Ressortforschungsberichte zum Strahlenschutz

5th International Workshop on the Causes of Childhood Leukemia - Vorhaben 3616I02233

Auftragnehmer:
Valentum Kommunikation gmbH

Das Vorhaben wurde mit Mitteln des Bundesministeriums für Umwelt, Naturschutz, Bau und Reaktorsicherheit (BMUB) und im Auftrag des Bundesamtes für Strahlenschutz (BfS) durchgeführt.

BfS-RESFOR-134/18

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urn:nbn:de:0221-2018050314817

Salzgitter, Mai 2018

Abschlussbericht

Vorhaben: 3616I02233

5th International Workshop on the Causes of Childhood Leukemia

Auftragnehmer:
Valentum Kommunikation gmbH
Bischof-von-Henle Straße 2b
93051 Regensburg

Laufzeit des Vorhabens: Juli bis Dezember 2016

Anlagen:
Veranstaltungsprogramm
Abstractband

1. Zielsetzung

Sowohl in der Umgebung von Kernkraftwerken als auch in der Nähe von niederfrequenten Magnetfeldern wird seit 2008 ein Trend für das Auftreten von Leukämie bei Kindern beobachtet. Es wird angenommen, dass diese beiden Faktoren, neben weiteren Umwelteinflüssen wie Infektionen oder Pestiziden, hierfür verantwortlich sind. Für beide Phänomene sind bisher keine zufriedenstellenden wissenschaftlich fundierten Erklärungen gefunden worden. Eine weitere Untersuchung auf Basis des derzeitigen Wissens über biologische Auswirkungen ionisierender und nicht–ionisierender Strahlung ist daher notwendig.
Zu diesem Zweck veranstaltete das Bundesamt für Strahlenschutz bereits zum fünften Mal einen internationalen Workshop mit Expertinnen und Experten aus der ganzen Welt.

Im Rahmen der 2,5–tägigen Veranstaltung wurde der aktuelle Forschungsstand zum Thema Ursachenforschung von Leukämie bei Kindern aus den unterschiedlichen Perspektiven der Forschungsfelder umfassend beleuchtet und durch neuen Input erweitert.

1.1 Einzelzielsetzung

Unmittelbares Ziel der Veranstaltung war es, einen Überblick über das derzeitige Wissen zum Thema Ursachenforschung der Leukämie bei Kindern zu erarbeiten und sich im vielfältig besetzten Plenum, darunter Kinderärzte, Strahlenschutz–Experten, Vertreter der Epidemiologie und Genetik, darüber auszutauschen.
Der Workshop wurde in sechs inhaltlich aufeinander abgestimmte Sessions eingeteilt. Internationale Rednerinnen und Redner aus verschiedenen Fachbereichen wurden nach München eingeladen, um folgende Fragestellungen zu beleuchten: Was sind die wichtigsten Themen in ihrem Forschungsbereich in Bezug auf die Ursachen von Leukämie bei Kindern? Mit welchem Ansatz könnte man die derzeitigen offenen Fragen lösen? Wird im eigenen Land eine wachsende Anzahl von Betroffenen festgestellt?

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 (Valentum Kommunikation GmbH).

1.2 Voraussetzungen für den Workshop

Der Workshop fand im Tagungssaal des Salesianums, Don Bosco in München Haidhausen–Au statt. Der Veranstaltungsort ist sowohl vom Flughafen als auch vom Hauptbahnhof mit einer direkten S–Bahn Verbindung innerhalb von ca. 30 Minuten zu erreichen. Das Tagungscatering wurde durch den Veranstaltungsort geleistet.

1.3 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
- Erstellung einer Website und eines Flyers
- Erstellung des Tagungsprogramms
- Einladung der Redner

- Einladung der Gäste
- Strukturierung des Programms
- Laufende Organisation und Vorbereitung des Workshops:
    - Registrierung der Teilnehmerinnen und Teilnehmer
    - Gestaltung und Druck der Namensschilder
    - Gestaltung und Druck des Programmhefts sowie weiterer relevanter Dokumente
    - Betreuung der Rednerinnen und Redner sowie Gäste
    - Koordination der Unterkunft der Rednerinnen und Redner
- Erstellung eines Abstractbandes

AP 2: Durchführung des Workshops

- Programmablauf siehe Anhang
- Koordination der Dienstleister vor Ort (Technik, Blumen, Catering, Social Dinner)
- Betreuung der Rednerinnen und Redner sowie Gäste

AP 3: Nachbereitung

- Bereitsstellung der fotografischen Dokumentation der Veranstaltung
- Reisekostenabrechnungen
- Abschluss

1.4 Organisatoren

Die 2,5-tägige Veranstaltung „5th International Workshop on the Causes of Childhood Leukemia" wurde vom Bundesamt für Strahlenschutz (Ingolstädter Landstrasse 1 | 85764 Neuherberg) veranstaltet.

Die Organisation und praktische Durchführung wurde durch die Agentur Valentum Kommunikation GmbH (Bischof-von-Henle Straße 2b | 93051 Regensburg) unterstützt.

2. Durchführung des Workshops

2.1 Hintergrund

Alleiniger Veranstalter des Workshops, der vom 14. bis 16. November 2016 in München stattfand, war das Bundesamt für Strahlenschutz im Auftrag des Bundesministeriums für Umwelt, Naturschutz, Bau und Reaktorsicherheit. Unmittelbar nach dem Auftaktgespräch am 14. Juli 2016 wurde die Veranstaltungswebseite www.leukemia-workshop.de eingerichtet. Hier wurden laufend aktuelle Informationen zum Programm und dem Ablauf der Tagung sowie die Möglichkeit zur Registrierung bereitgestellt. Zudem wurde der Einladungsflyer als Download bereitgestellt oder per E-Mail an interessierte Kontakte versandt.

2.2 Programm

Mit zweiundzwanzig Vorträgen von eingeladenen Rednern aus elf Ländern sowie acht Kurzvorträgen (short talks) wurde das Programm des 2,5-tägigen Workshops gestaltet. Das Programm wurde in sechs Einheiten gegliedert, die den Inhalt aufeinander aufbauend strukturierten:

- Session 1: Setting the Scene – From the radiation protection point of view
- Session 2: Childhood leukemia etiology – Mechanisms of clonal evolution
- Session 3: Old hypotheses get new support – Childhood Leukemia and the immune system
- Session 4: New Insights from animal models
- Session 5: Genetics, epigenetics and the environment
- Session 6: Ongoing studies and looking forward – What to do next?

Jede Einheit setzte sich aus den Präsentationen der Redner (20–35 Minuten) sowie so genannten short talks (10 Minuten) zusammen. Interessierte Teilnehmer konnten sich im Vorfeld durch die Einreichung eines Abstracts um einen Beitrag zur Veranstaltung in Form eines Short Talks bewerben. Insgesamt wurden acht Abstracts aus fünf Ländern eingereicht.

Zwischen den Programmpunkten konnte das Publikum Fragen zu den Präsentationen stellen und Probleme oder Streitpunkte diskutieren. In den Programmpausen sowie einem gemeinsamen Conference Dinner am zweiten Veranstaltungstag konnten die Gäste den Austausch sowie das Networking untereinander im lockeren Rahmen fortsetzen.

Insgesamt nahmen 68 Personen aus 15 Ländern am Workshop teil. Die Teilnahme war für die Mitarbeiterinnen und Mitarbeiter des Bundesamts für Strahlenschutz und des Bundesministeriums für Umwelt, Naturschutz, Bau und Reaktorsicherheit kostenlos.

2.3 Beteiligte Akteure im Workshop

Geladene Redner

Nachname	Vorname	Institution	Land
Auvinen	Anssi	University of Tampere School of Health Sciences	Finnland
Borkhardt	Arndt	Uniklinikum Düsseldorf	Deutschland
Cazzaniga	Giovanni	Clinica Pediatrica – Università di Milano Bicocca	Italien
Cobaleda	Cesar	CBMSO–Centro de Biologia Molecular Severo Ochoa	Spanien
Dywer	Terry	University of Oxford	Großbritannien
Erdmann	Friederike	International Agency for Research on Cancer (IARC)	Frankreich
Feychting	Maria	Karolinska Institutet	Schweden
Ghantous	Akram	International Agency for Research on Cancer (IARC)	Frankreich
Grosche	Bernd	Bundesamt für Strahlenschutz	Deutschland
Hauer	Julia	Uniklinikum Düsseldorf	Deutschland

Nachname	Vorname	Institution	Land
Kang	Alice	University of California, Berkeley	USA
Kesminiene	Ausrele	International Agency for Research on Cancer (IARC)	Frankreich
Kratz	Christian	Medizinische Hochschule Hannover	Deutschland
Lightfoot	Tracy	University of York – Departement of Health Science	Großbritannien
Müschen	Markus	UCSF School of Medicine	USA
Reid	Gregor	Departement of Pediactrics – University of British Columbia	Kanada
Saffery	Richard	Royal Children's Hospital – Murdoch Childrens Research Institute	Australien
Sanchez–Garcia	Isidro	CSIC/Universidad de Salamanca	Spanien
Schüz	Joachim	International Agency for Research on Cancer (IARC)	Frankreich
Spycher	Ben	Universität Bern – Institute of Social and Preventive Medicine	Schweiz
Stanulla	Martin	Medizinische Hochschule Hannover	Deutschland
Vergara	Ximena	Electric Power Research Institute	USA

Referenten der Kurzvorträge:

Nachname	Vorname	Institution	Land
Bucher	Martin	Bundesamt für Strahlenschutz	Deutschland
Jeremias	Irmela	Helmholtz Zentrum München Klinikum der Universität München	Deutschland
Fischer	Ute	University of Düsseldorf	Deutschland
Gomolka	Maria	Bundesamt für Strahlenschutz	Deutschland
Konstantinoudis	Garyfallos	University of Bern – Institute of Social and Preventive Medicine (ISPM)	Schweiz
Kreis	Christian	Institute of social and preventive medicine	Schweiz
Romeo	Paul–Henri	Commissariat à l´énergie atomique et aux énergies alternatives (CEA)	Frankreich
Søegaard	Signe	Statens Serum Institut	Dänemark

Wissenschaftliches Kommitee

Arndt Borkhardt | Chefarzt, Direktor der Klinik für Kinder–Onkologie, –Hämatologie und klinische Immunologie**,** Uniklinkum Düsseldorf, Deutschland

Bernd Grosche | Bundesamt für Strahlenschutz, Deutschland

Sabine Hornhardt | Bundesamt für Strahlenschutz, Deutschland

Joachim Schüz | International Agency for Research on Cancer, Frankreich

Martin Stanulla | Pädiatrische Hämatologie und Onkologie, Medizinische Hochschule Hannover, Deutschland

Gunde Ziegelberger | Bundesamt für Strahlenschutz, Deutschland

3. Ergebnisse

Für einen Überblick über die während des Workshops präsentierten Forschungsergebnisse sind im Anhang die eingereichten Abstracts beigefügt.

Im Nachgang des Workshops wird vom Bundesamt für Strahlenschutz ein gemeinsamer Report mit Input der Redner veröffentlicht werden.

Die Kosten– und Zeitplanungen wurden eingehalten.

Programme

Monday, November 14

from 11:00	Arrival, registration, welcome coffee & pretzels
12:30 – 12:45	Official welcome *Axel Böttger, Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety, Germany* Official welcome *Thomas Jung, Federal Office for Radiation Protection, Germany*

Session 1: Setting the Scene – From the radiation protection point of view
Chair: Federal Office for Radiation Protection

12:45 – 13:35	Looking back and lessons learned *Bernd Grosche, Germany*
13:35 – 13:55	Why a 5th workshop? Defining the goals *Gunde Ziegelberger, Germany*
13:55 – 14:45	Review of epidemiological studies of magnetic fields and childhood leukemia *Maria Feychting, Sweden*
14:45 – 15:15	Coffee break
15:15 – 16:05	Childhood leukemia and CT scans: overview of recent studies *Ausrele Kesminiene, France*
16:05 – 16:55	Environmental risk factors: natural background radiation and traffic–related air pollution *Ben Spycher, Switzerland*
16:55 – 17:45	New results from childhood leukemia international consortium CLIC *Alice Kang, USA*
17:45 – 18:00	Short break

Session 1: Short talks

18:00 – 18:15	New California study on the link of power lines and Childhood leukemia *Ximena Vergara, USA*
18:15 – 18:30	Very low doses of γ-rays decrease self-renewal and promote constitutive oxidative stress of Hematopoietic Stem Cells *Henri-Paul Romeo, France*
18:30 – 18:45	Increased radiation sensitivity of T lymphocytes in newborns and children under five years of age compared to adults after low dose in vitro CT exposure *Maria Gomolka, Germany*
18:45 – 19:00	Genome stability and DNA repair capacity after in vitro irradiation in a small collective of young Ataxia telangiectasia patients *Martin Bucher, Germany*
from 19:30	Informal get–together

Tuesday, November 15

from 08:00	Arrival & registration

Session 2: Childhood leukemia etiology – Mechanisms of clonal evolution
Chair: Federal Office for Radiation Protection

08:30 – 09:15	Risk factors of Childhood Leukemia *Anssi Auvinen, Finland*
09:15 – 10:00	Mechanisms of clonal evolution in childhood ALL – The clinical perspective *Arndt Borkhardt, Germany*
10:00 – 10:45	Molecular mechanisms *Giovanni Cazzaniga, Italy*
10:45 – 11:05	Coffee break
11:05 – 11:50	Metabolic gatekeeper function of B-lymphoid transcription factors *Markus Müschen, USA*

Session 2: Short talk

11:50 – 12:10	2% of healthy newborns reveal ETV6-RUNX1 Fusion by genomic inverse PCR for exploration of ligated breakpoints (GIPFEL) *Ute Fischer, Germany*
12:10 – 12:30	Preclinical mouse model of acute leukemia to study patients' primary tumor cells modified by genetic engineering *Irmela Jeremias, Germany*
12:30 – 13:30	Lunch break

Session 3: Old hypotheses get new support – Childhood Leukemia and the immune system
Chair: Federal Office for Radiation Protection

13:30 – 14:15	Childhood leukemia and infection *Tracy Lightfoot, United Kingdom*
14:15 – 15:00	Immune influence on ALL development *Gregor Reid, Canada*
15:00 – 15:45	Exposure to infection triggers pB-ALL on a genetically susceptible background *Julia Hauer, Germany*
15:45 – 16:15	Coffee break

Session 3: Short talks

16:15 – 16:30	Childhood vaccinations and risk of acute lymphoblastic leukemia in children *Signe Holst Søegaard, Denmark*
16:30 – 16:45	Space-time clustering of childhood leukemia: A systematic review and meta-analysis *Christian Kreis, Switzerland*

16:45 – 17:00	Spatial clustering of childhood leukemia in Switzerland: A nationwide study *Garyfallos Konstantinoudis, Switzerland*
17:00 – 17:15	Short break

Session 4: New Insights from animal models
Chair: Federal Office for Radiation Protection

17:15 – 18:00	The genetic basis of malignant transformation in ETV6–RUNX1 pB–ALL *Isidro Sanchez–Garcia, Spain*
18:00 – 18:45	Do electromagnetic fields contribute to B–ALL development in genetically predisposed mice? *Cesar Cobaleda, Spain*
from 19:30	Conference dinner at Klinglwirt (Balanstraße 16, 81669 Munich), walking distance 5 mins

Wednesday, November 16

from 08:30	Arrival & registration

Session 5: Genetics, epigenetics and the environment
Chair: Federal Office for Radiation Protection

09:00 – 09:50	Genetic predisposition: Genes and environment *Christian Kratz, Germany*
09:50 – 10:40	Sensitivity of the early human epigenome to environmental and genetic influences *Richard Saffery, Australia*
10:40 – 11:30	Epigenetic precursors of childhood cancer and associated early – life exposures *Akram Ghantous, France*
11:30 – 12:30	Early lunch

Session 6: Ongoing studies and looking forward – What to do next?
Chair: Federal Office for Radiation Protection

12:30 – 13:00	The International Childhood Cancer Consortium (I4C) *Terry Dwyer, United Kingdom/Australia*
13:00 – 13:30	Childhood leukemia around the globe – What can we learn from observations from developing countries? *Friederike Erdmann, Denmark*
13:30 – 14:00	Childhood leukemia epidemiology from a global perspective *Joachim Schüz, France*
14:00 – 15:00	Summing up *Martin Stanulla, Germany*

Looking back and lessons learned

Bernhard Grosche | (ex) Federal Office for Radiation Protection, Germany

Radiation protection covers the two broad areas of ionizing and non-ionizing radiation. Epidemiological findings from both areas leave us with unexplainable results. On one hand there are continuous reports on an increased risk of childhood leukaemia in the close vicinity of nuclear reactors, in particular among young children below the age of 5 [1].

On the other hand there are repeatable findings of a correlation between the risk of childhood leukaemia and the exposure to 40/50 Hz magnetic fields in the same age group [2]. While some studies around nuclear installations suggest a decreasing risk with increasing distance from the site [3] – with doses from the radioactive discharges which are by far too small to explain this observation based on current knowledge of radiation effects [4] – there are some indications for a dose-response relationship with regard to the magnetic fields but a lack of a biological model [5]. Starting from the latter observations BfS organized a series of meetings with varying partners to learn more about childhood leukaemia and its causes of which little is known.

This might in the end help getting a better understanding of the findings. In particular what has been learned from the first meeting [6] resulted two years later in a multidisciplinary long-term research agenda [7, 8], the initiation of some feasibility projects based upon this agenda, and another two years later in international recommendations for collaborative activities [9]. The presentation will touch the major lessons learned and their transformation into activities or respective recommendations.

References:

1. Laurier D, Grosche B, Hall P (2002) Risk of childhood leukaemia in the vicinity of nuclear installations-findings and recent controversies. Acta Oncol 41:14-24
2. Greenland S, Sheppard AR, Kaune WT, Poole C, Kelsh MA (2000) A pooled analysis of magnetic fields, wire codes, and childhood leukemia. Childhood Leukemia-EMF Study Group. Epidemiology 11:624-634
3. Kaatsch P, Spix C, Schulze-Rath R, Schmiedel S, Blettner M (2008) Leukaemia in young children living in the vicinity of German nuclear power plants. Int J Cancer 122:721-726
4. Grosche B (2011) Comment on 'a German storm affecting Britain: childhood leukaemia and nuclear power plants'. J Radiol Prot 31:503-504; author reply 505
5. Kheifets L, Afifi A, Monroe J, Swanson J (2011) Exploring exposure-response for magnetic fields and childhood leukemia. J Expo Sci Environ Epidemiol 21:625-633
6. Matthes R, Ziegelberger G (Eds.) (2008) Risk factors for childhood leukaemia. Proceedings of an ICNIRP Workshop, Berlin, May 5-7, 2008. Radiat Prot Dosimetry 132:107-275
7. Ziegelberger G, Baum C, Borkhardt A et al. (2011) Research recommendations toward a better understanding of the causes of childhood leukemia. Blood Cancer Journal 1
8. Ziegelberger G, Dehos A, Grosche B, Hornhardt S, Jung T, Weiss W (2011) Childhood leukemia-risk factors and the need for an interdisciplinary research agenda. Progress in biophysics and molecular biology 107:312-314
9. Laurier D, Grosche B, Auvinen A et al. (2014) Childhood leukaemia risks: from unexplained findings near nuclear installations to recommendations for future research. J Radiol Prot 34:R53-68

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Review of epidemiological studies of magnetic fields and childhood leukemia

Maria Feychting | Institute of Environmental Medicine, Karolinska Institutet, Sweden

Extremely low frequency (ELF) electromagnetic fields belong to the non-ionizing part of the electromagnetic spectrum, and there is currently no known biological mechanism for a carcinogenic effect of such fields at exposure levels in the general population. Epidemiologic research on childhood leukemia risk has been conducted on exposures to fields within frequencies around 50–60 Hz, i.e. magnetic fields generated in relation to the production, transmission, and use of electricity.

Research on the potential effects of residential exposure to ELF magnetic fields on childhood leukemia began in the late 1970s with the publication of the study by Wertheimer and Leeper in Colorado. Since then, the research field has developed considerably, both in terms of number of studies published, and regarding study quality, with major advancements achieved in exposure assessment methods and study designs. Exposure assessment in several early studies were simply based on distance from electrical installations such as power lines, or a combination of distance and a crude categorization of the electrical installations according to their potential to generate high magnetic field levels. More advanced exposure assessment methods were developed to include calculations of magnetic field levels from power lines based on detailed historical information about the power line configurations and load, or direct measurements of the magnetic fields in the children's homes over a longer period, both of which provide more accurate estimates of magnetic field levels inside the homes, although still with some limitations.

Individual studies have had limited statistical power as both high exposure levels and the outcome are rare, but have quite consistently reported risk estimates for childhood leukemia above unity. Therefore, the most informative studies are those that have pooled the data from the original studies, allowing harmonization of exposure estimation and cutpoints, and better statistical power. The two first pooled studies, with partly overlapping inclusion of original studies, were published in the year 2000 by Greenland et al. and Ahlbom et al., showing significantly increased risk estimates of 1.7 and 2.0, respectively, in the highest exposure category (0.3 ↔T and 0.4 ↔T). Despite the inclusion of a large number of subjects in the pooled analyses, the numbers with high exposure levels were still small, and the shape of a dose response pattern could not be adequately evaluated. In 2001, IARC classified ELF magnetic fields as "possibly carcinogenic to humans" primarily based on these data. In 2010, an update of the pooled analysis was published by Kheifets et al., including six studies published since 2000, largely confirming the previously pooled results, but with somewhat weaker, non-significant, risk estimates. Pooling only nighttime measurements have not provided different results. No effect of ELF magnetic fields on survival after ALL diagnosis has been found. Studies published during the most recent years have mainly used distance to power lines to estimate exposure, and provide limited information for the evaluation of the hypothesis that ELF magnetic field exposure increase childhood leukemia risk.

In summary, epidemiologic research published since the IARC evaluation in 2001 largely confirm the evidence available at that time, and does not give reason to alter the classification of these fields as "possibly carcinogenic to humans". Chance, bias and confounding cannot be ruled out as alternative explanations.

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Childhood leukaemia and CT scans: Overview of recent studies

Ausrele Kesminiene | International Agency for Research on Cancer, France

Developments in medical imaging, particularly in computed tomography (CT), have led to substantial increases in relatively high-dose x-ray examinations. In the USA alone, over 85 million CT examinations were performed in 2012, and the overall use per year, while slowing, continues to rise. Despite being highly beneficial, these procedures have also resulted in higher radiation exposures to patients compared with exposures from conventional radiography. Subsequently, this raised concerns about potential cancer risks related to radiation exposure from CTs, particularly in children, whose longer life expectancy renders into a higher number of years at risk of developing a radiation-induced malignancy. Further, children, due to their smaller body mass, tend to receive higher organ doses than adults if the CT protocol is not adjusted for smaller body size. It is also well known that exposure in childhood leads to higher risk of cancer, than similar exposure in adults.

Several epidemiological studies were set-up in the 2000s to assess the cancer risk associated with CT examinations performed on children. One of the endpoints of greatest concern was the risk of leukaemia because the red bone marrow is a particularly radiosensitive tissue, especially if exposure occurs in childhood. Moreover, bone marrow is also some of the most highly exposed tissues from childhood CT scans, and leukaemia is among the most common childhood cancers. Two studies, one conducted in the UK (Pearce at al. 2012) and another in Australia (Mathews et al, 2013) found increased risks for leukaemia associated with CT scan exposure. The later study, however, used very limited dosimetric methods leading potentially to substantial exposure misclassification.

Despite the consistent results, the two studies have received criticism for several reasons. There were concerns over potential organ-dose errors, and the possible inclusion of patients with cancer predisposing syndromes (such as Down syndrome or neurofibromatosis type1) and benign conditions with malignant transformation potential. Since information on the reason for CT scan was unavailable, reverse causation was raised as a likely explanation for the observed associations, i.e. that the malignancies were caused by the medical conditions prompting the CT scans rather than by the CT dose, although CT scanning is not the main diagnostic procedure leading to leukaemia diagnosis.

Recently, two smaller empirical studies, one in France (Journey et al, 2015) and another in Germany (Krille et al, 2015), tried to make adjustments for conditions that initiated the scan or other predisposing factors known to be associated with increased cancer and leukaemia risk. In the German study, the standardised incidence ratio (SIR) for lymphomas decreased substantially when subjects with conditions linked to increased risk of cancer were excluded from the analyses while the SIR for leukaemia changed only marginally. Interestingly, in the French study, the risk estimate for leukaemia among subjects without predisposing factors was higher than that in the entire cohort.

The observed risks per radiation dose are difficult to interpret based on substantial uncertainties in organ doses associated with type of CT scanner, type of procedure, changes in scanners and technique over calendar year and missed examinations. A large European prospective cohort study of cancer and leukaemia risks in children exposed to CT scans was launched in nine European countries in 2011 aiming to overcome the shortcomings of previously reported studies. EPI-CT largely invested in accurate dosimetry and thorough assessment of related uncertainties, and potential biases to ensure the validity of the resulting risk estimates.

1. Pearce MS, Salotti JA, Little MP, McHugh K, Lee C, Kim KP, et al. Radiation exposure from CT scans in childhood and subsequent risk of leukaemia and brain tumours: a retrospective cohort study. Lancet 2012; 380(9840):499–505.
2. Mathews JD, Forsythe AV, Brady Z, Butler MW, Goergen SK, Byrnes GB, et al. Cancer risk in 680 000 people exposed to computed tomography scans in childhood or adolescence: data linkage study of 11 million Australians. BMJ 2013; 346:f2360.
3. Journy N, Rehel JL, Ducou Le PH, Lee C, Brisse H, Chateil JF, et al. Are the studies on cancer risk from CT scans biased by indication? Elements of answer from a large-scale cohort study in France. Br J Cancer 2015; 112(1):185–93.
4. Krille L, Dreger S, Schindel R, Albrecht T, Asmussen M, Barkhausen J, et al. Risk of cancer incidence before the age of 15 years after exposure to ionising radiation from computed tomography: results from a German cohort study. Radiat Environ Biophys 2015; 54(1):1–12.

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Environmental risk factors: Natural background radiation and traffic-related air pollution

Ben Spycher | Institute of Social and Preventive Medicine, University of Bern, Switzerland

There is increasing evidence that exposure to low-dose ionising radiation and traffic-related air pollution may increase the leukaemia risks in children. Combining data from the Swiss National Cohort and the Swiss Childhood Cancer Registry, we recently investigated the risk of childhood leukaemia in relation to background ionising radiation and proximity to highways in Switzerland. In my presentation, I will discuss methods and results of our studies in the context of other recent studies.

A number of ecological and case-control studies have investigated whether natural background radiation is associated with increased risk of childhood cancers, particularly leukaemia. However, most of these studies focused on residential exposure to radon, which mainly effects the lung tissue. Fewer studies have looked at terrestrial gamma and cosmic radiation, which deliver comparatively higher doses to the red bone marrow. More recently, record-based case-control studies and cohort studies have investigated childhood leukaemia risks in relation to both radon and gamma ray exposure. In our studies, which included the entire resident childhood population in Switzerland, we found evidence of an increased risk for leukaemia associated with higher cumulative dose from terrestrial gamma and cosmic radiation but no evidence for exposure to radon. Two other recent record-based studies from the UK and Finland also reported positive associations for natural gamma radiation while a recent French found no evidence of an association.

Traffic-related air pollution contains a number of known carcinogens including benzene, a known risk factor for leukaemia in adults, particularly for AML. Numerous studies have investigated possible links between traffic-related air pollution and childhood leukaemia. Exposures were usually assessed based on proximity of roads, traffic density on nearby roads, or on modelled or measured air concentrations of NO2 or benzene. A recent review and meta-analysis supports a link between exposure to traffic pollution and childhood leukaemia, with the strongest association found for benzene and AML. Since that review, three new studies, including ours, have been published. In our study, we found a two-fold higher risk for leukaemia among 0-5 year old children living in the immediate proximity (<100m) of Swiss highways. Two studies from France and the US found an increased risk of childhood AML in association with traffic-related benzene and NO2, respectively.

A major challenge for future research is how to obtain improved exposure measurements on larger sample sizes while at the same time avoiding selection and participation biases. The pooling or joint analyses of different studies might allow to better investigate dose-response relationships, effects on different leukaemia subtypes including cytogenetic subtypes, and critical age windows of exposure.

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Session 1: Setting the Scene – from the radiation protection point of view

New results from childhood leukemia international consortium (CLIC)

Alice Kang Feychting | School of Public Health, University of California, Berkeley, USA

New California study of residential home distance to power lines and childhood leukemia

Ximena Vergara | Electric Power Research Institute, USA

We conducted a large-scale, records-based case-control study of childhood leukemia in California populations living near overhead electric power transmission lines, known as the California Power Line Study (CAPS). The CAPS study included 5,788 leukemia cases, and 3,308 central nervous system cancer cases for comparison, born in and diagnosed in California before age 16 years (yrs) (1986-2008). Cases were matched on age and sex to population-based controls. We geocoded address at birth and estimated distance from residence to transmission lines using several methods: geographic information systems, aerial imagery, and site visits, for a subset of residences of leukemia cases and controls closest to lines.

For leukemia, we noted a slightly elevated association between distances within 50 meters (m) of a transmission line over 200 kilovolts as compared to distances of 600 m or more (Odds ratio (OR) = 1.4, 95% confidence interval (CI) 0.7-2.7). We did not detect an increased risk for distances beyond 50 m, for lower voltage lines, or for CNS cancers. For acute lymphoblastic leukemia, we observed similar risk estimates for cases living within 50 m of a transmission line compared to those at 600 m or more [OR = 1.3, 95% CI 0.6-2.7]. When stratified by age of diagnosis, the OR for 0-50 m was 1.3 (95% CI: 0.6-2.7) among those less than 5 yrs compared to 0.8 (95% CI: 0.2-2.9) for those 5 yrs or greater.

When stratified by birth decade, the OR for 0-50m was lower for the earlier time period of 1986-1995 compared to the later time period. Our findings did not clearly support an increased childhood leukemia risk associated with close proximity (<50 m) to high voltage lines, but could be consistent with a small increased risk. Reports of increased risk for distances beyond 50 m, as was reported in a previous UK study, were not replicated. CAPS is an EPRI-funded epidemiologic study carried out by researchers at the UCLA Fielding School of Public Health and the University of Southern California.

Crespi CM, Vergara XP, Hooper C, Oksuzyan S, Wu S, Cockburn M, Kheifets L. Childhood leukaemia and distance from power lines in California: a population-based case-control study. Br J Cancer. 2016 Jun 28;115(1):122-8.

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Very low doses of γ–rays decrease self–renewal and promote constitutive oxidative stress of Hematopoietic Stem Cells

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Despite the increasingly frequent use of low–dose radiation (<0.1Gy) in medicine, the effects of low doses on somatic stem cells are poorly documented. Here, we show that adult hematopoietic stem cells (HSC) are hyper–radiosensitive to low dose of γ–irradiation. This hyper–radiosensitivity is not associated with activation of the DNA Damage Response pathway but rather with immediate production of Reactive Oxidative Species (ROS) for mitophagy and autophagy up–regulation, phosphorylation of p62 and activation of Nrf2 leading to protection of HSC. Twenty mGy to HSC causes long–term oxidative stress, decreased self–renewal capacity and a myeloid bias, all of which can be reversed by pretreatment with ROS scavenger NAC. Finally, we show that Total Body Irradiation at 0.02 Gy after AMD3100–induced mobilization of HSC decreases their self–renewal capacity. These results show that very low doses of γ–rays have detrimental effects on HSC and suggest that low–dose radiation exposure might have adverse effects on somatic stem cells.

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Session 1: Setting the Scene – from the radiation protection point of view

Increased radiation sensitivity of T lymphocytes in newborns and children under five years of age compared to adults after low dose in vitro CT exposure

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Computer tomography (CT) scans for diagnostic purposes deliver relatively high doses to the patient compared to usual X ray diagnostics. Approximately 60% of the annual medical diagnostic radiation exposure is due to CT scans in Germany. The radiation-related cancer risk from this technology, especially for children, is currently under discussion. Besides technical developments for dose reduction, the role of biological mechanisms counteracting damage induced by low dose exposures have to be clarified in cancer risk assessment and risk prevention strategies. Age dependent radiation sensitivity for cancer, especially in children, is a major factor that has to be considered. In several epidemiological reports, the increased radiation sensitivity in radiation-related cancer risks for children compared to adults was estimated to be a factor ranging from 0 to 10 depending on the type of cancer. For leukemia there is a clearly increased radiation-related risk for children but risk level and risk modification by age require further investigation, particularly in experimental settings. We investigated here in blood samples of male donors pertaining to three age groups (newborns, children under 5 years and adults) radiation biomarker levels after sham exposure (0 mGy), low dose (41 mGy) and high dose (978 mGy) in vitro CT exposure. For this purpose, levels of two radiation biomarkers that are either highly specific to radiation exposure (dicentric chromosomes) or very sensitive to low dose exposures (gammaH2AX foci) have been investigated.

Significantly higher levels of dicentric induction were found for the combined newborns/children group compared to adults, by a factor of 1.48 (95% CI 1.30-1.68), after exposure to 978 mGy. When we scored dicentrics in about 13,000 – 23 000 cells/dose point/group a statistically significant age variation was detected even in the low dose range of 41 mGy. Although a significant dose response and dose dependent repair efficiency was found, the gammaH2AX assay did not show an age dependent increase in DNA damage in children. This was the case for the gammaH2AX levels after repair time intervals of 30 minutes and 24 hours, after correcting for the underlying background damage.

This is the first study to report significantly increased DNA damage in lymphocytes of male children after in vitro radiation exposure down to 41 mGy, which may have an impact on leukemia induction.

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Session 1: Setting the Scene – from the radiation protection point of view

Genome stability and DNA repair capacity after in vitro irradiation in a small collective of young Ataxia telangiectasia patients

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Ataxia telangiectasia (AT) is an autosomal recessive multiorgan disorder based on a mutated ATM gene. AT causes neurodegeneration, immunodeficiency, chromosomal instability, abnormal sensitivity to ionizing radiation and cancer predisposition. The ATM gene product plays an important role in signaling of DNA double-strand breaks (DSB) and phosphorylates many proteins involved in DNA-repair, like the highly conserved histone variant H2AX. However, incorrect ATM protein leads to defects in the DNA damage response, unresolved DSB and genomic instability, all together contributing to the carcinogenic process.

In this ongoing project we analyse DNA repair capacity and chromosomal aberrations in a collective of 8 young AT patients with confirmed ATM mutation or clinical phenotype in comparison to a control group of 10 healthy children. After in vitro gamma-irradiation of isolated lymphocytes endogenous, induced DNA damage, repair kinetics and DNA repair capacity are detected by the alkaline comet assay and the accumulation of radiation induced foci (RIF) of γ-H2AX. Chromosomal aberrations are analysed with the mFISH technique.

Preliminary results show a frequency of aberrant metaphases in AT children after in vitro irradiation with 1 Gy (137Cs) more than three times higher compared to sham-irradiated cells (20%). Chromosomal instability is already detected in sham-irradiated cells with a high frequency of dicentric chromosomes (3-15‰), exchange aberrations (5-12‰) and non-exchange aberrations (7-10%). In comparison, normal rate for dicentrics and exchange aberrations is 1 per 1000 or 1-3 per 1000 cells, respectively. The frequency of all chromosomal aberrations in cells from AT children increases after irradiation: dicentric chromosomes (26-47‰), exchange aberrations (27-47‰) and non-exchange aberrations (24-50‰).

In summary, combined evaluation of all data will gain further information about the DNA damage response in young AT patients and validate RIF as a biomarker to detect the radiation sensitive phenotype.

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Risk factors of Childhood Leukemia – an overview

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The etiology of childhood leukemia remains largely elusive. The few well established risk factors include some congenital syndromes such as 21 trisomy and high doses of ionizing radiation.

A wealth of putative risk factor have also been reported, but the evidence remains inconclusive. Boys are at slightly higher risk than girls. Breast-feeding at least six months appears to lower the risk, and a protective effect has also been shown for early day care attendance. Studies of extremely low frequency (ELF) electromagnetic fields have shown increased risks reasonably consistently, and meta-analyses have confirmed a 1.5-fold risk at exposure levels above 0.3-0.4 uT. High birth weight has been reported as risk factor in several studies and small excess risk found also for first-born children. Maternal smoking has been suspected, but the findings are not entirely consistent. Some indications have been reported also for maternal coffee consumption during pregnancy. Some support has been found for the population mixing hypothesis, but the evidence is still conflicting. Also some reports have suggested increased risk related to traffic exhausts (or traffic density as a proxy) during childhood. Some evidence has also been fund for certain parental occupational exposures including benzene, solvents and pesticides, perhaps more for AML than ALL.

A nation-wide study was recently initiated in Finland, including all childhood leukemia cases diagnosed in 1990-2011, and population-based controls. The fist analyses have dealt with residential mobility as an indicator for population mixing, and background gamma radiation. The design and some planned analyses will also be presented.

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Mechanisms of clonal evolution in childhood ALL - the clinical perspective

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Over the last two decades, leukemia-risk adapted chemotherapy - mainly based on European-wide implementation of comprehensive measurements of minimal residual disease (MRD) - have revolutionized treatment of childhood acute lymphoblastic leukemia (ALL). Maintaining excellent initial treatment results but avoiding severe short-term toxicity as well as long term-term sequelae of multi-agent chemotherapy in childhood is the major challenge for the upcoming years.

In addition, curative treatment and definitive cure of children who had already been relapsed is still challenging and often requires allogeneic stem-cell transplantation (SCT). Notwithstanding, one third to 40% of children subsequently relapses again, even after SCT. Their prognosis is extremely poor. The unique situation after allogeneic stem-cell transplantation, namely the presence of donor-recipient chimerism offers the possibility to study clonal evolution and the mutational landscape of childhood ALL either after chemotherapy (relapse sample) or immunotherapy/SCT (subsequent post-allo-SCT relapse). Thus, post allo-SCT relapses comprise both donor hematopoietic cells as well as leukemic blasts. As the latter represent (at least partially) the individual genetic background, a tumor-germline analysis consequently requires two germline (i.e remission) samples, namely one before and one following the allo-SCT.

We analyzed ten children with post-allo relapses by exome sequencing. We found that the mutational load significantly increased from initial diagnosis to relapse in all ten children. One possible explanation is a therapy-induced effect by interfering with DNA replication and, thus, causing additional mutations. However, the mutational load did not futher increase in post-allo relapses but the individual genetic SNPs were highly changeable and differed dramatically between relapses after chemotherapy and relapses after SCT. Thus, we found considerable difference of the mutational landscape not only between initial diagnosis and relapse but likewise between the two types of relapses, either after chemotherapy or allogeneic transplantation.

Using this specific project as an example, the talk will emphasize that the selection pressure of specific therapy remains a major determinant of the individual mutational landscape in children with ALL.

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Session 2: Childhood leukemia etiology – Mechanisms of clonal evolution

Molecular mechanisms

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Session 2: Childhood leukemia etiology – Mechanisms of clonal evolution

Metabolic gatekeeper function of B-lymphoid transcription factors

Markus Müschen | University of California San Francisco and Howard Hughes Medical Institute, USA

B-lymphoid transcription factors (e.g. PAX5, IKZF1) are critical for early B-cell development, yet genetic lesions occur in >80% of cases of pre-B cell-derived acute lymphoblastic leukemia (ALL). Despite their high frequency in pre-B ALL, the significance of these lesions was unclear. Combining ChIP-seq and RNA-seq studies, we identified a novel B-lymphoid program for transcriptional repression of glucose utilization (INSR, GLUT1/3/6, HK2/3, G6PD) and activation of glucose transport inhibitors (NR3C1, TXNIP, CNR2). Our metabolic analyses revealed that PAX5 and IKZF1 enforce a state of chronic energy deprivation, resulting in constitutive activation of the energy-stress sensor AMPK in pre-B cells. Dominant-negative mutants of PAX5 and IKZF1 cloned from pre-B ALL patient samples relieved restrictions on glucose and energy supply. Studying a transgenic pre-B ALL mouse model, heterozygous deletion of Pax5 increased glucose uptake and ATP-levels by >25-fold. Reconstitution of PAX5 and IKZF1 in pre-B ALL patient samples carrying lesions of these transcription factors restored a non-permissive state and induced energy crisis and leukemia cell death. A CRISPR/Cas9-based screen of PAX5- and IKZF1- transcriptional targets identified NR3C1 (glucocorticoid receptor), TXNIP (glucose feedback sensor) and CNR2 (cannabinoid receptor) as central effectors of B-lymphoid restriction of glucose and energy supply. Interestingly, transport-independent lipophilic methyl-conjugates of pyruvate and TCA cycle metabolites bypassed the gatekeeper function of PAX5 and IKZF1, jumpstarted oncogenic signaling and readily enabled leukemic transformation. Conversely, pharmacological agonists of the glucose transport inhibitors TXNIP and CNR2 and a small molecule inhibitor of the energy-stress sensor AMPK strongly synergized with glucocorticoids, identifying TXNIP, CNR2 and AMPK as novel targets for the treatment of B-lymphoid leukemia.
Furthermore, our results provide a mechanistic explanation for the empiric finding that glucocorticoids are effective in the treatment of B-cell but not myeloid malignancies. We conclude that B-lymphoid transcription factors function as metabolic gatekeepers by limiting the amount of cellular ATP to levels that are insufficient for malignant transformation.

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Session 2: Childhood leukemia etiology – Mechanisms of clonal evolution

2% of healthy newborns reveal ETV6–RUNX1 fusion by genomic inverse PCR for exploration of ligated breakpoints (GIPFEL)

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Pediatric acute lymphoblastic leukemia (ALL) is characterized by preleukemic recurrent chromosomal translocations that emerge in utero. The translocation t (12;21) resulting in the formation of the chimeric transcription factor ETV6–RUNX1 is the most frequent structural aberration occurring in 25% of B–cell precursor patients. A previous study suggested that ETV6–RUNX1–positive preleukemic cells are present in every hundredth human newborn, thus exceeding the actually observed incidence of ETV6–RUNX1–positive ALL in children (1/10,000) by a factor of 100. This finding strongly indicated that secondary cooperating oncogenic hits were necessary for development of overt leukemia. However, later studies could not confirm this high frequency. To analyze the actual frequency of ETV6–RUNX1 preleukemic cells in newborns we developed a PCR–based method termed genomic inverse PCR for exploration of ligated breakpoints (GIPFEL) and applied this technique to a population–based retrospective screening of 300 cord blood samples from Danish newborns.

The GIPFEL method is capable of detecting the most common gene fusions associated with childhood leukemia without prior knowledge of the exact breakpoint. In contrast to previously used RNA–based methods, it relies on DNA as sample material, which is more stable than RNA. In the case of ETV6–RUNX1–positive leukemia GIPFEL exploits the unique presence of a genomic fragment joining material from chromosome 12 and 21. These fragments can be digested and re–circularized by ligation creating a junction across the restriction site whose sequence can be predicted from published genome data. The ligation site is independent of the translocation point within the individual DNA circle. Genomic DNA was prepared from mononuclear cells from cord blood samples of 300 newborns that were cryopreserved within 24 h from birth. After B cell enrichment and column purification of DNA, the DNA was subjected to screening by GIPFEL. Samples that screened positive underwent one further demultiplexed PCR, agarose gelelectrophoresis and Sanger sequencing to validate the result and to identify the breakpoint region. In previously published proof–of–principle blinded studies 64% for ETV6–RUNX1 fusion genes in samples from ALL patients were detected in that sample set. The sensitivity of the technique was estimated to be 10–4, i.e. one translocation carrying cell within 10,000 normal cells can theoretically be detected.

Within the analyzed cohort of 300 healthy newborns 6 screened positive for the ETV6–RUNX1 translocation (2%). Further 700 cord blood samples are currently screened.
Our results indicate that the actual incidence of ETV6–RUNX1–positive cells in healthy newborns might be even higher than previously assumed, potentially due to instability of the ETV6–RUNX1 RNA transcript in preserved cord blood samples. This would hint at a comparably low penetrance and leukemia inducing potential of the chimeric transcription factor ETV6–RUNX1 in human newborns and further strengthen the importance of secondary environmentally caused or spontaneously occurring cooperating oncogenic lesions for ETV6–RUNX1–positive childhood leukemia to emerge.

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Session 3: Old hypotheses get new support – Childhood leukemia and the immune system

Preclinical mouse models of acute leukemias to study patients' primary tumor cells modified by genetic engineering

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To study basic biologic processes in acute leukemias, we improved the individualized mouse model of acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML).

Primary tumor cells of patients with ALL or AML were xeno-transplanted into mice to generate patient-derived xenografted (PDX) cells. Lentiviral transduction resulted in transgenic PDX cells stably expressing recombinant proteins or knockdown constructs. To quality control our model, we characterized PDX cells after engraftment, serial transplantation, and molecular manipulation by targeted deep sequencing of 43 genes known to be recurrently mutated in myeloid malignancies. Targeted re-sequencing revealed that clonal and subclonal recurrent mutations of the primary specimens were largely preserved in the PDX samples tested, even after serial transplantation and molecular engineering. However, certain minor subclones had an engraftment and/or growth advantage, becoming the major clone or getting lost upon xenotransplantation or cell manipulation.

Mice harboring transgenic PDX cells expressing recombinant codon-optimized firefly luciferase were repetitively monitored by bioluminescence imaging (BLI). BLI was highly sensitive and reliably detecting as low as 1 transgenic PDX cell within more than 10,000 mouse bone marrow cells and thereby visualizing the clinically important stage of minimal disease. Growth of transgenic PDX cells in mice over time was exponential. Analysis of limiting dilution transplantation assays by BLI enabled convenient quantification of leukemia stem cells within as little as, e.g., 5 weeks.

Taken together, patients' ALL or AML cells growing in mice closely mimic the heterogeneity of the disease. Molecular manipulation and BLI on serially transplanted PDX AML cells facilitate reliable disease monitoring and preclinical in vivo trials. The technical improvements will allow detailed preclinical studies on patient-derived AML cells of diverse genetic backgrounds to study biology and therapy of acute leukemias in the future.

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Childhood leukemia and infection

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Session 3: Old hypotheses get new support – Childhood leukemia and the immune system

Immune influences on ALL development

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Support for the hypothesis that the early−life immune environment is a modifier of pediatric B cell precursor (BCP) acute lymphoblastic leukemia (ALL) progression comes from a variety sources. A large body of epidemiologic data implicates infection in the disease etiology; protective effects have been reported when surrogates of infection exposure, such as day are attendance, are analyzed, but apparently contradictory findings have emerged from studies of documented early infections. In addition, polymorphisms in several cytokine genes, including IL−10 and IFN−γ, and levels of IL−10 in neonatal blood spots have been correlated with ALL progression. The discovery that ALL−initiating genetic events often occur in utero indicated a potential cell target for the immune influence and pro−-- leukemic activity has been induced in ETV6−RUNX1−expressing BCP cells by immune signaling. Here, I will describe our studies using the E↔−ret and E2A−PBX1 mouse models of BCP ALL to further investigate the impact of immune environment on ALL development. Consistent with the observation that pre−--leukemic cells are detectable at birth in children who develop ALL several years later, early−occurring abnormal cells in E↔−ret transgenic mice are sufficient for disease. By targeting the IL−7 dependence of these leukemia−- initiating cells, we provide evidence that a reduction in the size of the population correlates with a significant delay in onset of disease. In the absence of IFN−γ, an expanded leukemia−-- initiating cell population is present early in life and this drives accelerated leukemia onset in IFN−γ−knockout E↔−ret mice. The leukemia−initiating cells from IFN−γ−knockout mice are more sensitive to FN−γ than their wild−type counterparts and this is associated with reduced expression of SOCS−1. In the context of infection, altering the pre−leukemic immune environment via toll−like receptor ligation induces production of interferons by innate immune cells that is sufficient to deplete E↔−ret and E2A−PBX1 leukemia−initiating cells and significantly delay disease progression. Furthermore, consistent with the identification of age as a significant variable in the epidemiologic infection studies, a mild self−limiting infection of neonatal mice is sufficient to ablate leukemia−initiating and leukemic cells while a comparable infection of adult mice has no effect. Overall, our studies provide mechanistic support for an influence of the immune environment on ALL progression. Specifically, our results demonstrate that both basal cytokine levels and infection−induced immune responses can directly affect leukemia development by altering the size of the leukemia−initiating cell population. Notably, in both the basal and stimulated immune environments, comparable effects were observed in the normal BCP cell population, suggesting that they were a consequence of normal BCP biology rather than the result of dysregulate.

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Exposure to infection triggers pB-ALL on a genetically susceptible background

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We have previously described an Ashkenazi Jewish family with germline predisposition to pB-ALL and reduced penetrance based on the germline variant PAX5 c.547G>A. Thorough characterization of a Pax5+/- murine model, which closely mimics the human phenotype of pB-ALL, especially the low penetrance observed in our family with the heterozygous PAX5 c.547G>A mutation indicates that exposure to common infectious environment can trigger leukemia at reduced penetrance.

Exposure of Pax5+/- mice to a common infectious environment produces pB-ALLs, which closely mimic the human disease. pB-ALL development was observed in 22% (9 out of 41) of Pax5+/- animals, with a CD19+/-B220+IgM-cKit+/-CD25+/- cell surface phenotype. All pB-ALLs displayed clonal immature BCR rearrangement and engrafted in secondary recipients with a phenotype identical to the primary disease. The majority of the murine Pax5+/- pB-ALL (5/9; 55.6%) did not express CD19 and in two mice we detected the Pax5 variants p.Pro80Arg and p.Pro80Leu, suggesting reduction of Pax5 activity, which is in agreement with gene expression analysis. In order to identify the second hit related to pB-ALL disease we next performed whole exome sequencing of three Pax5+/- tumors and corresponding germline on a HiSeq 2500 (Illumina) platform. We detected in 6/9 mice recurrent somatic SNVs in the pseudokinase domain of Jak3 causing constitutive active variants of Jak3R653H (4/9) (human homologue JAK3R657Q), Jak3R653C (1/9) (human homologue JAK3R657Q) and Jak3V670A (1/9) (human homologue JAK3V674A). Consistently, tumor pro-B-cells harboring Jak3V670A and Jak3R653H grew independent of IL7. We next performed deep sequencing with a depth between 600,000 and 2.5 x 106 reads per Jak3 SNV and observed the non-synonymous Jak3 variant only in tumor samples but not in BM cells of healthy Pax5+/- or wild-type mice, indicating that acquisition of the Jak3 variant is a rather late event.

Animals with Pax5+/- pB-ALL were exposed to murine noro virus, murine hepatitis virus and helicobacter pylori, according to strict health monitoring report whereas profiling of viral species using an NGS approach, did not reveal significant increase in viral load or species spectrum. Furthermore we set up to elucidate whether this mechanism applies to all childhood pB-ALL and exposed Sca1-ETV6-RUNX1 mice as well as Sc1-BCR/ABLp190 mice to a common infectious environment. Both mouse strains develop pB-ALL. However in Sca1-ETV6-RUNX1 mice pB-All development is restricted to animals, which were exposed to a common infectious environment.

Thus our data indicate that certain childhood pB-ALL subtypes are dependent on exposure to infection based on their molecular make up and this finding can be extended to ETV6-RUNX1 pB-ALL, the most common leukemia subtype in childhood but not to BCR/ABLp190 pB-ALL.

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Childhood vaccinations and risk of acute lymphoblastic leukemia in children

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Background: It has been proposed that childhood vaccinations protect against acute lymphoblastic leukemia (ALL) in children by modulation of future responses to common infections in childhood. However, the available studies provide conflicting findings, and population-based cohort studies with longitudinal information on vaccinations are lacking.

Methods: In a register-based cohort of all children born in Denmark from January 1, 1990, through December 31, 2008, followed up until age 15 or December 31, 2009 (n = 1 225 404), we evaluated exposure to childhood vaccination and risk of childhood ALL, including information on leukemia subtypes. Using Cox regression, we estimated hazard ratios (HRs) comparing vaccinated with unvaccinated children.

Results: Childhood ALL was diagnosed in 490 children during 10 829 194 person-years of follow-up. Neither the total number of vaccine doses received nor exposure to each vaccine given in childhood were associated with altered risk of ALL, including i) Haemophilus influenzae type b (HR, 1.04; 95% confidence interval (CI), 0.68-1.61), ii) measles, mumps and rubella (HR, 1.01; 95% CI, 0.76-1.34), iii) whole-cell pertussis (HR, 1.10; 95% CI, 0.51-2.39), and iv) diphtheria, tetanus and inactivated polio (HR, 1.14; 95% CI, 0.42-3.13). Analyses conducted according to leukemia subtypes defined by immunopheno- and karyotypes showed no statistically significant association with childhood vaccination.

Conclusions: This nationwide cohort study provides no support of the proposed protective effect of childhood vaccination against childhood ALL.

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Session 3: Old hypotheses get new support – Childhood leukemia and the immune system

Space–time clustering of childhood leukaemia: A systematic review and meta–analysis

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Childhood leukaemia (CL) is the most frequent paediatric cancer but its aetiology remains largely unknown. A number of studies have investigated whether CL cases cluster in space and time, but to date there has been no systematic synthesis of this evidence. We performed a systematic review and propose a method for meta–analysing studies using the Knox test, the most common test statistic to assess space–time clustering of CL.

We searched for studies assessing space–time clustering of CL based on residence at birth or diagnosis. The Knox test statistic T, i.e. the number of pairs of cases lying within a given spatial and temporal lag from each other, is assumed to be approximately Poisson distributed and its square root (√T) thus approximately normally distributed with constant variance. We propose meta–analysing √T/√E, where E is the expectation of T in the absence of clustering. We performed a random effects meta–analysis for spatial and temporal lags closest to the mean lag values over all included studies and subgroup analyses for different combinations of spatial and temporal lags for which a minimum of four studies could be included. Analyses were performed separately for residence at birth and diagnosis for children aged 0–15 and 0–5 years old.

We included 19 and 8 studies of residence at diagnosis and birth, respectively. There was evidence of space–time clustering of CL at diagnosis for ages 0–5 years ($p=0.039$). The clustering effect was strongest at space–time lags of 5 km and 6 months ($p<0.001$). At the time of birth, we observed marginally significant clustering for ages 0–5 years ($p=0.064$).

The results of our meta–analysis suggest that cases of CL aged 0–5 years tend to cluster in space and time due to an aetiological factor acting close to the time of diagnosis. However, due to selective reporting in the included studies, our findings must be interpreted with caution.

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Session 3: Old hypotheses get new support – Childhood leukemia and the immune system

Spatial clustering of childhood leukaemia in Switzerland: A nationwide study

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Introduction: Leukaemia is the most common cancer in childhood. The aetiology of leukaemia is largely unknown. Several hypotheses include environmental exposures and may implicate spatial clustering of cases. Results from previous studies investigating spatial clustering are inconclusive. Most previous studies used regional data and thus had limited spatial resolution.

Methods: We investigated whether there is spatial clustering of childhood leukaemia in Switzerland using exact geocodes of residence at diagnosis and at birth. We included 1871 leukaemia cases diagnosed at age 0–15 years during 1985–2015 from the Swiss Childhood Cancer Registry. Age and sex matched controls (10 per case) were randomly sampled from the national censuses (1990, 2000, 2010). We used k–functions, Cuzick–Edwards' test and Tango's index for point data to assess spatial clustering and Kulldorff's scan statistic to detect individual clusters. We also adjusted for multiple testing (different statistical tests and subgroups).

Results: Adjusting for the multiple tests performed, we found no evidence of spatial clustering around time of birth (p = 0.54) or diagnosis (p= 0.51). The strongest evidence from individual tests was for spatial clustering of leukaemia at time of diagnosis in children aged 5–15 years: p Cuzick–Edwards (one nearest neighbour) = 0.042, p k–functions (100m) = 0.049. The most significant cluster consisted of five cases living in a small rural area (p = 0.048, radius 500m).

Conclusion: This study does not provide evidence for spatial clustering of childhood leukaemia in Switzerland. If clustering did indeed occur, our study suggests that clusters were small in spatial extent (<1km).

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Session 4: New insights from animal models

The genetic basis of malignant transformation in ETV6-RUNX1 pB-ALL

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The ETV6-RUNX1 fusion gene is associated to the most common subtype of childhood pB-ALL. The underlying genetic basis explaining how the preleukemic clone evolves to pB-ALL remains to be identified. Here we show that human ETV6-RUNX1 pB-ALL is characterized by loss-of-function mutations in histone-modifying genes (42%of cases) especially of the KDM family. We model this in vivo by limiting ETV6-RUNX1 expression to murine HSPCs. Preleukemic ETV6-RUNX1 pro/preB cells show enrichment in histone modifying gene expression of the KDM family and high Rag1/2 expression, a hallmark of human ETV6-RUNX1 pB-ALL. Sca1-ETV6-RUNX1 mice develop a human-like pB-ALL only after exposure to postnatal infections. Mouse tumor exome sequencing revealed as a second hit similar secondary alterations found in human ETV6-RUNX1 pB-ALL. These results uncover how ETV6-RUNX1 promotes leukemogenesis by creating an aberrant progenitor that is susceptible to malignant transformation through acquisition of loss-of-function mutations in histone-modifying genes of the KDM family. Histone modification and concomitant high Rag1/2 expression alleviate Rag off target DNA cleavage, thereby offering the possibility of new therapeutic approaches.

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Session 4: New insights from animal models

Do electromagnetic fields contribute to B-ALL development in genetically predisposed mice?

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In the course of the EU FP7 ARIMMORA project (Advanced Research on Interaction Mechanisms of electroMagnetic exposures with Organisms for Risk Assessment), a new transgenic mouse model of childhood B acute lymphoblastic leukemia (B-ALL) was generated in which the human B-ALL-associated first genetic lesion, ETV6-RUNX1, is expressed in the stem/ progenitors compartment of the hematopoietic system. Breeding pairs of these transgenic mice, and their progeny, were exposed to a 50 Hz magnetic field of 1.5 mT with both fundamental and harmonic content, with and an on/off cycle of 10 min/5 min, 20 hours per day until three months of age. Analysis of the bone marrow of unexposed mice showed that, at 6 months of age, specific alterations in B-cell development could already be detected in the form of an increase in BM pro/pre-B-cells and immature B-cells. However, like in humans, the leukemic process in the mouse model does not develop very rapidly. Moreover, the appearance of this fusion protein in the mouse model does not commit the premalignant target cells to develop malignant disease, as a major proportion of the model animals developed no alterations, similar to observations in children who harbor the ETV6-RUNX1 fusion gene but never develop B-ALL, suggesting that secondary cooperative changes in the mouse genome seem to be necessary for disease expression. Thus, the transgenic mouse model generated is ideal for in vivo modelling of possible electromagnetic field effects in B-ALL.

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Session 5: Genetics, epigenetics and the environment

Genetic predisposition: Genes and environment

Christian Kratz | Pediatric Hematology and Oncology, Hannover Medical School, Germany

Approximately 2000 cases of childhood cancer are diagnosed annually in Germany. In contrast to adult oncology where environmental factors such as smoking and alcohol contribute in a significant manner to tumorigenesis, these external factors appear to be less contributory in pediatric cancers. The only known quantitatively relevant cause of childhood cancer is genetic cancer predisposition. Over 100 cancer predisposition genes (CPG) that are mutated in patients with cancer prone syndromes (CPS) have been identified and recent studies indicate that germline mutations in CPG occur more frequently than previously thought. Table 1 shows selected childhood cancers as well as examples of relevant CPG.

Table 1. Selected cancer types and predisposing genetic factors

CANCER TYPE	SELECTED PREDISPOSING FACTORS
Acute lymphoblastic Leukemia	Trisomy 21, TP53, ETV6, PAX5, ATM, NBS
Acute myeloid leukemia	Trisomy 21, RUNX1, CEBPA
Myelodysplastic syndrome	GATA2
Juvenile myelomonocyic Leukemia	NF1, CBL, PTPN11, KRAS
Medulloblastoma	SUFU, TP53, APC, BRCA2, PTCH1
Glioblastoma	MLH1, MSH2, MSH6, PMS2, TP53
Pilocytic astrocytoma	NF1
Osteosarcoma	TP53
Neuroblastoma	ALK, PHOX2B
Nephroblastoma	WT1, REST
Rhabdomyosarcoma	TP53, DICER1, HRAS, PTCH1
MPNST	NF1
Rhabdoid tumor	SMARCB1, SMARCA4
Pleuropulmonary blastoma	DICER1
Cystic nephroma	DICER1
Hepatoblastoma	APC
Retinoblastoma	RB1

In contrast, known childhood cancer environmental risk factors are rare and include ionizing radiation (e.g. Chernobyl radiation fallout: thyroid cancer), immunosuppressive therapy (e.g. Non−Hodgkin's lymphoma), treatment with diethylstilbestrol (adenocarcinoma of the vagina), and infections (e.g. Epstein Barr virus: Burkitt's lymphoma).

My talk will provide a concise overview on the causes of childhood cancer and I will discuss ways on how to move the field forward.

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Session 5: Genetics, epigenetics and the environment

Sensitivity of the early human epigenome to environmental and genetic influences

Richard Saffery | Royal Children's Hospital – Murdoch Children Research Institute, Australia

It has become increasingly clear that both the early life environment and genetic variation contribute to the risk of a range of non–communicable diseases, including childhood cancers such as leukemias. There is often a long latency between specific "exposures" *in utero* and later manifestation of disease. Several mechanisms have been proposed to mediate the 'biological embedding' of exposure information, including epigenetic variation induced early in development. Evidence for a direct link between environmentally–induced epigenetic variation and childhood cancer risk remains inconclusive.

Recent data have demonstrated an important role for genetic variation in 'shaping' the epigenetic profile throughout life and also suggest this plays a role in mediating the effects of environmental exposures on epigenetic variation. Many of the common translocations and mutations seen in childhood cancers disrupt the activity of genes regulating epigenetic processes. Further, specific *in utero* exposures (such as maternal smoking and folate insufficiency) induce defined epigenetic changes in newborns, some of which persist into adulthood. Mounting evidence also links early epigenetic variation to later onset phenotypes including cancer. In fact, all human cancers have a disrupted epigenetic profile in addition to genetic lesions, though the former appears especially prevalent in childhood tumours. Thus there appears to be a complex interplay between genes, environment and time in shaping the epigenome and risk of diseases such as cancer.

Epigenetic research is advancing at a rapid pace but remains in its infancy. The recent adoption of standardised platforms for analysis, the advent of several very large prospective birth cohorts, and the formation of large consortia to build sample sizes with power to detect small magnitude effects, represent major advances. Ultimately, a complementary approach encompassing, (i) well controlled animal studies, (ii) large longitudinal observational cohorts (including twins) with comprehensive environmental measures and biospecimens, and (iii) detailed molecular interrogation of clinical cases, represents the best way forward to firmly establish epigenetic variation as a mediator of cancer risk in children.

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Session 5: Genetics, epigenetics and the environment

Epigenetic precursors of childhood cancer and associated early-life exposure

Akram Ghantous | International Agency for Research on Cancer, France

Childhood cancer, though rare, remains the first cause of disease-related death in children, with increasing incidence worldwide. Its risk factors are largely unidentified but could be predetermined during in utero development. During embryogenesis, a global redistribution of DNA methylation occurs to enable tissue differentiation. Hence, DNA methylation is a potential sensor of environmental exposures during development and may persist later in life. We profiled the genome-wide methylation levels in cord blood samples from the International Childhood Cancer Cohort Consortium (I4C), the largest mother/child birth cohort of childhood cancer.

Starting with one of the largest I4C cohorts, the Norwegian Mother and Child Cohort (MoBa), DNA methylation levels of more than 450,000 cytosines were compared (using HM450-BeadChip) between nested cases of childhood cancer (n=80, representing similar proportions of leukemias, central nervous system tumors and other tumors) and control subjects followed-up for the same time period (n=160). We identified a differentially methylated 200-bp region in leukemias relative to controls (FDR<0.05). A mean difference of 5-10% methylation was consistently found across 8 CpG sites in this region and was validated using bisulfite pyrosequencing. The observed association was not influenced by covariates such as blood cell subtype distribution, gender or birth weight. This potential epigenetic signature of childhood leukemia is currently being replicated and analyzed in relevance to early-life exposure factors in other I4C cohorts. Preliminary findings suggest a role for early-life infection and maternal use of hormone contraception preceding pregnancy.

These findings may place DNA methylation in the causal pathways linking early-life exposures and childhood leukemia and may contribute to a 'leap forward' in deciphering mechanistic precursors of childhood cancer. [Acknowledgement: INSERM/INCA grant and the IARC Postdoctoral Fellowship-Marie-Curie-Actions-People-COFUND].

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Session 6: Ongoing studies and looking forward – what to do next?

The International Childhood Cancer Consortium (I4C)

Terry Dwyer | George Institute for Global Health & Nuffield Department of Obstetrics and Gynaecology, University of Oxford, United Kingdom/Australia

The International Childhood Cancer Cohort Consortium (I4C) was established in 2005. Its purpose is to bring together large birth cohorts globally to pool their data, providing the first adequately powered prospective evidence on many causes of childhood cancer (CC).

The consortium has now assembled data on almost 400,000 participants, among whom 670 childhood cancers have occurred, with almost 200 leukemias. The first publication in 2015 reported a positive association of birth weight with CC in accord with the findings of previous case–control study and record linkage studies.
The current focus concerns analysis of data on birth order, taking into account birth weight and other factors. Results of this will be discussed. In addition, work is planned on the testing of hypotheses related to maternal infection during pregnancy.
This will involve analysis of biospecimens as well as questionnaires from several of the participating cohorts. Plans for this work in which the International Agency for Research on Cancer will play a significant role will be presented.

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Session 6: Ongoing studies and looking forward – what to do next?

Childhood leukaemia around the globe – what can we learn from observations from developing countries?

Friederike Erdmann | International Agency for Research on Cancer (IARC), France & Childhood Cancer Survivorship Research Group, Danish Cancer Society Research Center, Denmark

Higher childhood cancer incidence rates, particularly for leukaemia, are reported from high-income countries versus lower income countries. Estimating childhood cancer incidence globally is however hampered by lack of reliable data from developing countries, including Latin America and especially Sub-Saharan Africa. Observed geographical differences in incidence rates may indicate that unique genetic or environmental exposures may affect the risk of childhood leukaemia and have been used to support several hypotheses related to aetiology. However, recent evidence from Brazil and India suggests that observed incidence differences across countries may also reflect under-ascertainment of cases in low- and middle-income countries. A better understanding of the true incidence in poor-resource countries and potential for under-diagnosis and under-reporting might contradict some aetiological hypotheses and contribute to a better understanding of the aetiology of childhood leukaemia.

Unique findings on childhood cancer incidence rates with a particular focus on leukaemia from the pathology - based National Cancer Registry of South Africa (SA) as well as from the nationwide population-based cancer registry of Costa Rica will be presented and discussed in a global perspective.

Incidence rates of childhood cancers in South Africa tended to be 3-4-fold higher in South African Whites compared to Blacks. With an age-standardised incidence rate (ASR) of 23.5/million for South African Whites and 6.9/million for South African Blacks, particularly incidence rates of leukaemia were considerably lower compared to incidence rates observed in economically developed populations. Genetic and environmental factors may explain only partly the substantial observed differences in incidence rates between racial groups in SA and compared to patterns of high-income countries. Socio-cultural factors related to access and utilization of health care services and health care seeking behaviour are likely to explain at least some of the differences. Childhood cancer incidence patterns in Costa Rica were closer to those reported from high-income countries than those reported from other developing countries. With an ASR of 58.1/million the observed leukaemia rate was among the highest in the world. Nevertheless in infants it was considerably lower than in high-income countries. Further research is recommended to explore which factors may drive the high overall leukaemia rate, the low leukaemia rate in infants as well as some low rates observed for some solid tumours. Overall the findings suggests applying caution when interpreting geographical variation, as this example of a developing country with established paediatric oncology and a well-functioning cancer registry showed small differences to childhood cancer incidence patterns in high-income countries.

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Session 6: Ongoing studies and looking forward – what to do next?

Childhood leukaemia epidemiology from a global perspective

Joachim Schüz | International Agency for Research on Cancer, Section of Environment and Radiation, France

With an estimated total of 215,000 new cases per year on a global scale, childhood cancer accounts for 1–4% of all cancers, depending on which continent you are. Childhood leukaemia is the most common cancer type diagnosed in children worldwide, accounting for about one third of all childhood cancers in economically developed countries to one fifth or less in some developing countries. Apart from this large geographical variation, there are other descriptive features that may pose hints on its aetiology. The most common type of leukaemia, acute lymphoblastic leukaemia (ALL), peaks in incidence at ages 2–5 years, with this peak most pronounced in developed countries and there increasing in prominence over the past century with economic development. Boys are slightly more affected than girls, with ratios normally between 1.2–1.4. Notably, pre–leukaemic cells were detected in neonatal blood spots suggesting a first hit in disease causation before birth, whereas data on how many healthy children have the same chromosomal damage varies by study, but common agreement is that a second hit is needed for overt leukaemia.

Especially the large geographical variation, driving some of the hypotheses suggested for ALL aetiology, is questioned of how much it is true underlying incidence difference as compared to incomplete registration or, even more important, lack of diagnosis of affected children. Detailed analyses of registry data in some developing countries but also reports from the local paediatric oncologists suggest such artefacts may play an important role, Hence, there is not only uncertainty on the majority of causes of childhood leukaemia but also on what the basic observational data tells us.

The IARC has recently established the collaboration network GALnet (Global Acute Leukaemia network) with 19 participating paediatric oncology units around the world, complemented by epidemiologists and biologists. One aim is to go global in aetiological research as it is believed learning from diversity in exposures and disease will overcome limitations faced with the main research restricted to Europe and the North Americas.

This review talk will give an overview on childhood leukaemia epidemiology from a global perspective and how networks such as GALnet can contribute to change.

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