.

We examined the impact of the COVID-19 pandemic on incidence, time of diagnosis and delivery of healthcare among paediatric oncology patients in Germany during the COVID-19 pandemic, with a particular focus on childhood leukaemia.

Material and methods: We analysed incident cancer diagnoses in children aged 0-17 years in Germany in 2020 and 2021 using data of the German Childhood Cancer Registry. Absolute numbers (monthly number of diagnoses throughout 2020) and age-standardised incidence rates (ASR) (standardised to the SEGI 1960 Standard World Population) were compared to the previous five years (2015–2019). Furthermore, we specifically analysed temporal changes in the age-specific incidence (ages 2-6 years vs 7-14 years) of childhood B-cell precursor ALL.

To complement the quantitative assessment, a survey with open-ended questions, gathering perceptions of the diagnostic process and healthcare delivery during 2020 was conducted.

Results: Noticeably more or similar numbers of paediatric cancer patients were newly diagnosed each month throughout 2020 in comparison to 2015-2019. Results from the qualitative survey indicated that diagnostic processes, timeliness of diagnosis, and delivery of treatment were hardly affected during 2020, but psychosocial supportive care and non-urgent appointments considerably reduced during the lockdown periods.

ASRs indicated a remarkable increase for childhood cancers overall (ages 0-14: 10.2%, ages 0-17: 11.5% increase) and across diagnostic groups for 2020 compared to 2015-2019. The percentage increase was highest for lymphomas and tumours of the central nervous system (CNS), but not much less pronounced for childhood leukaemia including ALL (ages 0-14: 10.4%, ages 0-17: 10.2% increase). Estimated ASRs for 2021 however suggested that this was followed by a decline, with the exception of CNS tumours for which the ASRs remained on the level of 2020. ASRs of leukaemia overall and ALL were still somewhat elevated compared to those in the five years before the pandemic, while ASRs for lymphomas and non-CNS solid tumours have dropped so drastically that they fell below the respective ASRs from 2015-2019.

Assessing the age-specific incidence of B-cell precursor ALL in 2-6-year-olds revealed an increase by 9.6% to 79.1 cases per million in 2020, followed by a remarkable regression in 2021. These temporal changes were less pronounced in the 7-14-year-olds (increase of 7.0% vs 9.6%), whereas the difference being still compatible with random variation.

Conclusion: Although it is reassuring that we found no signs of missed or delayed childhood cancer diagnoses throughout 2020-2021, the underlying reasons for the marked increase in incidence rates in 2020 remain speculative. Continued close monitoring of incidence patterns may shed light on the underlying reasons and contribute to understanding disease aetiology.

Denise Acunzo

Oncogene induced senescence in ETV6::RUNX1 pre-leukemia phase of childhood acute lymphoblastic leukemia

Centro Ricerca Tettamanti, Pediatrics, University of Milano-Bicocca, Monza, Italy

Background: ETV6::RUNX1 (E::R) is the most frequent alteration found in pediatric B cell precursor acute lymphoblastic leukemia. This fusion gene encodes for an aberrant transcription factor which leads to the formation of a clinical silent pre-leukemic progenitor, able to persist in the organism for many years and to be more susceptible to additional mutations (Wiemels J et al., 1999). It has been demonstrated that E::R expression in pro-B cells causes the slowdown of cell cycle progression(Ford AM et al., 2009), which is one characteristic of Oncogene Induced Senescence (OIS) phenotype. Nowadays there is an emerging evidence that OIS would promote cancer development inducing changes in the tissue microenvironment and on adjacent cells through secreted bioactive molecules (Davalos AR et al., 2010).

The aim is to demonstrate the ability of E::R to induce a senescent phenotype and understand if OIS is sustaining the pre-leukemic phase. This could make the rationale for developping novel therapeutic treatments to eradicate the pre-leukemic clone by avoiding the leukemia development.

Materials and methods: All the experiments were performed using an in vitro inducible E::R+pre leukemic mouse model, a gift of Dr A.M. Ford.

Results: Preliminary data, using an in vitro E::R+pre-leukemic model, showed that E::R expression causes the slow down of the cell cycle and the up-regulation of p21CIP1 (CDKN1A) and p27KIP1 (CDKN1B) genes, which encode for inhibitors of cyclin-dependant kinases involving in cell cycle progression (Ford AM et al., 2009). Using the same model, we demonstrated that E::R cells are positive to other cellular senescence markers, such as an altered morphology, up-regulation of β Galactosidase activity, up-regulation of ROS and increased γH2AX level.

Moreover, we analyzed the supernatant of the E::R and control cells using ELISA or Luminex assay and we detected the presence of several molecules in the supernatant of E::R cells but absent in control cells, such as PAI-1, CCL2, CXCL1, CXCL5, GM-CSF and IL4, which are proteins involved in inflammation and senescence response, resulting in a so called 'Senescence-Associated Secretory Phenotype' (SASP).

In addition, we observed a higher level of p53 protein in E::R+ cells, which is one of the principal proteins involved in senescence. We investigated the post-transcription modification of p53 in basal and apoptotic condition and, in pre-leukemic cells, we detected the absence of p53-serine 392 phosphorylation, a p53 regulation which promotes

its mitochondrial translocation and thus the induction of cellular apoptosis (Castrogiovanni C. et al., 2018). Consistently, we found a reduced level of CK2 kinase, which has been hypothesized to target p53-serine 392 (David M. Keller et al., 2002). To better elucidate the role of CK2 protein in p53 phosphorylation, we treated control cells with an inhibitor of CK2 kinase activity, and we observed a reduction of the p53-S392 phosphorylation.

Since resistance to apoptosis is another important feature of senescent cells, we measured the viability of E::R cells compared to control after exposure to apoptotic stimuli and we found that E::R cells were more resistant to apoptosis after stimulation with etoposide, a drug that induces DNA damage. In agreement with this result, we also observed that in a competitive growth assays, E::R cells showed growth advantage compared to control cells when subjected to stimuli leading to DNA damage (i.e. etoposide or X-rays).

However, γH2AX level, a marker of DNA double strand breaks, did not change after exposure to X-ray irradiation, indicating that pre-leukemic cells do not have an altered DNA damage repair pathway.

Conclusion: These results suggest that E::R expression is able to induce a senescence phenotype in our model and set the stage for further studies with the goal to contrast the apoptosis resistance. To achieve this goal, we will explore the possibility to use senolytic drugs, which are specific for senescent cells, to eradicate the pre-leukemic clone.

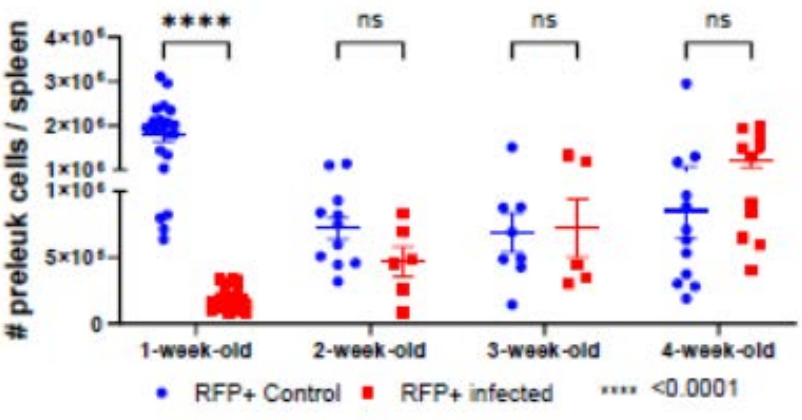
Ali Farrokhi

IL-12p40 homodimer/monomer dependent depletion of B cell precursor acute lymphoblastic leukemic cells following early-life infection in mice

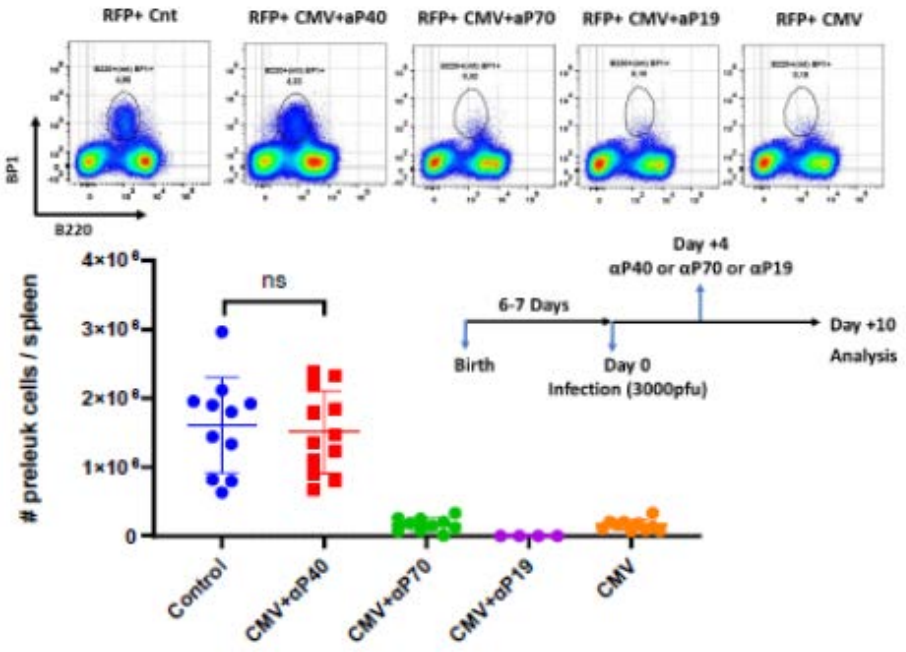
Michael Cuccione Childhood Cancer Research Program, BC Children's Hospital Research Institute, Vancouver, BC V5Z 4H4, Canada

Background: Precursor B cell acute lymphoblastic leukemia (B-ALL) is one of the most common forms of childhood cancer. Epidemiological studies have shown contrasting results for the role of infections on the risk of leukemia, and there is still no clear explanation for the pro- or anti-leukemia effects. The aim of this study is to investigate the influence of Cytomegalovirus (CMV) infection on the development of B-ALL and to identify the underlying immune mechanisms involved.

Materials and Methods: In this study, transgenic mice carrying a RFP/RET fusion gene (the amino terminal of the RFP protein, a transcriptional activator, and the membrane and tyrosine kinase domains of receptor tyrosine kinase (RET)) under the transcriptional control of the IgH enhancer (Eμ-ret) were used. For infection, luciferase tagged CMV (CMV-Luc) was administered by injecting 3000 pfu via intraperitenum. After euthanizing mice at different time points after infection, multicolor flowcytometry was used to identify different subsets of B cells and monitor depletion of preleukemic cells in bone marrow and spleen. To study the immune pathways involved in depletion of preleukemic cells, we generated Eμ-ret mice deficient in Rag1, Stat4, Stat6, or CD1d. In addition, we used neutralizing antibodies to evaluate the role of different cytokines/chemokines in preleukemia depletion in vivo.



Figue 1: Age dependent depletion of preleukemic cells



Figue 2: In vivo neutralization of p40, p19 (IL-23), p70 (IL-12) in infected neonates.

Results: Significant infection-induced depletion of preleukemic cells occurs in Eμ-ret mice on BALBc, BALBc/FVB (F1) and BALBc/C57 (F1) backgrounds. This depletion shows an age dependent pattern, with only infection in the first week after birth eliciting an immune response that reduces preleukemic B cell numbers. To investigate the role of cytotoxic lymphocytes in the reduction of preleukemia burden, we infected Rag1-deficient, CD1d-deficient, and NK cell-depleted Eμ-ret mice. Each of these strains achieved depletion of preleukemic cells after infection, suggesting that the depletion is unlikely to result from cytotoxic activity exerted by T cells, B cells, NKT cells, or NK cells. The preleukemia-depleting immune activity is dependent on Stat4 but not Stat6, suggesting a role for the IL-12 family of cytokines. Interestingly, antibody-mediated neutralization of p40 homodimer/monomers, but not p40 heterodimers (IL-12 and IL-23) inhibited the depletion of preleukemia cells. Further experiments have shown the significant role of other soluble factors, including a series of cytokines/chemokines, in induction of apoptosis in preleukemic cells.

Conclusion: To our knowledge, this is the first report of age-dependent depletion of preleukemic cells following virus infection. The identified Stat4- and IL-12p40 homodimer/monomer-driven activity could inform future treatment/prevention approaches for B-ALL.

3.2.4 Session 3: Environmental Risk Factors

Michael Hauptmann

Low dose ionizing radiation from medical imaging procedures

Institute of Biostatistics and Registry Research, Brandenburg Medical School Theodor Fontane, Neuruppin, Germany

Several comprehensive reviews, pooled analyses and meta-analyses have recently focused on the association between exposure to low doses of ionizing radiation at young age and cancer risk. Children are exposed to low doses in utero and postnatally, and the main sources of exposure are diagnostic medical procedures and natural background radiation.

Numerous epidemiologic studies support elevated cancer risks after exposure to low doses of radiation at young age. However, the situation for leukemia is complicated because the dose to the target, the active bone marrow, is generally low and assessment is challenging due to the age-dependent distribution of bone marrow throughout the body. Changing classifications of lymphohematopoietic neoplasms and heterogeneous grouping in studies further complicate the synthesis of the evidence.

The talk will summarize current evidence and integrate it with new results from the EPI-CT cohort, a pooling of European studies on CT-related radiation exposure.

Ben Spycher

Natural background radiation & air pollution

Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland

The cancer risks associated with low doses of ionizing radiation (<100mSv) are uncertain and difficult to quantify. Combined evidence from the studies of atomic bomb survivors (Life Span Study, LSS) and populations exposed to therapeutic radiation shows strong evidence of excess risks for both childhood ALL and AML after cumulative RBM doses <100 mSv and even some evidence when restricting to doses <50 mSv. Estimated relative risks are compatible with the modelled dose-response (based on the entire dose-range) in the LSS for leukaemia after exposure in childhood, suggesting excess relative risks in the order of 2-5% per mSv. Assuming that cumulative doses to the RBM received from ubiquitous exposure to natural background has similar effects on childhood leukaemia risks, these should be detectable in large population-based studies.

I provide an overview of studies on childhood leukaemia risks and exposure to natural background radiation with a focus on recent nationwide register-based studies of terrestrial gamma radiation, which contributes importantly to average RBM doses in children. Most of the recent large register-based studies either find evidence for increased risks in the expected order of magnitude or are compatible therewith. However, a large study from France reported no evidence of an association with narrow confidence intervals (RR per 1mSv 1.00, 95%-CI 0.99-1.01). Exposure misclassification has been suggested as a possible reason for these conflicting results. I will show some results from recent efforts to improve and validate exposure models for natural radiation. I will also provide an outlook of ongoing studies on background radiation and childhood leukaemia.

Outdoor air pollution has been classified by the IARC as carcinogenic to humans (Group 1). Specific agents in outdoor air pollution have also been classified as carcinogens including benzene, which is known cause leukaemia in occupationally exposed adults. Some studies have reported positive associations between childhood leukaemia and parental occupational exposure to benzene.

In the second part of the talk, I provide an overview of studies of outdoor air pollution and childhood leukaemia. Previous studies have mainly focused on traffic related air pollution using a variety of exposure measure including traffic density and modelled or measured levels of specific air contaminants such as NO2, benzene, or particulate matter (PM). A recent review and meta-analysis of 26 cases control and 3 cohort studies published up to 2017 reported a positive association between leukaemia and benzene exposure but no evidence of an association for NO2. The association with benzene was strongest for AML with no indication of any threshold effect. Among 5 studies published since this review, only 1 investigated benzene exposure adding further support for an association with AML, while 3 studies investigated NO2 with conflicting results. Other notable findings from these recent studies include a positive association between PM2.5 and Non-Hodgkin lymphoma and between ultrafine particle exposure during the first trimester of pregnancy and overall childhood cancer.

Anssi Auvinen

Residential radon as a risk factor for childhood leukemia – What is the evidence?

Tampere University, Tampere Finland

STUK – Radiation and Nuclear Safety Authority, Vantaa, Finland

Residential radon is a naturally occurring radioactive noble gas that has also several isotopes and radioactive progeny. The main isotope, radon-22 is formed in the decay chain of uranium-238. It is a major source of radiation exposure globally and the most important one for natural radiation in many populations. Radon and its daughter isotopes emit mainly alpha radiation, which is particulate radiation with a very short range of penetration in tissue. Radon causes radiation dose primarily to the bronchial epithelium and is an established cause of lung cancer. A fraction of inhaled radon is absorbed to the blood stream and results in doses to other tissues than lung, though substantially lower, at least by a factor of ten.

Radon concentrations can be measured with simple technology. In most epidemiological studies, long-term measurements with passive alpha track detectors have been used. Critical issues in such approach include measuring all previous dwellings and ensuring adequate participation to avoid selection bias. An easier approach is to use prediction models to calculate estimated radon levels instead of actual measurements. This can be achieved for large numbers of dwellings without active participation of the inhabitants. However, such prediction models have rarely performed adequately. This is due to the fact that residential radon concentrations depend on a number of factors, some of which are hard to evaluate. Uranium concentration in the ground defines the availability of radon. Yet, it can enter a building only if the ground is permeable and the building structures facing the ground are not airtight. In addition, ventilation affects the exit of radon from a building.

Five case-control studies have evaluated the risk of childhood leukemia from residential radon based on direct radon measurements. They have not shown increased risks with radon level. They are, however, limited by numbers of participants, low radon levels, and incomplete coverage of residential histories by the measurements.

Another set of five studies have evaluated risks from residential radon using modelled or predicted radon levels without actual measurements. Their results are less consistent, with a Danish study reporting significantly increased risks and a study performed in the UK showing odds ratios non-significantly above unity. The advantages of these studies include large study populations and several studies have tracked the full residential histories of cases and controls. However, the estimated radon

exposure is a major source of uncertainty, as the performance of the evaluated models cannot be described as more than suboptimal (Andersen et al. 2007, Nikkilä et al. 2020). The proportion of variance explained has been generally close t0‘0.2, with some values up to 0.4. Generally, identification of dwellings with low levels is relatively easy, but for the most informative high concentrations, the predictions are rarely superior to a coin toss.

In addition, several ecological studies have evaluated the relation between average radon levels and incidence of leukemia by region, but they are uninformative due to very large variation in radon levels even in small geographical areas. This means that area-based measures of radon levels are not meaningful indicators of exposure at individual level.

Overall, the evidence concerning potential risk of childhood leukemia from residential radon is weak, despite reasonable number of published studies (Laurier et al. 2001, Raaschou-Nielsen 2008, Lu et al. 2020). A meta-analysis has nevertheless reported a small but significant increase in risk (3% per 100Bq/m^3), but the methods were not described very clearly (Moon & Yoo 2021). The studies with lowest risk of bias have not indicated increased risks, but they have limitations. The only signals suggesting elevated risk are from studies with shortcomings in exposure assessment. Ideally, large studies with radon measurements conducted at individual level and performed in radon-prone areas with high exposure levels might increase the certainty of the evidence, but the feasibility of such studies is questionable.

References:

1. Andersen CE, Raaschou-Nielsen O, Andersen HP, et al. Prediction of 222Rn in Danish dwellings using geology and house construction information from central databases. Radiat Prot Dosim 2007;123:83-94
2. Kendall G, Smith TJ. Doses to organs and tissues from radon and its decay products. J Radiol Prot 2002;22:389-406
3. Laurier D, Valenty M, Tirmarche M. Radon exposure and the risk of leukemia. Health Phys 2001;81:272-288
4. Lu Y, Liu L, Chen Q, Wei J, Cao G, Zhang J. Domestic radon exposure and risk of childhood leukemia. J BUON 2020:25:1035-41
5. Moon J, Yoo HK. Residential radon and leukemia. Environ Res 2021;202:111714
6. Nikkilä A, Arvela H, Mehtonen J, Raitanen J, Heinäniemi M, Lohi O, Auvinen A. Predicting residential radon concentrations in Finland. Scand J Work Environ Health 2020;46:278-292
7. Raaschou-Nielsen O. Indoor radon and childhood leukemia. Radiat Prot Dosim 2008;132:175-181

Joachim Schüz

Extremely low-frequency magnetic fields and childhood leukaemia: an overview

International Agency for Research on Cancer (IARC/WHO), Environment and Lifestyle Epidemiology Branch, Lyon, France

Background: In 1979, a case-control study from Denver, USA, by Wertheimer and Leeper, showed a higher risk of leukaemia in children who lived in houses that were closer to wiring configurations assumed to produce higher-than-background domestic extremely low-frequency (ELF) electric and magnetic fields. This finding received a lot of attention and in the following 20 years many similar case-control studies were carried out mainly in Europe and in North America to confirm or refute this finding, accompanied by large-scale measurement surveys to constantly improve exposure assessment. Those studies were synthesized in meta-analyses and systematic reviews, and led to the classification of ELF magnetic fields as possibly carcinogenic to humans by the IARC/WHO Monograph program in 2001. Further studies were conducted, but with little impact on the overall assessment of the evidence.

Results: A positive association between residential exposure to ELF magnetic fields (MF) and childhood leukaemia has been observed in several epidemiological studies in different settings at different points in time; combining those studies in pooled analysis showed summary relative risks of 1.5-2 at daily average exposure levels exceeding 0.3/0.4μT. ELF-MF of elevated levels occur mainly in close vicinity of high-voltage power lines or other major electrical installations, but exceptionally to indoor wiring; electric devices emit higher fields but are used only for short time periods and therefore hardly contribute to the daily average. Despite data from >20 studies, there numbers of highly exposed children remain small, hence, the precision of exposure-response relationships is limited. Methodological shortcomings of the studies hamper a causal interpretation; especially selection bias is a concern. Novel ideas are needed to explain the observed association.

Parental occupational ELF-MF exposures before conception (both parents) or during pregnancy (mother) have also been looked at in several studies, but show overall no association. No association was seen when investigation the ELF electric field.

Conclusion: Recent reviews come to conclusions well compatible with the IARC classification from 20 years ago, namely a possibly causal association, or possibly an artifact. There is no convincing support from mechanistic studies and some impact of bias in the epidemiological studies has been clearly demonstrated. Whether it explains the observed association in its entirety remains an open question. It was estimated that if the association was causal, about 1-2% of childhood leukaemia cases would be attributable to ELF-MF exposure in Western European countries, perhaps even lower in the Nordic countries but slightly higher in Northern America.

Jacqueline Clavel, MD, PhD and Stéphanie Goujon, PhD

The GEOCAP study

Epidemiology of childhood and adolescent cancers research team and National registry of childhood cancer; CRESS, UMRS 1153, Inserm, Université Paris-Cité; Villejuif, France

The GEOCAP study is a permanent nationwide study based on the French National registry of childhood cancers. Since 2002, all the cases are included at diagnosis as well as 5,000 controls per year representative of the same-aged general population. All the addresses at inclusion are geocoded blind to the case or control status. Precision is <50 m for 83% of the addresses, similar for cases and controls. Since 2010 we have started a parallel study on residences at birth, in order to better approach prenatal exposures. Addresses at birth are geocoded for all the cases born from 2010 forward and for yearly samples of 2000 controls representative of French births.

The GEOCAP study is used to investigate environmental exposures that can be assessed by location with respect to geocoded sources (eg: industrial sites, roads, high-voltage power lines, crops), maps (eg: background ionizing radiation, UV radiation, air pollution) or models of exposure around sources (eg: ELF-EMF close to power lines, agricultural use of pesticides, radioactive discharges from nuclear plants). When variations of exposures cannot be detected at a level smaller than municipalities (eg: background ionizing radiation), ecological studies are used in complement.

Because of power considerations, most of our results to date concern leukemias (≈500 cases per year in France) and residence at diagnosis/ inclusion. We will present some of the results, particularly on background ionizing radiation, vicinity of main roads, of very high voltage power lines, and of viticulture areas.

Dan Baaken

Childhood Leukemia Environmental Risk Factors (CLERF) Project

Division of Epidemiological Methodology and Radiation Research, Institute of Medical Biostatistics, Epidemiology and Informatics (IMBEI), University Medical Center Mainz, Mainz, Germany

1. Cancer is the most common cause for disease-related mortality in children in high-income countries
2. Approximately 1,850 children under the age of 15 are diagnosed with cancer in Germany every year
3. Acute lymphoblastic leukemia (ALL) is the most common type of childhood leukemia. More than 80% of ALL cases are classified as B-lineage ALL
4. Regarding the development of ALL in general, it is hypothesized that a first initial genetic alteration occurs in-utero ("first hit", e.g. TEL/AML1 or IKFZ1+), which is followed by further postnatal genetic alterations (second hit), possibly triggered by environmental exposures
5. Beside the established environmental risk factor ionizing radiation, other factors such as infections, pesticides, air pollution, and extremely low frequency magnetic fields (ELF- MF) are discussed
6. ELF-MF can be identified in the vicinity of power transmission lines by the public electricity grid and electric wiring in general
7. The International Agency for Research on Cancer (IARC) classified exposure to ELF-MF as "possibly carcinogenic" to humans (group 2B) 9, which was confirmed by the European Commission's Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR)
8. Despite numerous epidemiological studies that indicated a statistical association of ELF-MF and childhood leukemia 11 12, this association has not been confirmed convincingly in in vivo studies so far
9. Similarly, evidence of a plausible mechanism is also still lacking to date
10. Based on the current body of evidence, it is unclear whether genetic factors are associated with susceptibility to different environmental risk factors, such as the exposure to ELF-MF.

This research project investigated the feasibility of an interdisciplinary study on the joint role of environmental and genetic risk factors for developing B-Cell ALL in children. Such an interdisciplinary study is expected to advance the understanding of the etiology of pediatric B-Cell ALL and could have implications for radiation protection. In this feasibility study it was evaluated whether such an interdisciplinary study could be integrated into an ongoing or starting clinical study on pediatric ALL in Germany as a pilot study.

In the feasibility study, we drafted and obtained approval of a preliminary study protocol and ethics application, and established contact with the clinical ALL studies in Germany – CoALL and ALL-BFM. Furthermore, we analyzed exposure assessment methods in collaboration with national and international experts on ELF-MF, including the development of a questionnaire to retrospectively assess environmental risk factors and the draft of different exposure assessment scenarios for ELF-MF including on-site measurements. Finally, we conducted sample size as well as budget planning.

Results showed that a pilot study in Germany (CLERF-Pilot) on environmental and genetic risk factors on B-Cell ALL in children would be feasible in cooperation with the ALL-BFM study group. For the pilot, we are advising the participation of three institutions. 1) A scientific institute to coordinate, conduct and evaluate CLERF-Pilot, including data analysis and reporting. 2) The ALL-BFM study center for enrollment and recruitment of B-cell ALL patients in clinical trials. Information on routinely collected information in the course of the clinical trials such as patient characteristics, and particular genetic factors will be made available for CLERF-Pilot by the study center. In addition, the ALL-BFM study center would coordinate the questionnaire survey on retrospective exposure and on-site exposure measurements. 3) A measurement institute for on-site exposure measurements of ELF-MF and potentially other factors at the residence of the B-Cell ALL patient. The pilot study should have a total duration of 2.5 years, of which 12 months are planned for the main phase including recruitment of study participants. The total costs of a pilot would be around 430,000 €, including a retrospective exposure assessment via questionnaire, genetic analyses carried out by the ALL-BFM study group, and on-site exposure measurements of ELF-MF. During 12 months of recruiting of patients, approximately 330 cases could be included assuming a participation of 70% for the questionnaire survey. For the evaluation of differential exposure by environmental factors in groups of genetic subtypes, a much higher number of cases is needed to reach sufficient statistical power. For detecting an association of an odds ratio (OR) of 2 for TEL/AML1 and ELF-MF at a power of 70 % 3,000 cases would be required. For the differential association of IKZF1+ with ELF-MF at a power of 70% and an OR of 2, as many as 7,500 subjects need to be included. The number of cases is considerably larger than can be collected during 12 months in a pilot. Nevertheless, the pilot can provide useful information on participation rates for the questionnaire survey and the on-site measurements, as well as on the workflow between the three participating institutions.

The pilot study (CLERF-Pilot) would provide important scientific insights into response rates regarding study participation, into exposure assessment, as well lead to first results on the possible difference of environmental exposure, in particular ELF-MF, in different genetic ALL subgroups. Based on the results of this feasibility study, the conduct of a pilot study is evaluated as promising. However, restricting such a study to Germany would result in a limited number of participants, especially in high exposure groups for factors such as ELF-MF. An international perspective extending the pilot study to the European region could increase the number of cases to include. However, this would require a separate feasibility study.

References:

1. Force LM, Abdollahpour I, Advani SM, et al. The global burden of childhood and adolescent cancer in 2017: an analysis of the Global Burden of Disease Study 2017. The Lancet Oncology 2019;20(9):1211-25. doi: https://doi.org/10.1016/S1470-2045(19)30339-0
2. Johnston WT, Erdmann F, Newton R, et al. Childhood cancer: Estimating regional and global incidence. Cancer Epidemiol 2021;71(Pt B):101662. doi: 10.1016/j.canep.2019.101662 [published Online First: 2020/01/12]
3. Erdmann F, Kaatsch P, Grabow D, et al. German Childhood Cancer Registry- Annual Report 2019 (1980-2018). Institute of Medical Biostatistics, Epidemiology and Informatics (IMBEI) at the University Medical Center of the Johannes Gutenberg University 2020
4. Huang F-L, Liao E-C, Li C-L, et al. Pathogenesis of pediatric B-cell acute lymphoblastic leukemia: Molecular pathways and disease treatments. Oncology letters 2020;20(1):448-54. doi: 10.3892/ol.2020.11583 [published Online First: 2020/05/04]
5. Greaves M. A causal mechanism for childhood acute lymphoblastic leukaemia. Nature Reviews Cancer 2018;18(8):471-84. doi: 10.1038/s41568-018-0015-6
6. Greaves MF. Aetiology of acute leukaemia. Lancet 1997;349(9048):344-9. [published Online First: 1997/02/01]
7. Onyije FM, Olsson A, Baaken D, et al. Environmental Risk Factors for Childhood Acute Lymphoblastic Leukemia: An Umbrella Review. Cancers 2022;14(2):382.
8. Bundesamt für Strahlenschutz. Was sind elektromagnetische Felder? 2018 [Available from: http://www.bfs.de/DE/themen/emf/einfuehrung/einfuehrung_node.html.
9. Iarc Working Group on the Evaluation of Carcinogenic Risks to Humans. Non-ionizing radiation, Part 1: Static and extremely low-frequency (ELF) electric and magnetic fields. IARC monographs on the evaluation of carcinogenic risks to humans 2002;80:1-395.
10. SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks). Potential health effects of exposure to electromagnetic fields (EMF). 2015 doi: 10.2772/75635
11. Ahlbom A, Day N, Feychting M, et al. A pooled analysis of magnetic fields and childhood leukaemia. British Journal Of Cancer 2000;83:692. doi: 10.1054/bjoc.2000.1376
12. Amoon AT, Crespi CM, Ahlbom A, et al. Proximity to overhead power lines and childhood leukaemia: an international pooled analysis. British Journal of Cancer 2018;119(3):364-73. doi: 10.1038/s41416-018-0097-7
13. Campos-Sanchez E, Vicente-Dueñas C, Rodríguez-Hernández G, et al. Novel ETV6-RUNX1 Mouse Model to Study the Role of ELF-MF in Childhood B-Acute Lymphoblastic Leukemia: a Pilot Study. Bioelectromagnetics 2019;40(5):343-53. doi: 10.1002/bem.22193
14. Swanson J, Kheifets L. Biophysical mechanisms: a component in the weight of evidence for health effects of power-frequency electric and magnetic fields. Radiat Res 2006;165(4):470-8. doi: 10.1667/rr3522.1 [published Online First: 2006/04/04]

Felix M. Onyije

Parental occupational exposure to combustion products, metals, silica and asbestos and risk of childhood leukaemia

Environment and Lifestyle Epidemiology Branch, International Agency for Research on Cancer (IARC/WHO), Lyon, France

Background: Leukaemia is the most common cancer diagnosis among children aged 0-14 years. The main subtypes of leukaemia are acute lymphoblastic leukaemia (ALL) which accounts for ~80% of leukaemia and acute myeloid leukaemia (AML) ~17%. Parental occupational exposures around conception (father) or during pregnancy (mother) have been hypothesized as potential predisposing factors for childhood leukaemia. We investigated parental exposure to several known occupational carcinogens and childhood leukaemia risk.

Materials and methods: We conducted a pooled analysis using case-control data included in the Childhood Cancer and Leukemia International Consortium (CLIC) from France, Germany, Greece and Italy (3362 childhood leukemia cases and 6268 controls). Parental occupational exposures to polycyclic aromatic hydrocarbons (PAH), diesel engine exhaust (DEE), chromium, nickel, crystalline silica, and asbestos were assessed by a general population job-exposure matrix (DOM-JEM). We estimated odd ratios (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression models for all childhood leukaemia combined, by leukaemia type (ALL and AML) and by ALL subtype (B-lineage and T-lineage).

Results: We found an association between high paternal occupational exposure to crystalline silica and childhood ALL (OR 2.20, CI 1.60-3.01) with increasing trend from no exposure to high exposure (P = <0.001), and also for AML (OR 2.03, CI 1.04-3.97; P for trend = 0.008). ORs were similar for B- and T-lineage ALL. For ALL, ORs were also slightly elevated with wide confidence intervals for high paternal occupational exposure to chromium (OR 1.23, CI 0.77-1.96), and DEE (OR 1.21, CI 0.82-1.77). No associations were observed for paternal exposures to nickel, PAH and asbestos. For maternal occupational exposure we found several slightly elevated odds ratios but mostly with very wide confidence intervals due to low numbers of exposed mothers.

Conclusion: This is a first study suggesting an association between fathers' occupational exposure to crystalline silica and an increased risk of childhood leukaemia in their offspring. As this association was driven by certain occupations (field crop farmers and miners) where other potentially relevant exposures like pesticides and radon may also occur, more research is needed to confirm our findings of an association with silica, and if so, mechanistic studies to understand the pathways.

Phung Tran

Overview of EPRI Research on Powerlines, Plant Nurseries, and Childhood Leukemia

Electric Power Research Institute (EPRI), Palo Alto, California, USA

Background: An overview of two published studies related to powerlines, plant nurseries, and childhood leukemia will be presented. The two studies are noted below:

1. Andrew Nguyen, Catherine M Crespi, Ximena Vergara, Nicholas Chun, Leeka Kheifets, 2021. Residential proximity to plant nurseries and risk of childhood leukemia. Environmental Research. https://doi.org/10.1016/j.envres.2021.111388 [doi.org]

2. A.Nguyen, C.M.Crespi, X.Vergara, L.Kheifets. 2022. Commercial outdoor plant nurseries as a confounder for electromagnetic fields and childhood leukemia risk. Environmental Research https://doi.org/10.1016/j.envres.2022.113446

These studies were partly funded by EPRI as part of a series of research devoted to understanding other potential alternative causes for childhood leukemia that may be associated with powerlines. It has been observed that pesticides are a potential risk factor for childhood leukemia. Pesticides and herbicides are often used in plant nurseries that could be part of powerline easements. Additionally, a utility's vegetation management program for the powerline easements may also include the use of pesticides/herbicides.

Materials and methods: A large statewide, record-based case-control study of childhood leukemia in California, which includes 5788 cases and an equal number of controls was used in both published studies. Pesticide, powerline, and magnetic field exposure assessment utilized models that incorporated geographical information systems, aerial satellite images, site visits and other historical information.

Results:

1st study results: Overall, the results supported an increased childhood leukemia risk only for birth residences very close to nurseries. For birth residences less than 75 m from plant nurseries, we found an increased risk of childhood leukemia (odds ratio (OR) 2.40, 95% confidence interval (CI) 0.99-5.82) that was stronger for acute lymphocytic leukemia (OR 3.09, 95% CI 1.14-8.34).

2nd study results: The relationship for calculated fields with childhood leukemia (odds ratio (OR) 1.51, 95% confidence interval (CI) 0.70–3.23) slightly attenuated when controlling for nursery proximity (OR 1.43, 95% CI 0.65–3.16) or restricting analysis to subjects living far (>300 m) from nurseries (OR 1.43, 95% CI 0.79–2.60). A similar association pattern was observed between distance to high-voltage powerlines and childhood leukemia.

The association between nursery proximity and childhood leukemia was unchanged or only slightly attenuated when controlling for calculated fields or powerline distance; ORs remained above 2 when excluding subjects with high calculated fields or close powerline proximity (OR 2.16, 95% CI 0.82–5.67 and OR 2.15, 95% CI 0.82–5.64, respectively). The observed relationships were robust to different time periods, reference categories, and cut points.

Conclusion: Overall, the findings did not support proximity to plant nurseries as an explanation for the MF and childhood leukemia association, however the authors were not able to fully assess possible confounding due to small numbers. The findings suggest that close proximity to plant nurseries is an independent risk factor for childhood leukemia. Further research on the specific pesticide types applied at plant nurseries and childhood leukemia should be performed, as plant nurseries are potential sources of chronic pesticide exposure.

Christina-Evmorfia Kampitsi

Mode of delivery and childhood leukemia risk— a Swedish population-based cohort study

Unit of Epidemiology, Institute of Environmental Medicine, Karolinska Institutet, Sweden

Background: Worldwide cesarean section (CS) rates continue to rise beyond medically warranted thresholds, despite the link to several long-term immune-related adverse outcomes. Previous research on delivery via CS and childhood leukemia is conflicting; where associations emerged, they pointed towards an increased acute lymphoblastic leukemia (ALL) risk. Additionally, studies distinguishing between planned and unplanned CS found increased risks only for the former. As maternal and pregnancy conditions predisposing to birthing difficulties might confound such an association, we aimed to elucidate the relationship between mode of delivery and leukemia in Swedish children.

Materials and methods: This population-based cohort study included children born in Sweden and registered in the National Medical Birth Register (MBR), who had information about mode of delivery (n=2,369,804). Childhood leukemia diagnoses were retrieved from the National Cancer Register and the Swedish Childhood Cancer Registry, whereas delivery mode, maternal and pregnancy conditions, and perinatal factors were retrieved from the MBR; these nationwide population and health data registers were linked through the unique personal identity number assigned to all Swedish residents. The association between mode of delivery and childhood leukemia and subtypes (<20 years at diagnosis) was evaluated using Cox proportional hazards regression models, adjusting for offspring sex, birth decade, birth weight by gestational age, region of residence at birth, and maternal age, education, preeclampsia, and diabetes.

Results: We observed an increased risk of leukemia among children delivered via planned CS (HR=1.16, 95% CI 0.93–1.44), driven by ALL (HR=1.23, 95% CI 0.97–1.56) and specifically b-cell precursor ALL (HR=1.31, 95% CI 1.02–1.70). The associations persisted after adjustment for maternal and pregnancy conditions, as well as offspring perinatal factors (HR for leukemia=1.14, 95% CI 0.91–1.42; HR for ALL=1.21, 95% CI 0.95–1.54; HR for b-cell ALL=1.28, 95% CI 0.99–1.65). Delivery via unplanned CS was not associated with increased risk of childhood leukemia.

Conclusion: Children delivered via planned, rather than unplanned, CS have an increased risk of b-cell precursor ALL, irrespective of maternal conditions that may increase the likelihood of a planned CS. It stands to reason that other biological mechanisms may underlie the relationship between mode of delivery and childhood ALL. These mechanisms might include a lack of exposure to maternal vaginal microbiota, or decreased levels of stress hormones at birth. Regardless, the results of this study support reconsidering non-medically warranted CS.

Maike Wellbrock

28-year incidence and time trends of childhood leukaemia in former East Germany compared to West Germany after German reunification: A study from the German Childhood Cancer Registry

Division of Childhood Cancer Epidemiology, Institute for Medical Biostatistics, Epidemiology and Informatics (IMBEI), University Medical Center of the Johannes Gutenberg University Mainz, Germany

Background: The aetiology of childhood leukaemia is largely unknown. Analyses of geographical differences may enhance aetiologic insights. The reunification of Germany in 1990 provides a unique opportunity to evaluate incidence patterns and time trends in two merging countries with substantial lifestyle, social and socioeconomic differences.

Materials and methods: For this descriptive study, we identified all children diagnosed with a lymphoid leukaemia (LL) or acute myeloid leukaemia (AML) before the age of 15 years between 1991 and 2018 using the German Childhood Cancer Registry (N=14,922), and evaluated the incidence pattern and temporal trends in former East Germany compared to West Germany by subtype, sex and age at diagnosis.

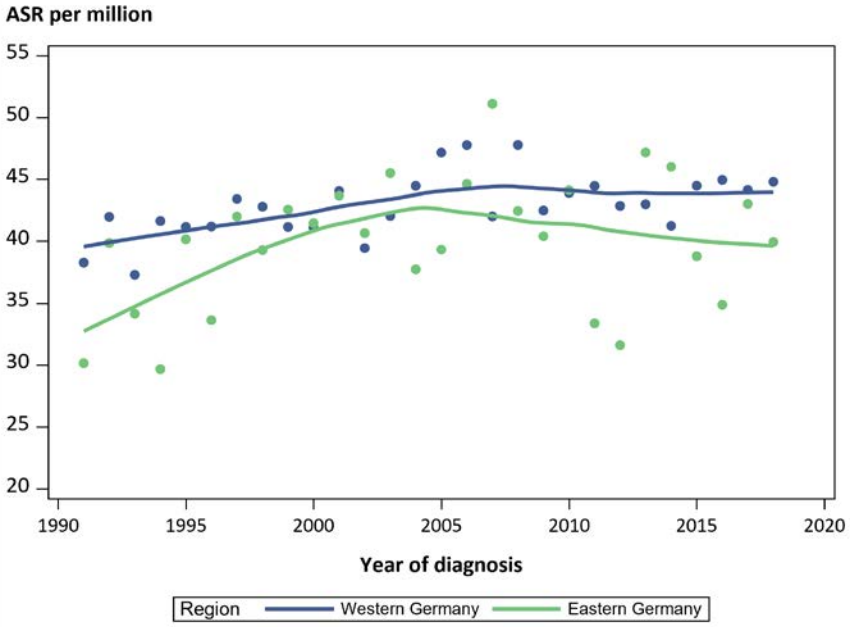


Figure1: Age-standardised incidence rates of lymphoid leukaemia in Eastern and Western Germany over time

Results: Incidence rates of LL were substantially lower (around 20%) in Eastern Germany compared to Western Germany at the time of reunification. This was followed by a remarkable increase in Eastern Germany across both sexes and age groups until around 2000, when incidence rates reached the same levels as those in Western German federal states. Thereafter, incidence rates remained rather stable with some indications of a slightly decreasing tendency in both Eastern and Western Germany (estimated annual percentage changes (EAPC) 2005-2018: East Germany =-0.8%; West Germany = -0.4%), driven by the 0- to 4-year olds. Overall, AML incidence rates were stable over time in Western Germany, while EAPC for Eastern Germany indicated an increasing tendency (EAPC 1991-2018 = 1.3%) driven by the older children, mostly during the early 2000s and in most recent years.

Conclusion: The underlying mechanisms driving the childhood leukaemia rates remain inconclusive. Linkage studies including individual and clinical data would be valuable in evaluating the impact of a population's social, socioeconomic and lifestyle changes on the risk of childhood leukaemia and disease aetiology overall.

Shai Izraeli

Role of signaling through the TSLP/IL7R in initiation and propagation of acute lymphoblastic leukemia

Schneider Children's Medical Center and Tel Aviv University Israel

We have previously discovered a subtype of B B-cell precursor acute lymphoblastic leukemia (BCP-ALL) that is characterized by aberrant expression of the TSLP receptor comprised from CRLF2 and IL7RA coupled with activating mutations either in the receptors themselves or downstream in JAK-STAT pathway. While originally discovered in children with Down Syndrome, it was subsequently shown to cause 5-10% of childhood ALL and to be associated with bad prognosis. We have recently shown in primary human hematopoietic progenitors that that constitutive activation of IL7RA can initiate preleukemia in primary human hematopoietic progenitors and cooperates with CDKN2A silencing in progression into BCP-ALL. This is the first time of mechanistic demonstration that an activation of a signaling pathway has a role in initiation of human ALL from human hematopoietic progenitors. Our studies also suggest that targeting the receptor combined with targeting the proteins regulated by CDKN2A may be a relevant therapeutic approach for these poor prognosis BCP-ALLs.

3.2.6 Session 4: Inflammation and the Bone Marrow Microenvironment

Salvatore Nicola Bertuccio

Early hematopoietic stages of pediatric acute megakaryoblastic leukemia

Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna, Italy. IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy

Non Down syndrome acute megakaryoblastic leukemia (Non-DS-AMKL) accounts 4-15% of pediatric acute myeloid leukemia (AML). A third of non-DS-AMKL harbors particular fusion genes: ETO2-GLIS2, NUP98-KDM5A, KMT2A-MLLT3 (as known as MLL-AF9). The presence of these fusion genes predicts a very poor prognosis and high risk of relapse.

While KMT2A-MLLT3 (as known as MLL-AF9) and NUP98-KDM5A fusion genes share the upregulation of the HOXA genes family, thus suggesting a similar mechanism of leukemic activation, gene expression analysis revealed that ETO2-GLIS2 display a distinct transcriptome and peculiar upregulated pathways.

Currently a high-fidelity preclinical model elucidating the ontogenesis and reproducing the disease cellular and molecular features is absent. Thus, there is an urgent need to increase the biological knowledge of non-DS-AMKL as well as to have a high-fidelity preclinical model suitable to test new innovative target therapies.

It has been demonstrated that pediatric non-DS-AMKL are diagnosed at a significantly younger age compared with patients with other AML. Moreover, recent research studies have demonstrated that specific hematopoietic developmental stages or identities are presumably more permissive to transformation than others. In particular, fetal hematopoiesis is more susceptible to AMKL transformation. Indeed, during embryonic development, at least three different ways of hematopoiesis can be distinguished: extraembryonic hematopoiesis derived from yolk sac giving primitive and EMP (Erythro-myeloid progenitors) followed by intra-embryonic definitive program giving rise to hematopoietic stem cells and to all the blood cell lineages found in the adult. Progenitors from each program move to the fetal liver to complete their maturation. Recent studies confirmed that in the fetal liver different hematopoietic precursor reside and there are two distinct populations of megakaryocyte-erythroid-mast progenitor cells, one of which is a highly cycling population, suggesting a greater susceptibility to malignant transformation.

Our hypothesis is that hematopoietic precursors derived from different ways of hematopoietic development display different susceptibility to AMKL transformation. Given the recent knowledge on mouse model and recent sequencing data in the fetal liver's hematopoietic progenitors we hypothesize that extra-embryonic hematopoiesis, due to its properties in term of self-renewal, could contribute significantly to AMKL transformation.

We exploited differentiation into the hematopoietic lineage of induced pluripotent stem cells engineered to express ETO2-GLIS2 and NUP98-KDM5A. As compared to controls, ETO2-GLIS2 expression induced an increased proportion of CD41+42+ megakaryocytes at several differentiation timepoints and generated a CD41low42low population that is absent in controls. In addition, expression of ETO2-GLIS2 and NUP98-KDM5A enhanced proliferation and self-renewal capacities in methylcellulose assays. Compared to wild-type CD41+42+ profiles, ETO2-GLIS2-expressing CD41+42+ profiles were enriched for several ETO2-GLIS2 AMKL patients blasts signatures and an ETO2-GLIS2-dependent enhancer-associated genes signature. In conclusion, this human ETO2-GLIS2 iPSC model recapitulates differentiation alterations, increased self-renewal and transcriptional signatures observed in human AMKL and should therefore represent an interesting platform to perform future molecular and preclinical investigations.

Mayla Bertagna

ETV6::RUNX1 pre-leukemic niche: role of infections and bone marrow microenvironment in leukemic onset

Centro Ricerca Tettamanti, Pediatrics, University of Milano-Bicocca, Monza, Italy

ETV6::RUNX1 fusion gene, arising in utero from t(12;21), is the most frequent alteration in childhood acute lymphoblastic leukemia (ALL). However, ETV6::RUNX1 is insufficient to overt disease since it generates a clinically silent pre-leukemic clone which persists in the bone marrow (BM) and fails to out-compete normal progenitors. ETV6::RUNX1 pre-leukemic cells show increased susceptibility to transformation following additional genetic insults which can trigger leukemia development in 1% of ETV6::RUNX1 cases. Dysregulated inflammatory and immune response to common infection is thought to be the major player in ETV6::RUNX1 malignant transformation, driving the acquisition of secondary mutations. Comprehend pre-leukemic cells localization and crosstalk with BM supportive populations remains a crucial point to understand the mechanisms which sustain pre-leukemic clone survival in the BM. Recently, it has been demonstrated that shifting Sca1-ETV6-RUNX1 transgenic mice from a specific pathogen free facility (SPF) to a conventional facility (CF) can lead to B-ALL onset in 8% of the cases (Guillermo Rodríguez-Hernandez et al. 2017).

Taking advantage of Sca1-ETV6-RUNX1 transgenic model, we dissected the events preceding leukemia onset and evaluated pre-leukemic cells' behavior in infective conditions. Notably, we did not find any significant difference between ETV6::RUNX1 and WT cells in SPF, but we found that common pathogens induced HSPCs mobilization in the PB of Sca1-ETV6-RUNX1 and their subsequent accumulation, suggesting an aberrant response to infective stimuli of pre-leukemic cells compared to their normal counterpart. Alteration in frequency and distribution led us to investigate the cytokines profile of pre-leukemic mice to identify possible molecules involved in retention and mobilization of HPSCs. Among the cytokines analyzed we found that common pathogens induced an upregulation, in the BM, of M-CSF and IL-4 in Sca1-ETV6-RUNX1 pre-leukemic mice. The functional effect of both cytokines in pre-leukemic phase alteration still needs to be elucidated and further studies are necessary to understand the contribution of infections in pre-leukemic phase, a crucial event to lead overt leukemia.

Jill McKay

Exploring a potential mechanistic role of CpG-specific methylation in the relationship between environmental exposures and childhood acute lymphoblastic leukaemia

Faculty of Health and Life Sciences, Department of Applied Sciences, Northumbria University, UK

Background: The aetiology of childhood acute lymphoblastic leukaemia (ALL), the most common form of childhood cancer, remains unclear. Whilst genetic aberrations have been suggested to be initiating events, these mutations alone are not sufficient for disease onset and additional spontaneous or environmentally induced factors likely play a role in leukaemia development. Epigenetic alteration, such as changes in DNA methylation, plays a key role in human health. Our previous gene-level analysis provided evidence suggesting DNA methylation may be a mediating mechanism through which some environmental factors may contribute to ALL manifestation [1]. Here we have used our previously established, meet in the middle approach, to perform a more in depth CpG-level analysis to investigate DNA methylation as a mediating mechanism between potential risk exposures and ALL.

Materials and methods: We utilised data from our previously published epigenome wide association studies and from published meta-analysis to identify differentially methylated CpGs (DMC's) associated with environmental risk factors related to ALL [1-3]. We selected data from the most comprehensive study to date measuring DNA methylation in ALL, where DMC's conserved across all the ALL subtypes were termed constitutive CpGs [4]. DMCs associated with constitutive ALL were integrated with those DMCs with altered methylation associated with risk exposures to determine overlapping DMCs. Hypergeometric tests were used to assess the probability of relationships between exposure-associated and ALL-associated methylation for any overlapping methylation change, and also when considering the directionality of methylation. Where exposures are hypothesised to increase ALL risk (i.e. maternal smoking, radiation and alcohol), we hypothesise observing the same directionality for exposure-related methylation and methylation in ALL. However, where exposures are hypothesised to be protective (i.e. maternal folate status, day care attendance, reported cold) it is more appropriate to assess directionally opposing methylation changes.

Results: In support of our previously performed gene-level analysis, this more sensitive CpG analysis reinforced earlier findings indicative of significant overlap between radiation and alcohol exposure-related methylation and methylation changes observed in ALL itself, and lack of associations for methylation associated with maternal folate supplementation and coffee consumption during pregnancy. Using data from a meta-analysis assessing the relationship between maternal plasma folate and DNA methylation, our CpG-level approach corroborated previous gene-level findings of a significant number of CpGs with directional overlap in response to maternal plasma folate and

in ALL itself. Previously, sugary caffeinated drinks intake during pregnancy was observed to have significant overlapping gene methylation, however, overlaps for CpG methylation were not significant. For smoking related methylation, previous findings at the gene-level suggested significant overlap with ALL methylation, but not for concordance of directionality, whereas at CpG-level neither overall nor concordant methylation was found to be significant. Where previously gene-level methylation associated with day care attendance had shown a significant overlap with ALL methylation, there were no CpGs found in common, suggesting that different gene regions have altered methylation in response to the exposure than in disease. Conversely, for reported colds, where overlapping gene methylation was previously found to be non-significant between exposure and ALL, the level of overlapping CpG methylation was found to be significant.

Conclusion: Following our previous study of exposure-associated and ALL-associated methylation at gene-level, using a more sensitive CpG-level analysis, we corroborated earlier findings suggesting that DNA methylation associated with maternal radiation exposure, alcohol intake and plasma folate environmental exposures is also present in overt disease at a rate that is higher than expected by chance, therefore these exposures may contribute to disease aetiology through this mechanism. We suggest that our updated CpG-level analysis may be more robust due to the increased sensitively to pinpoint the exact genomic position in which methylation has been altered, in comparison with our previous gene-level approach.

Acknowledgements: We would like to thank Dr Jessica Nordlund, Uppsala University, for providing the ALL subtype data used in this analysis.

References:

1. Timms, J.A., et al., Exploring a potential mechanistic role of DNA methylation in the relationship between in utero and post-natal environmental exposures and risk of childhood acute lymphoblastic leukaemia. Int J Cancer, 2019. 145(11): p. 2933-2943.
2. Richmond, R.C. and B.R. Joubert, Contrasting the effects of intra-uterine smoking and one-carbon micronutrient exposures on offspring DNA methylation. Epigenomics, 2017. 9(3): p. 351- 367.
3. Joubert, B.R., et al., Maternal plasma folate impacts differential DNA methylation in an epigenome-wide meta-analysis of newborns. Nat Commun, 2016. 7: p. 10577.
4. Nordlund, J., et al., Genome-wide signatures of differential DNA methylation.

Jessica Saville

Exploring the potential role of environmentally-associated DNA methylation to contribute to risk of different subtypes of childhood acute lymphoblastic leukaemia

Faculty of Health and Life Sciences, Department of Applied Sciences, Northumbria University, UK

Background: The most common form of childhood cancer, acute lymphoblastic leukaemia (ALL), is a heterogenous disease with unclear aetiology. Various genetic aberrations, such as chromosomal translocations, have been retrospectively detected at birth and are suggested to be initiating events in disease development. However, these mutations alone are not sufficient for disease progression and additional spontaneous or environmentally induced factors or 'hits', such as epigenetic modifications, are required. Previously we provided evidence to suggest that DNA methylation may be a mediating mechanism through which some environmental factors may contribute to ALL manifestation [1]. ALL can be categorised into cytogenetic subtypes based on the leukaemia-initiating chromosomal alterations they possess. Due to the differing genetic profiles of the subtypes, it is plausible that different exposures may pose differing levels of risk for each given subtype. Here we have employed our previously established, meet in the middle approach, to perform CpG-based analysis to investigate DNA methylation as a mediating mechanism between potential risk exposures and specific ALL-subtypes.

Materials and methods: Differentially methylated CpGs (DMC's) associated with ALL environmental risk factors were identified using previously published epigenome wide association studies and meta-analysis [1-3]. We then selected data from the most comprehensive study to date, measuring DNA methylation in ALL, where analysis of 10 cytogenetic subtypes were considered [4]. DMCs associated with cytogenetic subtypes were integrated with those DMCs with altered methylation associated with risk exposures. Hypergeometric tests were used to assess the probability of relationships between exposure-associated and ALL-associated methylation for any overlapping methylation change, and also when considering the directionality of methylation. Where exposures are hypothesised to increase risk (i.e. maternal smoking, radiation and alcohol), we hypothesise observing the same directionality for exposure related methylation and methylation in ALL. However, where exposures are hypothesised to be protective (i.e. maternal folate status, day care attendance, reported cold) it is more appropriate to assess opposing methylation changes.

Results: Significant overlapping methylation was observed across multiple subtypes with both maternal radiation and alcohol intake. However, taking concordance for directionality of methylation change into account, significant overlapping methylation was only observed in MLL with radiation exposure and T-ALL and ETV6/RUNX1 with maternal alcohol intake. For methylation associated with reported colds at 6 months of age, only the MLL subtype was observed to have statistically significant overlapping methylation, where all CpGs

were observed to be discordant for the direction of methylation change, which would be the anticipated observation for exposures considered protective. Methylation change associated with day care attendance significantly overlapped with methylation patterns associated with 6 of the 10 subtypes examined, however the relationships were observed for concordant methylation. Methylation associated with maternal plasma folate significantly overlapped with methylation patterns across all 10 subtypes, with 8 of 10 of these relationships remaining significant after taking into account the direction of methylation change i.e. methylation being discordant, which would be anticipated since maternal folate exposure is protective. Methylation associated with maternal smoking significantly overlapped with methylation across all 10 subtypes, however there was no significant overlaps when taking into account directionality of methylation change. There was no significant overlap between any subtype specific methylation and methylation changes associated with maternal sugary caffeinated drinks intake or coffee intake.

Conclusion: Whilst the potential influence of maternal folate exposure during pregnancy on methylation patterns that may contribute to ALL appears to be fairly consistent across most subtypes, the epigenetic effect of other exposures may be more likely to contribute to the development of specific subtypes. This analysis is therefore useful in understanding which risk factors may contribute to specific subtypes of leukaemia and those which more generally influence ALL risk. Such knowledge may be useful to influence public health policy to aid and tailor prevention strategies.

Acknowledgements: We would like to thank Dr Jessica Nordlund, Uppsala University, for providing the ALL subtype data used in this analysis.

References:

1. Timms, J.A., et al., Exploring a potential mechanistic role of DNA methylation in the relationship between in utero and post-natal environmental exposures and risk of childhood acute lymphoblastic leukaemia. Int J Cancer, 2019. 145(11): p. 2933-2943.
2. Richmond, R.C. and B.R. Joubert, Contrasting the effects of intra-uterine smoking and one-carbon micronutrient exposures on offspring DNA methylation. Epigenomics, 2017. 9(3): p. 351-367.
3. Joubert, B.R., et al., Maternal plasma folate impacts differential DNA methylation in an epigenome-wide meta-analysis of newborns. Nat Commun, 2016. 7: p. 10577.
4. Nordlund, J., et al., Genome-wide signatures of differential DNA methylation in pediatric acute lymphoblastic leukemia. Genome Biol, 2013. 14(9): p. r105.

Erin Marcotte

Breakpoint-agnostic screening for childhood leukemia

Department of Pediatrics, University of Minnesota, USA

Background: Childhood acute lymphoblastic leukemia (ALL) is a heterogeneous genetic disease; a t(12;21) translocation that creates an ETV6/RUNX1 gene fusion is the most common genetic event, present in approximately 25% of cases. Retrospective studies have identified this signature translocation in the newborn dried blood spots (DBS) of patients with leukemia, and it is estimated that at least 2% of healthy newborns have leukemia translocations at birth. Our hypothesis is that these rare somatically altered cells define a subset of newborns with pre-leukemia, defined as the presence of leukemia-specific translocation in the absence of overt disease. Translating this knowledge to epidemiologic investigation and clinical impact requires a method that can detect individually unique translocations in DBS.

Materials and methods: We developed a novel RNA method that can be applied to newborn DBS: Breakpoint Agnostic Translocation Screening (BATS). Our method uses reverse transcriptase droplet digital PCR (RT-ddPCR) to detect RNA that is specifically generated from a translocation event. The Reh leukemia cell line, which is ETV6/RUNX1 translocation-positive, served as positive control. Amplified DNA from positive reactions was extracted using chloroform and Sanger sequenced. Cell mixing experiments were used to establish limit of detection. A range of 10-10,000 Reh translocation positive cells were mixed with 10,000 translocation negative cells (the SupB-15 leukemia cell line). Given the degraded quality of RNA from the DBS, we also evaluated the impact of RIN score on BATS performance. RNA from the Reh:SupB15 cell mixtures was degraded to a RIN level of 4-5 using heat (75C for 20 minutes) or extended exposure to room temperature (3 days).

Results: Dilution curve experiments using a range of positive control input (10- 10,000 cells) demonstrated linearity of the assay (r2=0.9996). Sequencing products aligned with the expected translocation sequence. This experiment demonstrated our ability to detect the translocation when present at a 1:1000 dilution, which is in the expected range of cells in both children who are later diagnosed ALL and healthy newborns. Lowering the quality of RNA closer to that expected in our newborn DBS samples, we were still able to positively detect the ETV6/RUNX1 translocation at a cell dilution of 1:1000. Across the cell dilution experiments, BATS sensitivity was 88% in identifying presence of the ETV6/RUNX1 translocation down to a frequency of 1:1000. Our preliminary data using BATS and unselected newborn DBS suggests the population prevalence at birth may be as high as 15%.

Conclusion: We have developed a method to detect ETV6/RUNX1 translocations in newborn dried blood spots. In future work we will (1) identify factors associated with pre-leukemia at birth, and (2) characterize expansion/persistence of pre-leukemia in early childhood. Our long-term goal is to reduce the burden of ALL, by identifying high risk populations with pre-leukemia and using our recently developed method to monitor ALL risk and progression.

Franziska Auer

Clinical criteria for genetic testing in pediatric oncology show a low specificity and miss every 4th child carrying a cancer predisposition

Technical University of Munich, Germany; School of Medicine; Department of Pediatrics, Munich, Germany

Background: The topic of genetic predisposition in pediatric cancer is gaining increasing interest and attention. Up to 5% of leukemias have been shown to be due to de-novo or inherited cancer predispositions (Zhang et al., 2015). In order to identify children at risk, a thorough analysis for potential germline susceptibilities is essential. In this regard, clinical checklists are the current gold standard to determine whether a child with cancer shows indications for genetic testing. Nevertheless, the efficacy of these tests to reliably detect genetic predisposition in children with cancer is still insufficiently investigated.

Materials and methods: To assess the validity of clinically recognizable signs to identify cancer predisposition, we correlated a state-of-the-art clinical checklist to the corresponding whole exome sequencing analysis in an unselected cohort of 139 child-parent datasets. Thereby, we applied a strict testing to only include autosomal dominant or compound heterozygous cancer-related variants.

Results: Our study reflects a high patient satisfaction with an acceptance rate of >90%. The evaluation of the study cohort using the predefined checklist shows a high indication for cancer predisposition. Overall, genetic counseling was indicated for 36% (n=50) of the children, which was mainly due to the two criteria “tumor diagnosis” and “phenotypic abnormalities”. Exome analysis confirmed the presence of a known cancer predisposition in 15 children (11%), four of whom were clinically unremarkable (checklist negative). Therefore, our data show a low checklist specificity of 68.5%, and a likewise low sensitivity score of 73.3% missing every 4th child with genetic predisposition.

Interestingly, we detected a high correlation between indicated genetic counseling and predisposition in children with myelodysplastic syndrome (MDS). Here, 50% of all MDS patients (5 out of 10) showed a disease-relevant germline mutation. Moreover, the leukemia group (n=33) displayed genetic predisposition in 9% of the cases, with DNA-damage related variants being the most prominent.

Conclusion: Our data highlight the drawbacks of sole clinical evaluation to accurately identify all children at risk and underlines the needs for routine germline sequencing in pediatric leukemias. Identifying germline mutations early on is of extreme importance, since their presence in every cell of the body can lead to an increased intrinsic risk for secondary aberrations and cancer development, particularly when additional stressors, e.g. environmental factors, are taken into account.

Julia Vogt

Characterization of a novel JAK3 variant in a family with a history of leukemia and lymphoma

Technical University of Munich, Germany; School of Medicine; Department of Pediatrics, Munich, Germany

Background: Recent studies report an involvement of germline predispositions in the development of childhood cancer in up to 15% (Byrjalsen et al., 2020), although their actual contribution is believed to be much higher. Being able to identify children at risk, opens up new possibilities in the form of disease prevention rather than treatment. Moreover, predisposing germline variants, particularly in ubiquitously expressed genes, might considerably affect treatment related toxicity. Therefore, individualized genomic patient analyses, in the context of the respective familial background, are essential to elucidate the impact of genetic predisposition.

Methods and Results: Here, we present a family with a strong history of cancer, harboring a novel germline variant in the Janus Kinase 3 (JAK3) gene (p.Y399C). JAK proteins belong to a family of intracellular, non-receptor tyrosine kinases, consisting of the four members JAK1, JAK2, JAK3 and TYK2. They exhibit essential roles as signal transducers downstream of cytokine receptors, by generating docking sites for SH2-domain-containing adaptors and effectors, including the signal transducers and activators of transcription (STAT) proteins. Subsequently, after being phosphorylated by JAK, STAT proteins translocate into the nucleus, where they activate a variety of transcriptional programs corresponding to cell proliferation, differentiation and survival. Thus, acquired dysregulation of the JAK-STAT pathway is a key event in a various cancers, including hematological malignancies.

The here identified JAK3 p.Y399C germline variant was found in a 16 year old girl with EBV-positive Hodgkin lymphoma and was transmitted from the father, who had presented with chronic lymphocytic leukemia (CLL) at the age of 43. Additionally, the father's sister and mother had been reported with CLL and acute leukemia, respectively. Cloning and subsequent in-vitro validation of JAK3 p.Y399C, using an IL-3 dependent murine pro-B cell line (BaF3), confirmed weak constitutive activating proliferative properties of the variant. While the oncogenic potential was not strong enough to achieve long-lasting IL-3 independent growth, signaling analysis confirmed strong pSTAT3 upregulation after inducible JAK3 p.Y399C expression.

Conclusion: Taken together, germline sequencing is a strong tool in pediatric oncology to identify potentially disease-causing variants and our work emphasizes the rising need for validated functional testing strategies.

Katharina Gößling

Virus-specific functional hyperresponsiveness in children with ETV6::RUNX1 positive acute lymphoblastic leukemia (ALL)

University Hospital Duesseldorf, Department of pediatric oncology, hematology and clinical immunology, Duesseldorf, Germany

Background: B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is the most common subtype of childhood leukemia with a peak during early childhood when children are exposed to viral infections. About 5% of all newborns carry a preleukemic clone, but only about 0,2% of them develop BCP-ALL later in life. It has been assumed that a dysregulated, altered immune response underlies the outgrowth of a pre-leukemic clone in infection-triggered B-cell precursor acute lymphoblastic leukemia (BCP-ALL). We investigated whether children with BCP-ALL differ in their cytokine response from healthy, age-matched, non-leukemic children, after immune cells were challenged with viral, fungal or bacterial stimuli.

Materials and Methods: We set up a functional experimental platform with 73 stimulus-cytokine pairs to comprehensively characterize the immune profile of pediatric patients with BCP-ALL with the two most common genetic subtypes, either carrying the ETV6::RUNX1 gene fusion or the high-hyperdiploid karyotype in comparison with an age- and gender-matched healthy control cohort without BCP-ALL and their healthy parents (in total n = 101 individuals). Children were in stable first remission, at least two years after the end of therapy and had a fully reconstituted immune system. Blood was taken of patients and controls in parallel and peripheral blood mononuclear cells (PBMCs) were isolated using a density gradient centrifugation. Additionally, PBMCs of ETV6::RUNX1 positive and negative cord blood cells had been isolated. Cells had been stimulated with various fungal, bacterial and viral pathogens, such as Candida albicans, Staphylococcus aureus, Influenza virus, Respiratory Syncytial virus and toll-like receptor ligands (TLR), such as lipopolysaccharide (TLR4), R848 (TLR7/8) or CpG (TLR9); subsequently cytokines (IFNα, IL-1β, IL-1Ra, IL-10, IL-12p70, IL-17, IFNγ) had been measured in the cell culture supernatant.

Results: Cytokine base line level did not differ between the groups, while the challenge with various infectious antigens resulted in an overall elevated cytokine production of the BCP-ALL patients with ETV6::RUNX1 fusion (n=11) ($p < 0.01$, Mann-Whitney-Wilcoxon test two-sided with Benjamini-Hochberg correction). By deciphering the characteristics of the immune response, a remarkable difference for the anti-viral immune response ($p < 0.01$) was identified, while the anti-bacterial and anti-fungal response did not differ. Further analysis of the cytokine profile of the ETV6::RUNX1 parents compared with an age- and gender-matched healthy control group revealed an overall significantly elevated cytokine response ($p < 0.05$) after stimulation, but the different sub-features, such as the fungal, bacterial and viral immune responses were not different.

Most strikingly, we could identify this pattern specifically for the ETV6::RUNX1 cohort. Comparing the cytokine responses of the patients cured from the high-hyperdiploid BCP-ALL (n=8) with the healthy controls (n=22) did not result in any significant differences. Children carrying a pre-leukemic ETV6::RUNX1-positive clone in their cord blood who remained disease free did not show this specifically elevated anti-viral cytokine pattern.

Conclusion: Our study suggests that an altered pro-inflammatory anti-viral cytokine response pattern in children with ETV6::RUNX1-BCP-ALL is specific to those children, who later developed the BCP-ALL. It is still present years after chemotherapy and possibly contributes to the outgrowth of a pre-leukemic clone, consistently generated in utero during fetal hematopoiesis.

Gregor Reid

Pathogen sensor engagement and host immune responsiveness dictate the outcome of TLR ligand exposure on precursor B-ALL cell populations

Michael Cuccione Childhood Cancer Research Program, BC Children's Hospital Research Institute, Vancouver, BC V5Z 4H4, Canada

Department of Pediatrics, University of British Columbia, Vancouver, BC V5Z 4H4, Canada

Background: Exposure to common infection has long been proposed to play a role in the development of pediatric B cell acute lymphoblastic leukemia (B-ALL). While several epidemiological studies of early-life infection indicate a subsequent increased risk for ALL, protective effects of infection exposure against ALL have also been reported. An infectious agent responsible for pro- or anti-leukemic effects has not been identified and a mechanistic explanation for the seemingly contradictory effects of early-life infection has not been defined. We have previously observed that stimulation via infection-associated toll-like receptor (TLR) ligands can induce expansion (polyI:C) or depletion (e.g. CpG ODN) of preleukemic and leukemic B-ALL cell populations. Here we further investigate the variables that contribute to these outcomes.

Materials and methods: Emu-ret mice, which express a ret fusion protein under the control of the IgH enhancer, possess an abnormally expanded B cell precursor (BCP) population at birth and develop B-ALL characterized by non-random hyperdiploidy (with >90% penetrance) between 8 and 52 weeks of age. Early-occurring preleukemic BCP cells are readily identifiable based on cell surface phenotype (CD19, B220int, CD43int, BP-1hi), enabling simple quantification even at low burden. Preleukemic and leukemic cells from Emu-ret mice, as well as patient-derived B-ALL cells, were treated in vitro with TLR agonists in the presence or absence of immune effector cells, and cell viability assessed after 48 hours. In vitro responses were validated by in vivo delivery of TLR agonists to Emu-ret mice of varying ages, with preleukemic cell burden in organs assessed one week later by flow cytometry.

Results: In our previous studies, the expansion of preleukemic and leukemic cells observed after exposure to polyI:C was in stark contrast to the depletion achieved with other TLR ligands. As polyI:C binds both endosomal (TLR3) and cytoplasmic (RIG-I and MDA-5) receptors, we evaluated these distinct signaling pathways independently. Exposure of purified Emu-ret preleukemic cells to polyA:U (TLR3) or 5'ppp-dsRNA (RIG-I) revealed that the signal driving expansion of the BCP cell population was mediated only via TLR3. A similar pattern of response was observed with leukemia cells from Emu-ret mice and B-ALL patients, revealing that the relevant biologic activity is retained at

distinct disease stages and shared between mouse and human B-ALL. To assess the impact of simultaneously induced immune responses on the potentially pro-leukemic TLR3 signaling, we treated bulk spleen and bone marrow cells from Emu-ret mice of different ages with TLR ligands (Figure 1). CpG ODN consistently reduced the number of viable preleukemic cells in all settings. In contrast, a significant expansion was observed in response to polyA:U in the presence of spleen or bone marrow cells from 1-2 week-old, but not older, Emu-ret mice. PolyI:C induced an intermediate response, consistent with the opposing effects of TLR3 and RIG-I signaling. The same pattern of age- and TLR-dependent changes in preleukemia burden were observed following in vivo treatment of healthy Emuret mice. This finding reveals that the overall outcome of infection-related immune stimulation on B-ALL preleukemia is determined by the balance of multiple, often conflicting, signals that can change with age. To identify whether it is the age of the immune effector cells or of the preleukemic cells that influences outcome, we incubated purified preleukemic cells from 2-week-old Emu-ret mice with immune effector cells from 2- or 4-week-old BALB/c mice. Notably, expansion of the preleukemic cell population was achieved by polyA:U treatment only in the presence of 2-week old immune effector cells; the same stimulation with 4-week old effector cells achieved a significant switch to preleukemic cell depletion.

Conclusion: Our results reveal previously unrecognized pro-leukemic signalling through TLR3 that could contribute to infection-driven progression of B-ALL. Further, we demonstrate that age-dependent host immune responsiveness and the specific pathogen-sensing pathways stimulated combine to determine the outcome of infection-related immune modulation on early-occurring preleukemic cell burden. Our study supports the hypothesis that early life infection can influence pre-leukemia cell fate and provides a potential mechanistic explanation for reports of infection promoting B-ALL progression.

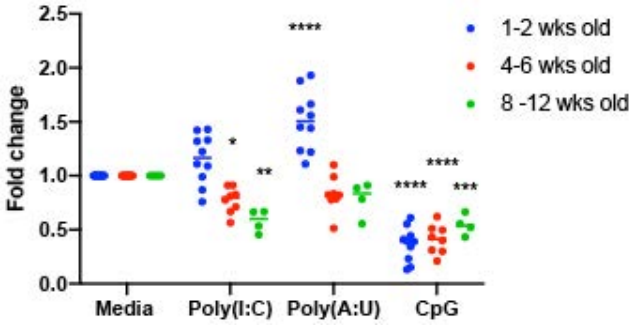


Figure 1: Changes in viable pre-leukemia cell numbers 48hrs after in vitro stimulation of bulk bone marrow cultures from variously aged Emu-ret mice.

3.2.8 Session 5: Genetics and Epigenetics

Giovanni Cazzaniga

Identification, functional characterization and management of genetic variants associated to predisposition to childhood Acute Lymphoblastic Leukemia

Centro Ricerca Tettamanti, Pediatrics, University of Milano-Bicocca, Monza, Italy

Medical Genetics, University of Milano-Bicocca, Monza, Italy

State of the art

Genetic predisposition occurs in 5-10 % of pediatric cancer, including hematological malignancies, but it is still largely uncharacterized. Among genes known to be involved in leukemogenesis, we focused our attention on three subsets: 1) Cohesin genes, to functionally dissect germline variants with a possible role in leukemia predisposition; 2) TP53 in hypodiploid childhood ALL, a clinical manifestation of Li-Fraumeni Syndrome; 3) PAX5, with a new variant identified in a family with recurrence of ALL in Italy.

1. Germline mutations of cohesin genes lead to Cohesinopathies, while somatic mutations are known in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML), as well as solid tumors. The report of three Cornelia de Lange patients (CdLS) affected by acute megakaryoblastic leukemia (AMKL), acute lymphoblastic leukemia (ALL) and MDS, suggested that germline mutations in Cohesins could have a role in predisposition to hematological disorders. Indeed, we demonstrated that germline STAG1 mutation associated to hematological disorders lead to an increased number of Sister Chromatid Exchanges and reduced DNA repair.

2. We analyzed TP53 variants in the Italian cohort of hypodiploid pediatric ALL patients diagnosed between 2000 and 2019 and we identified 19 TP53 variants in 20/40 (50%) hypodiploid ALL patients, with 19/20 being low-hypodiploid ALL cases. Notably, 13 patients out of 20 (65%) were found to carry a germline variant; among them, 9/13 were pathogenic whereas 4/13 were classified as VUS. A family history of cancer at <45 years of age was observed in 5/13 (38%) patients with a germline variant; secondary malignancies were reported in 2 patients with/without an associated family history of cancer. Mutational testing of TP53 in these patients is highly recommended, to ensure a proper genetic counseling for patients with germline mutations and their families, with tailored clinical surveillance, specific attention during familiar donor selection in case of HSCT and the selection of a proper therapeutic regimen.

3. A family with two siblings both affected by BCP-ALL was referred to our attention. Parents were healthy and unrelated; no history of hematological malignancies was reported across the pedigree. Through NGS we showed that both patients shared a novel germline

heterozygous PAX5 variant (c.548delG, p.Gly183AlafsTer84). Interestingly, both patients carried the additional somatic p.Pro80Arg PAX5 variant. The familiar segregation showed a paternal origin of the germline PAX5 variant, which was also found in the DNA sample of the patients' grandmother. Interestingly, the carrier father had a significant reduction on mature IgD+/CD27+ B-cells with a concomitant accumulation of IgD+/CD27- naïve B-cells. In contrast to the previously described cases that carry an inherited missense variant resulting in a modest attenuation of PAX5 activity, our patients present a germline frameshift variant that brings to premature stop of the protein, suggesting a more destructive impact on PAX5 activity. Consent-dependent characterization of additional family members is ongoing to assess the penetrance of leukemia associated to this new variant and possible additional features.

Logan Spector

Lessons from a low incidence population: The Admixture and Risk of Acute Leukemia (ADMIRAL) study

Department of Pediatrics, Division of Epidemiology & Clinical Research,
University of Minnesota, Minneapolis, USA

The risk of B-cell acute lymphoblastic leukemia (B-ALL) is lower in children with substantial African ancestry than in most other populations. There is longstanding debate as to whether this low incidence is due to environment, genetics, or underascertainment. However several lines of evidence have converged which collectively suggest genetics are the major explanation for lower B-ALL incidence among African children and children of the African diaspora. These include: 1) the establishment of gold-standard, population-based cancer registries in sub-Saharan African which confirm low incidence, 2) the similarity of rates of B-ALL in both Africa and African diaspora populations in different settings, and 3) lower incidence of B-ALL among African-American (AA) children in all strata of socioeconomic position in the United States. We therefore established the Admixture and Risk of Acute Leukemia (ADMIRAL) study, which to date has genotyped 641 AA children with B-ALL and 411 AA controls using the Illumina Global Diversity Array. After imputation using the TOPMED server we examined 13,077,899 single nucleotide variants. Local ancestry inference was conducted using RFMix and aggregated to obtain global ancestry estimates, which we compared in cases and controls. In addition we conducted genomewide association analysis (GWAS) and admixture mapping. Mean African ancestry (%AFR) was lower in cases than controls, particularly in the subset of data from a population-based sample of cases (N = 108; %AFR = 72.2) and controls (N = 377; %AFR = 80.2) nested within the birth cohort of Michigan state (two-sample t-test p-value = 5.625e-05). This difference was however not seen in a similarly constituted sample from the California birth cohort, in which mean %AFR was 71.8 in both cases and controls. GWAS confirmed that loci discovered in European children replicate in AA but revealed no new loci. Admixture mapping revealed a suggestive peak on chromosome 15 which contained a number of plausible candidate genes. Preliminary results from the ADMIRAL study suggest that global African ancestry is associated with lower risk of B-ALL, but this difference cannot be explained by currently known common variants. Admixture mapping is a promising approach to gene or variant discovery in this population which should yield new loci as the sample size grows.

Thomas Mercher

Role of ontogeny and transcriptional alterations in pediatric acute megakaryoblastic leukemia

Institut Gustave Roussy, INSERM U1170, Université Paris-Saclay, 94800 Villejuif, France

De novo pediatric acute megakaryoblastic leukemia (AMKL) is a representative subtype of fusion oncogene-driven pediatric myeloid leukemia as this single aggressive morphological subtype presents with a wide variety of fusion oncogenes; including ETO2-GLIS2, NUP98-KDM5A, MLL and HOX fusions. This genetics-based classification has important implications. Mechanistically, it suggests different requirement for oncogenic cooperation in pediatric AMKL. Indeed, some subgroups show recurrent associations of genetic alterations. For example, NUP98-KDM5A or HOX fusions have a remarkable association with RB1 inactivating mutations and MPL activating mutations, respectively. On the other hand, OTT-MAL and ETO2-GLIS2 fusions are rarely associated with known mutations. The genetic heterogeneity of AMKL raises the question of the mechanism of transformation by AMKL oncogenes and their pediatric specificities. Functional insights are emerging from modelling approaches. Mostly from studies in patient-derived cell lines and xenograft amplified cells, molecular analyses of ETO2-GLIS2 showed that strong transcriptional deregulations result, in part, from the interaction with enhancer regulatory regions leading to pleiotropic effects, including an imbalanced activity within the ETO2 transcriptional complexes (e.g. ERG and GATA1) and the control of signaling pathways (e.g. KIT). The establishment of an ETO2-GLIS2 inducible expression murine in vivo model indicated that the aggressiveness and phenotypes in leukemia result from an ontogeny-related differential susceptibility to transformation by fusion oncogenes. Indeed, the most aggressive disease and the megakaryoblastic phenotype is mostly reproduced upon expression of the fusion oncogene in hematopoietic stem cells from fetal liver hematopoietic development as compared to adult cells. The differential susceptibility was associated with both intrinsic and ETO2–GLIS2-induced differences in the activities of key transcription factors, including ERG, SPI1, GATA1, and CEBPA. Ongoing studies aim at obtaining and characterizing transformation models starting from human cells, including human induced pluripotent stem cells and primary human fetal cells. As translational perspectives, the activity of several domains of the fusion is required to maintain the ETO2-GLIS2 gene expression program and the leukemia cell survival and proliferation. However, no current strategy allows to directly target the ETO2-GLIS2 fusion. Using transcriptome data and functional studies, we have obtained evidences that ETO2-GLIS2 induces both cell death and pro-survival processes leading to a dependency on the BCL2 anti-apoptotic family, which can be efficiently targeted in patient-derived xenograft models.

Hélène Cavé

Childhood ALL in the context of RASopathies – What does it tell us about leukaemogenesis?

Genetics department, Hôpital Robert Debré

INSERM U1131, Institut de Recherche Saint-Louis – Université Paris Cité, France

Acute lymphoblastic leukaemia (ALL) is the most common malignant tumor in children and uncovers a constellation of different entities, each of which is considered to be defined by a founding lesion. The founding genetic alterations consist in aneupoidies, translocations and point mutations, which are usually assumed to be initiating events of leukaemogenesis and determine their biology. More than 30 founder lesions are now recognized, the most common being the ETV6-RUNX1 fusion and high hyperdiploidy (Heh) in B-cell precursor (BCP)-ALL. Being mutually exclusive, these entities define ALL subclasses but also shape biological entities with similar gene expression profiles. Yet, these genetic lesions are not sufficient to induce leukaemia and various recurrent mutations involved in classical oncogenic pathway or lymphoid development genes are also found in ALL. Contrary to classifying lesions, these cooperative alterations do not appear to confer a prominent biological signature to ALL. However, nonrandom association between classifying alterations and secondary alterations (e.g. RAS pathway activating mutations in Heh BCP-ALL) suggests a privileged cooperation between some oncogenic alterations, with unique oncogenic trajectories. In addition to somatically acquired mutation, there is increasing evidence that some characteristics of the host genome also contribute to developing ALL.

RASopathies are inherited developmental disorders caused by activating mutations in components of the RAS pathway. Noonan syndrome, the most common RASopathy (about 1/200 births) is the consequence of germline mutations in PTPN11 or more rarely in other components of the RAS/MAPK pathway. Children with NS associate short stature, cardiac defect, lymphoedema and a variable spectrum of developmental disorders. They have been reported to be prone to developing cancer and leukemia, among which ALL.

Cross-referencing a prospective cohort of RASopathy patients with the French childhood cancer registry (coll. J Clavel) allowed us to identify ALL in 6/1176 (0.5%) patients with NS which is above the expected frequency. Strikingly, somatic and germline PTPN11mut ALL shared similar features. All were BCP-ALL and 76% of them had Heh compared to about 30% expected. This non-random association suggests a functional link between PTPN11 and Heh.

More recently, LZTR1, an adapter for Cullin 3 (CUL3) ubiquitin ligase complexes, was identified as a negative regulator promoting RAS proteasomal degradation. LZTR1 germline mutations result in dominant or recessive forms of NS. The report of several cases of ALL in LZTR1-mutated NS patients

prompted us to sequence LZTR1 in a cohort of childhood ALL. A pathogenic germline variant was found in 0.8% patients. This frequency does not appear to be significantly increased compared to the frequency in the general population. However, 70% patients with a germline variant acquired a somatic LZTR1 variant as compared with 1% in other patients, which is in favor of a driver effect of LZTR1 loss. Moreover, Abdel-Wahab's group recently shown that mice reconstituted with Lztr1 KO fetal liver cells developed fatal hematologic malignancies, consistent in about half of cases with an immature B-cell lymphoid malignancy.

These observations highlight the complexity of assessing a causal effect of germline variants on the development of leukaemia. They also raise questions about the role of germline mutations in the oncogenic process.

Like other malignancies, leukaemia evolve as a consequence of the accumulation of mutations in cooperating oncogenic drivers. GWAS as well as analyses of familial ALL cases have identified a variety of susceptibility variants targeting mainly genes known to be targets of somatic alterations as well. The penetrance of germline variants is difficult to assess and probably vary from one to another, but overall appear to be quite low, suggesting that other genetic or environmental events are required for the development of leukaemia. Although pre-existing to the founder alterations, germline variants usually do not shape the biology of the leukaemia but can provide 'guidance', as evidenced by the preferential link between some germline mutations and specific ALL subgroups (e.g. PTPN11 and Heh). Although technically pre-existing the founding lesion, germline variants such as RAS activating ones seem to be functionally equivalent to secondary alterations in the leukaemogenic process, thus highlighting how much germline and somatic alterations are intricated in driving leukemogenesis. To obtain a complete picture, it is therefore essential that both somatic and germline events are studied.

Kim E. Nichols

ETV6 Regulates Hematopoietic Stem Cell Function and Represses TNF During Stress Hematopoiesis

Department of Oncology, St. Jude Children's Research Hospital, Memphis TN

ETS Variant 6 (ETV6) encodes a transcriptional repressor highly expressed in hematopoietic stem and progenitor cells (HSPCs), where it is essential for adult hematopoiesis. Heterozygous germline ETV6 variants are associated with Thrombocytopenia 5 (T5), a genetic condition predisposing to thrombocytopenia and hematologic malignancies. To elucidate how germline ETV6 variants impact the HSPC compartment and contribute to disease, we generated a knock-in mouse harboring Etv6R355X, the murine equivalent to a T5-associated variant ETV6R359X. Frequencies of lineage-sca1+cKit+ (LSK) cells were normal in young Etv6R355X/+ mice but declined significantly as animals were aged to 12-months. Functionally, Etv6R355X/+ bone marrow (BM) cells exhibited reduced colony formation in later rounds of serial replating and Etv6R355X/+ LSK cells exhibited significantly impaired engraftment in serial competitive transplantation. RNA-seq of Etv6R355X/+ LSK cells and/or sort-purified HSPC subsets revealed upregulation of genes associated with an active stem cell phenotype and pro-inflammatory signaling, including increased expression of the cytokine Tnf. CUT&RUN analysis showed that ETV6 binds to the Tnf locus at regions of open chromatin in Etv6+/+ LSK cells and to genes that regulate TNF in normal donor human CD34+ cells. In line with these findings, Etv6R355X/+ HSPCs exhibited increased cell-cycling and increased TNF production post-transplantation. Genetic deletion of Tnf restored the serial replating capacity of Etv6R355X/+ BM cells and related in vivo studies are ongoing. From these studies, we conclude that ETV6 inhibits the expression of Tnf, particularly under conditions of aging and/or hematopoietic stress. The studied heterozygous T5-associated Etv6R355X variant compromises Tnf repression and is associated with impaired HSC function.

Pablo Menéndez

Biological insights into the pathogenesis of aneuploidies in childhood B-ALL

Josep Carreras Leukaemia Research Institute, Department of Biomedicine, School of Medicine, University of Barcelona, Spain

Arndt Borkhardt

Hyperdiploidy: the longest known, most prevalent but still most enigmatic form of acute lymphoblastic leukemia in children

Department for Pediatric Oncology, Hematology and Clinical Immunology, Medical Faculty, Heinrich-Heine-University, Düsseldorf, Germany

Hyperdiploidy is the largest genetic entity B-cell precursor acute lymphoblastic leukemia in children. The diagnostic hallmark of its two variants that will be discussed in detail herein is a chromosome count between 52 and 67, respectively. The classical HD form consists of heterozygous di-, tri-, and tetrasomies, whereas the nonclassical one (usually viewed as “duplicated hyperhaploid”) contains only disomies and tetrasomies. Despite their apparently different clinical behavior, we show that these two sub-forms can in principle be produced by the same chromosomal maldistribution mechanism. Moreover, their respective array, gene expression, and mutation patterns also indicate that they are biologically more similar than hitherto appreciated. Even though in-depth analyses of the genomic intricacies of classical HD leukemias are indispensable for the elucidation of the disease process, the ensuing results play at present surprisingly little role in treatment stratification, a fact that can be attributed to the overall good prognoses and low relapse rates of the concerned patients and, consequently, their excellent treatment outcome. Irrespective of this underutilization, however, the detailed genetic characterization of HD leukemias may, especially in planned treatment reduction trials, eventually become important for further treatment stratification, patient management, and the clinical elucidation of outcome data. It should therefore become an integral part of all upcoming treatment studies.