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Childhood Leukaemia — Mechanisms and Causes

Proceedings of a scientific seminar held in Luxembourg on 3 November 2009

Working Party on Research Implications on Health and Safety Standards of the Article 31 Group of Experts

Directorate-General for Energy
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This publication contains the contributions made by leading scientists in a seminar organised by the European Commission on childhood leukaemia, its mechanisms and causes. The views and opinions of originators expressed herein do not necessarily state or reflect those of the European Commission and should not be relied upon as a statement of the Commission's views.

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FOREWORD

Luxembourg, June 2010

Under the terms of the Treaty establishing the European Atomic Energy Community, the Commission, amongst other things, establishes uniform safety standards to protect the health of workers and of the general public against the dangers arising from ionizing radiation. The standards are approved by the Council, on a proposal from the Commission, taking into account the opinion of the Group of Experts referred to in Article 31 of the Treaty. The most recent version of such standards is contained in Council Directive 96/29/Euratom of 13 May 1996 laying down basic safety standards for the protection of the health of workers and the general public against the dangers arising from ionizing radiation.

The European Commission organises every year, in cooperation with the Group of Experts referred to in Article 31 of the Euratom Treaty, a Scientific Seminar on emerging issues in Radiation Protection – generally addressing new research findings with potential policy and/or regulatory implications. Leading scientists are invited to present the status of scientific knowledge in the selected topic. Based on the outcome of the Scientific Seminar, the Group of Experts referred to in Article 31 of the Euratom Treaty may recommend research, regulatory or legislative initiatives. The European Commission takes into account the conclusions of the Experts when setting up its radiation protection programme. The Experts' conclusions are valuable input to the process of reviewing and potentially revising European radiation protection legislation.

The 2009 Scientific Seminar discussed *Childhood Leukaemia – mechanisms and causes*. Five internationally renowned scientists working in the field of childhood leukaemia presented current knowledge. The speakers offered a general overview on advances in childhood acute leukaemia, followed by a presentation on risk factors of childhood leukaemia – the French research programme, a review of identified and possible aetiologies of childhood leukaemia, a summary of the ionising radiation epidemiology of childhood leukaemia, and a report on childhood leukaemia around nuclear installations. The presentations were followed by a round table discussion, in which the speakers and invited additional experts discussed potential *policy implications and research needs*.

The Group of Experts discussed this information and drew conclusions that are offered for consideration by the European Commission and other international bodies.

Augustin Janssens
Head of Radiation Protection Unit

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1 INTRODUCTION

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Leukaemias are a haematological cancer of the leukocytes, the cells that defend the body against infectious diseases. There are two main types of white blood cells, myelocytes and lymphocytes, to which respectively correspond myeloid leukaemias and lymphoid leukaemias. These illnesses result in a dramatically excessive proliferation of white blood cells, which leads to an invasion of the bone marrow, the lymph nodes and the spleen with an excessive accumulation of cells in the blood flow, ending with an extension of malignant colonisation in various tissues of the body, especially the liver and the central nervous system. Leukaemias can appear as an overpopulation of moderately differentiated cells and develop in a chronic manner or, quite the opposite, result in an invasion of young, poorly differentiated cells, with acute evolution.

Childhood leukaemias are leukaemias with a proliferation of young cells, which evolve in an acute manner. The yearly incidence is around 4 per 100,000 children. They mainly involve lymphoblastic leukaemias, which account for more than 80% of cases, whereas acute myeloblastic leukaemias account for less than 20% of cases. A peak in frequency has been observed between the ages of two and five years, with a slight predominance in boys compared to girls (ratio of 1.2) for lymphoblastic leukaemias, whereas the age distribution is uniform for myeloblastic leukaemias. In terms of public health, leukaemias represent the predominant malignant disease in children, namely around 1/3 of all paediatric cancers. They can appear in the form of geographic groups or clusters[1]. It is precisely the occasional association of such clusters with the presence of a nuclear installation and the fact that ionising radiation is capable of causing leukaemias that has led to the role of radioactive releases from such installations being called into question at various times, for example in England (Seascale – Sellafield nuclear plant), Scotland (Thurso – Dounreay nuclear plant), France (Beaumont – La Hague reprocessing plant) and Germany (Elbmarsch – Kruemmel power plant). The purpose of the 2009 seminar organised by the Group of Experts referred to in Article 31 of the Euratom Treaty was to review the state of knowledge of the risk of leukaemia in children, to deliver advice on the likelihood of the relation with nuclear installations and to draw conclusions regarding the regulatory implications of radiation protection and research aimed at further elucidating the aetiology of this disease.

Several factors, some harmful, others protective, have been evoked with regard to the onset of childhood leukaemias. None of these factors results in a specific signature and consequently the assumed relations between these factors and leukaemias are based on statistical criteria and a possible likelihood with regard to the physical, chemical and biological mechanisms that can logically be invoked. The basis of the malignant transformation is a modification of the genome, generally multifocal, accompanied by an adaptive epigenetic evolution. This process as a whole leads to the emergence of a long-lasting clone in which the barriers that normally limit the expansion of a cell line are no longer working. In childhood leukaemias, chromosomal translocations have been identified in a recurrent manner that generate, through fusion, chimeric genes specific to the type of leukaemia and its prognosis. As regard lymphoblastic leukaemias, the translocation t(12,21) → TEL-AML1 fusion is predominantly observed and, less frequently, the translocations t(4,11)

[1] The expected number of cases is low so that data collection has to be made on several years and the statistical analysis is sophisticated.

→ MLL-AF4 fusion, t(1,19) → E2A-PBX1 fusion and t(9,22) → BCR-ABL fusion. In the case of myeloblastic leukaemias, the translocations t(8,21) → AML1-ETO fusion, t(9,11) → MLL-AF9 fusion and t(15,17) → PML-RARα fusion are mainly found. It has been established that many of these chromosomal rearrangements, in particular TEL-AML1, take place during intrauterine life. They can be detected in the blood of the umbilical cord in around 1% of newborn babies. Genes of interest can also be transformed through chromosomal inversions and point mutations. Added to that are aneuploidies, deletions (TEL gene) or on the contrary amplifications (AML1 gene). Complementary oncogenic mutations, adjuvant of the process, have been identified or are strongly suspected. Such mutations involve in particular the constitutive activation of receptor tyrosine kinases (FLT3 and KIT), of the G protein RAS and of the transcription factor MYC, which contribute to initiate clonal proliferation.

The leukaemia genes, which act upstream in the functional tree of the genome, interfere with other genes and control the expression of a great number of them, in particular the morphogenetic homeobox genes. When they are transformed, they redirect the economy of the cell towards clonal expansion. Some bring about a loss of differentiation (for example TEL-AML1), while others cause a mitotic overpressure (for example BCR-ABL or amplification of AML1). Nevertheless, in all cases, it is the constitutive expression of an oncogene that is the "match" that actually sets off the cancerous process. Young cells have high mitotic ability (Fig.1). This property, necessary for tissue construction and the regeneration of cell lines, is also the Achilles' heal in cancer terms since it facilitates the action of oncogenes. One of the characteristics of the infantile leukaemia genome is the blockage of differentiation, leading to a population of young, ready to multiply cells. Consequently, the over-expression of an oncogene, established with success in one of the cells, creates a clone of poorly differentiated cells, lymphoblasts or myeloblasts, which invade the body. Thus, for example, among children who have the differentiation inhibitor fusion gene TEL-AML1 in the cells of their umbilical cord, around 1% develop a lymphoblastic leukaemia in the early years of their life. Although the non-differentiation may be explained by relating it to the chromosomal lesions affecting the genome, it is on the other hand not known why it is these lesions that are precisely privileged in children.

As is generally the case in cancers, all events likely to lead to such anomalies of the genome may potentially be involved in leukaemic transformation. Consequently, the following factors may be considered as a risk:

- **The immunological context**. Lymphocytes are antibody factories and the genes involved in the production of immunoglobulins (B lymphocytes) and antigen receptors (T cells) undergo, in a physiological way, rearrangements through the action of VDJ[2] recombinases. Certain chromosomal translocations, which occur in a characteristic manner in lymphoblasts, could be linked to an inappropriate action of recombinases. Clonotypic rearrangements of immunoglobulin chains and T cell antigen receptors are present in leukaemic clones.
- **Common infections**. Concerning common infections, it seems that the period of exposure of the infant to pathogenic agents could play a role. At an early stage, could it protect the child (like the protective effect of being placed in a day nursery) whereas, at a later stage, could it be unfavourable? One explanation may be based on a differential proliferative advantage between normal and preleukaemic clones, as follows: early stimulation of the immune system will promote normal, producing immunoglobulins lymphocytic clones, the expansion of which will stifle the not immunologically efficient preleukaemic clone, thus reducing the number of target cells for the subsequent leukaemogenic hit. Conversely, if exposure to infections occurs late, the preleukeamic clone will have enough time to significantly expand, thus presenting a lot of favourable target cells for the subsequent hit. Similarly,

[2] VDJ = immunoglobulin gene domains, V = variable, D = diversity, J = junction.

extended breast-feeding could be protective, in this case via substances present in the mother's milk that could have a stimulating effect on the synthesis of immunoglobulins, as for instance non virulent antigenic substances.

- **Viral infections**. This is a very relevant hypothesis but at this time no such leukaemogenic virus for childhood leukaemias has been identified. However, if the virus operates through a hit-and-run mechanism, the question is far from being solved.
- **The effect of ionising radiation**, particularly through the creation of double-strand breaks in the DNA, conductive to the risk of illegitimate chromosomal recombinations.
- **Inhibitors of topoisomerase II**, an enzyme involved in the topology of DNA by modifying the degree of coiling through double-strand breaks and joins. This mechanism concerns, in a ubiquitous manner, the various processes (transcription, replication, repair) involving variations in DNA configuration. Topoisomerase inhibitors are present in certain antibiotics, certain cancer chemotherapies and in some natural foodstuffs (fruit and vegetables rich in flavonoids).
- **Folate deficiency**. Folates are vitaminic substances (vitamin B9) that act in the synthesis of nucleotides destined for DNA and RNA.
- **Exposure to toxic chemicals**. These include, for example, pesticides, insecticides and various organic molecules like benzene that can alter the genome in various ways.
- **Hereditary chromosomal anomalies**, in particular Down syndrome (trisomy 21), Fanconi's disease (mutations of genes involved in reparation of DNA double strand breaks, chromosomal instability), ataxia telangiectasia (mutation of the ATM gene that manages reparation of DNA double strand breaks) and the Bloom syndrome (mutation of the BLM gene that participates to DNA replication, substantial rate of chromatin sister exchanges).
- **A tendency towards overweight and obesity**.

To summarise, childhood leukaemias present a highly diversified genetic typology[3] that determines their evolution and prognosis. However for the moment there is no causal typology, in other words no etiological signature. In comparison with endogenous causes, whether natural or spontaneous, inherent in the hazards of biological processes, a lot of exogenous causes have been evoked and either clearly established or simply suspected. Faced with clusters of leukaemia cases, a statistical analysis associated with likelihood or unlikelihood criteria of a suspected cause may simply be used as a basis. For example, if radioactivity is suspected, the likelihood needs to be evaluated from estimated doses and dose-effect relations that have been established in sufficiently well-consolidated epidemiological studies. In fact, for the moment, it is not possible to explain all the links in the chain, from exposure to a suspected nuisance to the genomic modification producing the leukaemia. Many elements of a biological nature, which would enable a more deterministic and thus a less contestable approach to the causality, are still lacking.

[3] In addition to polymorphism and complexity of the genetic anomalies, it is noteworthy that AML1 gene has an ambivalent action, participating to the loss of differentiation when it is combined with TEL gene whereas playing the role of an oncogen when it is overexpressed through amplification.

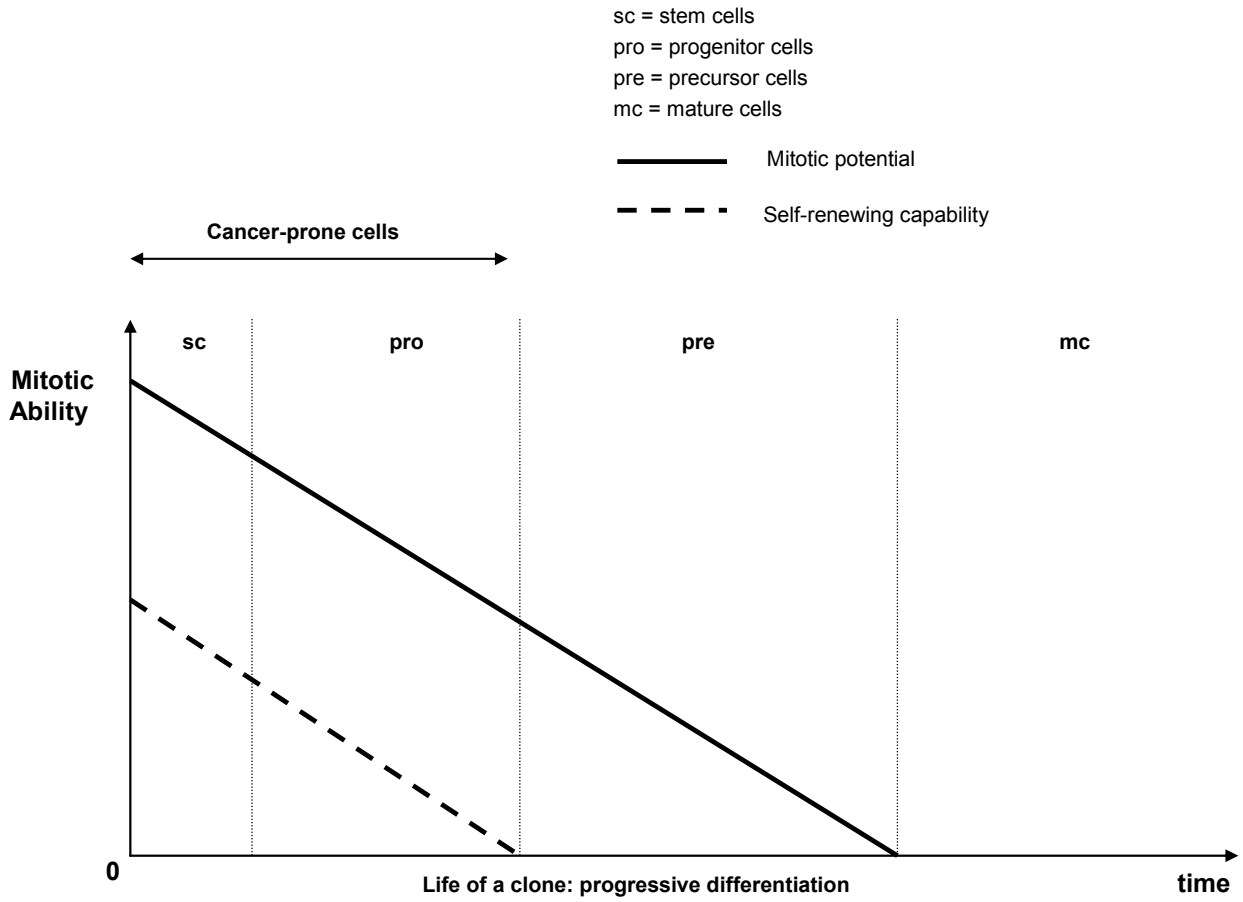

Figure 1: Downward trend in mitotic ability over the lifetime of a clone. The blockage of differentiation creates a young, self-renewable population with high mitotic potential. This provides an extremely favourable environment for the onset of cancer in the case of constitutive expression of an oncogene.

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2 CHILDHOOD LEUKAEMIA – GENERAL OVERVIEW AND ONGOING STUDIES

Danièle Sommelet / Jacqueline Clavel

2.1 Acute leukemia in childhood: a multitude of diseases

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2.1.1 Summary

The stratification of acute leukemias, notably the lymphoblastic acute leukemias in childhood is based on clinical and biological conventional criteria, but mainly on the genetic abnormalities involved in the leukaemia genesis along its multistep development.

The heterogeneity of this disease should be taken into account, not only as prognostic factors to adapt the best therapeutic program, but also in the epidemiological research studies, aiming to establish potential relationships between some molecular subtypes of ALL and AML and some genetic and/or environmental factors.

International large prospective studies should be needed to obtain statistically significant findings.

2.1.2 Introduction

Acute leukaemia is the consequence of a malignant process coming from a stem-cell possessing multilineage development or from a single hematopoietic lymphoid (85 %) or myeloid (15 %) precursor cell, combined with a dysregulation of the following programs, due to acquired genetic abnormalities with consequences on proliferation, differentiation, senescence and apoptosis.

It is the most frequent childhood cancer in developed countries (30 % of cases) and represents 470 new cases per year in France between 0 and19 yrs of age, 420 in 0-15 yrs patients and 50 in 15-19 yrs patients. The peak age of 2 to 4 yrs in acute lymphoblastic leukemias (ALL) is well known, while there is no peak age in acute myeloid leukemias (AML) (Clavel *et al,* 2004).

Previous and current studies have been focused on the improvement of treatment based on a better knowledge of prognostic factors, notably on the molecular events responsible of the origin and the development of the disease. However, the causes of childhood leukemias remain questionable, in spite of a lot of recent epidemiological studies (Rossig and Juergens, 2008). It is important to observe that most of them are focused on the potential etiological factors, without a precise correlation with leukemic genotypes.

2.1.3 Interactions between the patient and his disease

They contribute to the clinical and biological characteristics of the leukaemia and influence also their epidemiological correlations.

2.1.3.1 As regard to the patient

Predisposing genetic syndromes are known in about 5 % of cases, as Down'syndrome, immunodeficiencies, chromosomal instability syndromes.

Other factors are also potential, **as genetic variability in xenobiotic metabolism** (drugs, environmental agents)**,** DNA repair pathways, cell-cycle checkpoint function or **HLA D class II alleles** (Taylor *et al.,* 2008).

Subtle genetic alterations or polymorphisms may affect the response to specific environment exposures. So, the combination of parents and children genotypes have to be better studied.

2.1.3.2 As regard to the leukemic cell

Cytology and immunophenotype allow, not only the diagnosis and the lineage of the leukemias, but they contribute also to the prognosis, the evaluation of response to therapy (residual disease) and may lead to a targeted treatment.

The most impressive data are brought by the development of conventional and molecular cytogenetics, target molecular biology, global approaches (FISH, RT-PCR, single nucleotide polymorphism microarrays, genomic DNA sequencing, proteome) allowing : - to refine the prognosis at diagnosis and relapses ; - to adapt the treatment according to minimal residual disease ; - to approach the sequential genomic and signalling pathway alterations.

40 % of BP-ALL present associated genome abnormalities (PAX5, IKAROS, EBF1) confirming the relationship between leukemogenesis and troubles of differentiation (Mullighan *et al.* 2009).

So, it becomes possible to better understand the process of leukemogenesis and the subsequent consequences of genetic abnormalities. The knowledge of the role of some immunological responses and of neoangiogenesis underlines the additional role of bone-marrow environment.

2.1.4 Advances in acute leukemias

The best important advance consists in the increase of survival rate, notably along the last twenty years, from 1 % in 1960 to 80-85 % presently in ALL and 60 % in AML. That is explained : - by the therapeutic progresses due to the activation of (inter) national clinical research associated with improvement of supportive care ; - and by a better knowledge of prognostic factors, aiming to stratify the patients, with the decrease of secondary effects in low-risk patients and the reinforcement of treatment in high risk patients. Nevertheless, present and future advances depend on the improved understanding of genetic abnormalities involved in leukemogenesis, the follow-up of response rate to chemotherapy and the development of new target drugs aiming to cure more and better (Pui *et al.,* 2008).

Obviously, we must also underline the impact, since the seventies, of a **strict organisation of pediatric onco-hematology**, everywhere, with the same objectives, the same rules, the development and the recognition of referent clinical and biological units/centres, with appropriate interdisciplinary skills and technical equipment.

Currently, more **and more sophisticated biological studie**s allow: - to contribute to understand the mechanisms of treatment resistance and therapeutic innovations; - to propose epidemiological studies combined with precise genomic definition of leukemic cells; - and perhaps in the future to prevent the diseases.

2.1.5 Current risk stratification of acute leukemias

Whatever its type (ALL, AML), the stratification is based on the following criteria: cytology, clinical variables (age, white cell count), immunophenotype, cytogenetic and molecular typing, response to chemotherapy) (Pui *et al*., 2008, Vrooman *et al.,* 2009).

To illustrate the large variety of leukemias in every type of AL, we will take the example of the prognostic factors at diagnosis of **B precursor cell ALL (BPC)** used to define risk groups and to adapt the treatment.

In spite of several differences in therapeutic protocols, there is an agreement on the **conventional following prognostic criteria**, leading to be used currently : - the **age** less than one year (and more than 10 yrs in some trials) with a 5 yr and event-free-survival (EFS) of 30-50 % ; - the white cell count (less of more than 50 000/mm^3) ; - there is no difference between L1 and L2 (FAB) cell type, but L3 (Burkitt type) must be separated and treated differently; - as regard to the immunophenotype, the 5 yr EFS in BPC-ALL remains better than in T precursor cell ALL (85 % versus 75 %) in spite of recent improvements in their treatment.

In summary, cooperative groups consider presently the conventional following four types of lymphoblastic leukemias.

▪ In infancy (less than one year of age, 2 % of ALL), 70 to 80 % of children present a severe ALL, proB (CD19+, CD10-) with a rearrangement of MLL gene located in 11q23.

▪ The children presenting a standard risk preB ALL (55 %) : 1 to 10 yrs of age, white cell count less than 50 000/mm^3, absence of poor prognosis cytogenetic or molecular criteria ; hyperploïdy > 50 chromosomes, trisomies associated with chromosomes 4, 10, 17, translocation 7 (12,21)/TEL-AML1.(Rubnitz *et al*., 2008).

▪ The children with high risk ALL (30 %): more than 10 yrs of age or leucocytosis more than 50 000/m^3; among them, a very poor prognosis is related to r(9,22)/BCR-ABL, t(4;11)/MLL-AF4 and other rearrangements of MLL, hypodiploïdy < 45 chromosomes.

▪ The children with T cell leukemias (15 %) ; specific therapeutic protocols have improved their prognosis.

Evidently, new sophisticated genetic studies will refine the conventional prognostic stratification and will impact on the therapeutic decision and the knowledge of epidemiology. More or less combined with previous conventional risk factors, **cytogenetic and molecular genetics,** appear presently the most important criteria.

Some high risk groups of patients with unfavourable genetic criteria represent 10 % of BPC-ALL (Table 1).

Table 1: Unfavourable genetic criteria (BPC-ALL)

	Incidence	5 yr EFS
• t (9;22) (q34; q11) / BCR – ABL fusion	2-3 %	20 > 40 %
• IKZF1 mutation or del, without BCR – ABL		20 %
• t (4;11) (q21;q23) MLL – AF4 fusion	2-3 %	30-50 %
• hypodiploïdy ≤ 44 chromosomes	1-2 %	40-50 %
• intrachromosomal amplification of chr.21		29 %

These patients affected by poor prognostic leukemias need for a more aggressive treatment and in the future a targeted treatment.

Deletion of IKZF1 which encodes the lymphoid transcription factor IKAROS is a frequent event in BCR-ABL1-positive ALL and in the lymphoid blast crisis of chronic myeloid

leukaemia. Deletion of mutation of IKZF1 is a predictor of very poor risk in BCP-ALL, independently of BCR-ABL/fusion. This genetic alteration may also emerge at the time of relapse (Mullighan *et al.,* 2009).

On the contrary, **patients without genetic unfavourable criteria** may perhaps receive a less aggressive treatment, at least in some of them (Table 2).

Table 2: Non unfavorable genetic criteria (BPC-ALL)

	5 yr EFS
• Hyperdiploidy (> 50 chromosomes)	85 – 90 %
• t (12;21) TEL – AML1 fusion (RUNX1)	85 – 90 %
• t (1;19) E2A – PBX1 fusion	85 – 90 %

In T cell ALL, cytogenetics seems of less prognostic value than in precursor B-ALL. However, TLX3/HOX11 expression is considered as responsible of a poor outcome (in Fralle 93 protocol). Cryptic molecular changes influence also negatively the prognosis of some T cell ALL (Borowitz *et al*., 2008).

2.1.6 Response to treatment. Minimal residual disease: a major criteria of therapeutic decisional value in ALL

Early (or not) sensitivity to treatment is evaluated in blood and bone–marrow by cytology and mainly by molecular and flow-cytometric methods (Borowitz *et al.,* 2008). Minimal residual disease (MRD) is detectable at a very low level, less that 0,01 %). Two early points are usually determined at d21/29 and at day 35/42, allowing to define :- low risk (MRD $\leq 10^{-4}$) ;- high-risk (MRD > 10^{-2}) - and intermediate risk (MRD < 10^{-2} and > 10^{-4}).

In Germany, it is recommended to test also the cortico-sensitivity at day 8 on peripheral blood. Recent studies insist on the value of late monitoring at 12 months, 24 months after previous disappearance, to detect a relapse, sometimes several months before conventional symptoms.

As published by Bhojwani *et al.* (2008), the gene expression signature may be predictive of early response and outcome in a sample of high-risk patients (bone-marrow evaluated at day 7 after onset of chemotherapy) : apoptosis-facilitated genes were upregulated in rapid-responders, while multiple genes involved in cell adhesion, proliferation, anti-apoptosis were upregulated in slow-responders.

So, it is useful to underline the importance of the analysis of gene expression profiles that contributes to a good approach of biologic understanding of why clinical and laboratory variables are associated with outcome. Nevertheless, until now, no potential links with some precise genetic or environmental factors are described.(Sikic *et al*., 2008).

Once more, this is a demonstration of the fact that childhood AL (notably ALL), include a multitude of diseases, product of alterations to the germline genetic and epigenetic code ; this clonal disease is linked mainly to translocations (fusion transcription factors or activated signalling kinases) and other genetic anomalies: aneuploïdy, deletions in cell-cycle checkpoint genes and mutated genes.

2.1.7 Multistep development of acute leukemias

A lot of studies conducted since more than ten years conclude that the origin of leukemias (ALL and AML) happens **during the prenatal life** (Wiemels, 2008, Wiemels *et al*., 2008). The reasons are based on the following points:

- the short latency between foetal life and post natal onset of AL diagnosed in infancy or mainly between 2 to 5 yrs; - the extreme developmental and cellular kinetic stress of the foetus; - the concordance of leukaemia in twins; - the discovery of preleukemic clones (about 1 %) in archived newborn bloods.

The described preleukemic clones concern:

- some rearrangements of MLL genes at 11q23 with chromosomes 4,9,19 (observed in 80 % of AML and 60 % of ALL in infants). MLL rearrangement is also associated with secondary ALL after previous exposure to topoisomerase II inhibitor; so dietary, medical and environmental exposures to some substances that inhibit topoisomerases combined with the reduced ability of foetuses or their mothers to detoxify such agents could lead to infant ALL;
- some rearrangements of ETV6 at chromosome 12 with RUNX1 on chromosome 21 (TEL-AML1) in 25 % of ALL; (Hong *et al*., 2008);
- some rearrangements of RUNX1/ETO at chromosome 8 in 15 % of AML;
- trisomy 21;
- NOTCH1 mutation in T-ALL.(Armstrong *et al.,* 2009 ; Eguchi-Ishimae *et al.,* 2008).

So, human embryonic stem-cell differentiation may become a promising human system for studying leukemogenesis and to assess the role of in utero exposure to some mutagen agents.(Wiemels *et al.,* 2008).

Except in some cases (infancy), after a first hit, **secondary oncogenic events** appear as obviously needed ; only 1 % of the children presenting prenatal clone cells (ex : positive for TEL-AML1) will become leukemic patients (role of mixed populations ? role of infections ? role of immunological abnormal stimulations ?) (Kinlen, 2004 ; Greaves and Buffler, 2009)). In Down's syndrome, the acquired mutation of GATA1 leads in 5-10 % of children to a transient "leukaemia" at birth ; a leukaemia (often M7 type) may follow further mutations. After a Notch1 mutation, the SIL-TAL fusion may explain the appearance of a T.ALL (Eguchi-Ishimae *et al*. 2008).

We will come back to TEL-AML1 leukaemia fusion, ETV6-RUNX1 (t 12,21), the most common chimeric fusion gene of B precursor cell ALL. It constitutes a preleukemic phenotype, predominantly in utero. TEL-AML1 induces a population of self-renewing human cord blood cells at a very early B cell stage (first hit) to sustain a persistent preleukemic state and interferes with the TGFβ pathway.

Responsible of the inhibition of responses to TGFβ, TEL-AML1 cells proliferate slowly until the 2nd hit. Dysregulation of TGFβ signalling by TEL-AML1 protein blocks cell differentiation, suppresses proliferation of cells, interferes also with the regulation of immunologic and inflammatory reactions. So, it is an argument in favour of a **dysregulated immune response to infection** (2nd hit) and can explain the malignant evolution of the TEL-AML1 preleukemic clone, usually after 12p deletion (Wiemels *et al*., 2008 ; Ford *et al*., 2009)

Pharmacogenetics

We have also to take into account the influence of **polymorphisms of genes involved in several metabolic pathways**. It is known that some of them may alter the activity of drug metabolizing enzymes, with some consequences on the efficacy and the toxicity of therapy. Other genes may influence the risk for ALL. We can give these examples: - genes involved in folate metabolism pathways (DNA synthesis and repair, methylation processus); - genes P450 and glutathione-S-transferase enzymes; - role of multidrug resistance gene (MDR1); - genes preventing oxidative stress and toxic metabolites.

2.1.8 Perspectives

Face to the present advances in the field of leukemias, we need for a better evaluation and understanding of the heterogeneity and complexity of acute leukemias:

- development of new molecular techniques, animal models, human embryonic cells;
- research on the role of leukemic cells and of the stroma;
- impact of pharmacogenomic studies in children and their parents.

The challenge is also to understand the biology of blast stem cells in childhood ALL and their differences with normal hematopoïetic stem cells. It has been demonstrated that NOD/scid mice engrafted with human leukemic bone-marrow are able to reconstitute in vivo the different maturational stages and to re-establish the complete leukemic phenotypes. The animal models may be applied both to AML and ALL and will lead to a better knowledge of drug resistance, risk of relapses and to develop drugs rendering leukemic cells more susceptible to attack and lead to the suppression of dormant cells (Vormoor, 2009; Le Viseur *et al*. 2008; Bernt and Armstrong, 2009 ; Sipkins, 2009; Barber *et al*., 2009). Moreover, it should be possible to test the effects of some etiological factors on these models.

New epidemiological approaches have to be proposed, taking account the multitude of the diseases and their multi-step development. That means a lot of potential causes, a lot of case-control studies and probably a lot of controversial data.

Future epidemiological studies have to be designed around the characterization of the childhood acute leukemias, with the aim to better approach the relationships between the causes and the consequences of the development of the (pre)leukemic cells and notably the role of post-natal factors.

It has been written in many papers that cooperation among consortia and major centres are necessary to make genomic studies in homogeneous groups of patients (prognostic factors and modalities of treatment), but relationships between the genomic heterogeneity of acute leukemias and the responsiveness of environmental agents could take a long time, due to the complexicity of this new molecular medicine, and the difficulties to demonstrate the significativity of the results in a rare disease. Perhaps, in the future, this type of researches could be restricted to patients with a common primary genetic lesion (first hit).

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2.2 Risk factors of childhood leukaemia: the French research program

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The French epidemiological program on childhood leukaemia was set up in 1995, in the National Institute of Health and Medical Research, Inserm. We created the National Registry of Childhood Hematopoietic Malignancy (RNHE) as a tool for etiological research, and made it begin the registration in 1990. The RNHE complies with the international rules for registration [Jensen, 1991] and has to apply for national qualification every 4 years. According to the RNHE, leukaemia affects around 470 children less than 15 years old each year in France [Clavel et al, 2004; Lacour et al, 2010]. The research program includes various complementary studies, mostly conducted at a national scale.

2.2.1 Specific infections

Although viruses are responsible for leukaemia in many animal species, no infectious agent has been evidenced to date in human childhood leukaemia. Evidences of space-time clustering could be indirect clues of underlying infections, as signs of local microepidemics. We tested the hypotheses of extra-Poisson heterogeneity and space-time interactions on the data collected in the national registry. We observed a very slight trend toward spatiotemporal case clustering [Bellec et al, 2006], more pronounced for children less than 5 years old living in rural isolated places. However, the available methods used to test clustering still suffer from many weaknesses and this issue remains debatable.

If a virus is responsible for childhood leukaemia, extreme population mixing is liable to alter the immunity of the population, unbalancing the proportion of healthy carrier subjects and subjects at risk, thus increasing the risk of micro-epidemics of leukaemia. Thus Kinlen postulated that leukaemia could be a rare response to a common infectious agent that may give rise to a cluster of leukaemia cases under particular conditions of imbalance of the herd immunity, and reported a number of findings of local increases in incidence in places that had encountered extreme population mixing [Kinlen, 1995]. We tested the issue of more common degrees of population mixing systematically at a national scale. Incidence of acute lymphoblastic leukaemia was increased in the cohorts of children less than 6 years old born in isolated municipalities which encountered more migrations from another region between the last censuses. The relative risks increased with the proportion of migrants from other regions, from 1.4 [1.0-2.0] to 1.8 [1.1-2.9] and 2.4 [1.1-4.7] for influx of 10%, 20% and 30%, respectively [Rudant et al, 2006]. Incidence of acute lymphoblastic leukaemia was increased (RR=1.4 [1.1,1.8]) in children less than 5 years old residing in isolated municipalities where the influx from distant places had been the highest between the 1990 and 1999 censuses [Bellec et al, 2008].

2.2.2 Ionizing radiation – ecological studies

- **Radon exposure**

The French geological contrasts allow investigating the role of radon by an ecological approach, with mean radon concentration of 85 Bq/m^3 [range 15-387 Bq/m^3]. The estimates of radon concentrations derived from measurement campaigns (13,240 measurements) performed in the 70s [Billon et al, 2005]. The geographical units were ‘employment areas’

which corresponds to a partition of the French territory into 343 divisions. Domestic exposure to radon was significantly associated with the incidence of acute myeloblastic leukaemia, with a positive linear trend (SIR=1.24 [1.08-1.44] for 100 Bq/m^3), and the relationship was not explained by the estimated exposures to telluric and cosmic gamma radiation [Evrard et al, 2005; Evrard et al, 2006].

Also supporting the hypothesis of a role of radon was the finding that a large cluster of AML was evidenced using an elliptic scan statistics [Kulldorff Nagarwalla, 1995] in an area covering a large part of the granite Massif Central. This unpublished result is currently being verified with several methods of cluster detection.

- **Proximity of nuclear sites**

We studied the risk of leukaemia in the vicinity of the French civil nuclear sites. We first considered municipalities of residence located within 5 km, 10 km, 15 km, 20 km around the sites. We observed no increase in incidence overall (SIR=0.92 [0.85 – 0.99]), and at any distance, whatever the type of leukaemia and the age of occurrence, the kind of site (power or reprocessing plant, research site), the date the plan started, and the power of the reactors for power plants [White-Koning et al, 2004]. Then, the exposure of the municipalities was estimated by quantitative modelling of radioactive discharges based on the type of site and the local wind patterns. Again, we observed no increase (SIR=0.94 [0.88-1.01] in incidence in the exposed municipalities [Evrard et al, 2006].

2.2.3 The Geocap project

The GEOCAP project is an ongoing national case-control study that uses a geographic information system to systematically link the address of all childhood cancer cases and of population controls with several georeferenced exposure sources such as power lines, roads, industrial sites. The addresses at diagnosis of all the cases registered since 1990 and, since 2002, that of 5000 controls per year representative of the French children are geocoded with an uncertainty of 15m for 80% of them and less than 100 meters for the other children. The project is devoted to both research and surveillance of children population and is conceived as a long-term program.

- **Ionizing radiation**

In the GEOCAP project, children are attributed the mean local radon concentrations derived from the measurement campaigns, but in addition they are classified according to a semi–quantitative indicator of local exhalation rate of radon. This works are collaborative with the IRSN.

GEOCAP will also include a new analysis of leukaemia risk around nuclear sites, using individual distances between residence and sites, and individual location on the grid of estimates of modelled radioactive discharges.

- **Extremely low frequency electromagnetic fields (ELF-EMF)**

The IARC classification of ELF-EMF exposures as possible human carcinogens [IARC, 2002] is still considered valid in view of more recent observations; although no underlying biological mechanism has been identified. Our program only addresses the exposure due to powerlines. The geocoded addresses of the GEOCAP cases and controls are individually located on the vector map of high-voltage power lines, providing the distance to the lines as a first proxy of the exposure. Subsequently, the exposure to ELF-EMF will be individually

assessed by experts, using the distance to the lines, the kind of lines and pylons, the voltage of the lines and the mean annual current they carry.

- **Traffic**

Literature on traffic and childhood leukaemia is still little developed, and heterogeneous. Indicators of distance to main roads and density of main roads within 500 meters are derived from Navteq maps. Estimated of NO2 and benzene concentration are modelled by the ADEME based on a national network of monitoring stations.

2.2.4 Individual data - Case-control studies

We conducted 3 case-control studies investigating environmental and genetic risk factors of leukaemia. The ADELE study included 280 cases and 288 hospital-based controls recruited in 4 cities between 1995 and 1999, with face-to-face interview; the ELECTRE study was carried out at the same period in 14 more rural regions and included 473 cases and 567 population-based controls; the ESCALE study, nation-wide and population-based, included 763 cases and 1681 controls in 2003-2004. The ADELE and ESCALE studies include biobanks. The ESTELLE study, currently ongoing, is very similar to ESCALE so that some pooled analyses could be done.

- **Insecticides**

The use of household insecticides by mothers during pregnancy was investigated in the ADELE and ESCALE studies and both evidenced positive associations with ORs of 1.8 [1.2-2.8] [Menegaux et al, 2006] and 2.1 [1.7-2.5] [Rudant et al, 2007], also consistent to date with most of the published studies. Reducing the time since pregnancy by considering only the children less than 5 years tended to reinforce the associations. The relationships were confounded by none of the factors investigated.

- **Garages and gas stations**

Another environmental source of exposure that was associated with leukaemia in both studies was the immediate proximity of garages or gas stations, with OR of 4.0[1.5-10.3] [Steffen et al, 2004] and 1.6 [1.2-2.2] [Brosselin et al, 2009], suggesting a role of low-dose benzene exposure in the aetiology of childhood leukaemia.

- **Early common infections**

All our studies showed that having had repeated common infections in the first year of life was negatively associated with acute lymphoblastic leukaemia [Perrillat et al, 2002; Jourdan-Da Silva et al, 2004; Rudant et al, 2010]. These findings support the hygiene hypothesis proposed by Greaves [Greaves, 2006] that delayed common infections promotes the risk of B-cell precursor lymphoblastic leukaemia in children who carry preleukemic cells generated in the course of a prenatal event. The negative relationship with breastfeeding, which also intervene in the early maturation of the immune system, was observed in the ADELE and ESCALE study but not in the ELECTRE study.

- **Genetic factors and gene-environment interactions**

In line with literature, we observed no link with maternal smoking during pregnancy in our 3 case-control studies [Menegaux et al, 2005; Menegaux et al, 2007; Rudant et al, 2008]. However, the findings of the ADELE study suggested that passive smoking could nonetheless contribute to leukaemia risk in children with predisposing genotypes of CYP1A1 [Clavel et al, 2005]. Similar results were also reported in the Quebec study [Infante-Rivard et al, 2000]. We are testing again the hypothesis of gene-smoking interactions on the ESCALE data.

A genome wide analysis is currently in process.

2.2.5 Conclusion and perspectives

The French epidemiological program on childhood leukaemia relies on a powerful tool, the RNHE, and on the national collaborative network of paediatric oncology. Its national scale allows contrasts and large size studies, although numbers can still be insufficient given the rarity of some types of childhood leukaemia. Its complementary approaches may give a chance to improve our understanding of the natural history and aetiology of childhood leukaemia.

The program also contributes to the childhood leukaemia international consortium 'CLIC' created at the initiative of P. Buffler (Berkeley). The CLIC offers a considerable opportunity for stimulating data pooling and discussions, which should lead to substantial findings for the next decade.

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3 ETIOLOGY OF CHILDHOOD ACUTE LEUKEMIAS – CURRENT STATUS OF KNOWLEDGE

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3.1 Abstract

Acute leukemia is a consequence of malignant transformation of a hematopoietic progenitor cell. Molecular studies have revealed a prenatal origin of many childhood leukemias. According to current models, a preleukemic stem cell clone is generated by a first mutation in utero and, in a minority of children, progresses to leukemia after receiving further postnatal genetic hits. The nature of pre- and postnatal events involved in leukemogenesis in children is not well understood. While genetic predisposition and specific environmental exposures may account for individual cases, the bulk of childhood leukemia can not be explained by any of these factors. The higher incidence of the most common leukemia subtype in affluent societies, as well as the age peak between 2-5 years suggest a contributory role of socioeconomic factors. An abnormal immune response during delayed exposure to common infections provides a plausible mechanism for malignant progression of preleukemic clones in a subgroup of children. As highlighted in this review, a common cause for all types and subtypes of childhood leukemia is highly unlikely. Deeper insights into the pathogenesis of childhood leukemia will rely on large-scale and combined epidemiological and biomolecular studies.

Acute leukemia is the most common childhood cancer in developed societies, where it accounts for one third of all malignancies in this age group. Due to the development of effective treatment regimens since the 1970s, the survival of children with leukemia has increased considerably, and acute lymphoblastic leukemia (ALL), the most common leukemia in children, has become curable in 70-80% of cases. Current efforts primarily are focussing on further improvements of treatment especially for high-risk subtypes of this disease. The other critical question regards potential causes and triggers involved in the process that leads to childhood leukemia.

3.2 Natural History of the Disease

Leukemia is a consequence of malignant transformation of a single hematopoietic progenitor cell. A sequence of molecular events disrupts the process of differentiation and limited proliferation that characterizes normal hematopoiesis and generates a leukemic clone capable to expand by indefinite self-renewal. Recent molecular insights into childhood leukemia have provided an opportunity to study the origins and natural development of the disease. In particular, many acute leukemias of childhood have consistent chromosomal translocations leading to abnormal fusion genes, which result in genetic dysregulation and are involved in the leukemogenesis. In the most common of these translocations, the oncogene AML1 on chr 21 is aberrantly expressed under the regulatory control of the TEL gene promoter on chr 12 (TEL/AML-1 gene-rearrangement). Another example are rearrangements involving the MLL gene on chromosome 11, e.g. translocation t(4;11). Besides their role in leukemia initiation and progression, specific molecular abnormalities represent stable and unique markers of the

disease and thus have been used as tools for tracking preleukemic and leukemic cells in vivo. Based on these molecular markers, twin studies have been performed and provided important clues about etiology. The concordance rate of childhood ALL among monozygotic twins is 5-10%. This may be suggestive of a genetic predisposition, however, non-twin siblings have not been consistently been reported to have an increased risk for the disease. The presence of potential leukemogenic factors of the shared intrauterine environment also fail to explain the concordance rates among monozygotic twins, since dizygotic twins with separate placentas do not seem to have an elevated risk of leukemia. These considerations have led to the hypothesis that the leukemia may originate prenatally in one twin and then be transmitted to the co-twin through anastomosing placental vessels. And indeed, in 1993, Mel Greaves and coworkers found evidence for this hypothesis[1]. Molecular studies of the leukemic cells from three pairs of monozygotic twins concordant for infant leukemia identified identical breakpoints at chromosome 11q23 in all three, confirming a common clonal origin of the leukemia from one transformed cell in utero.

Further evidence for a prenatal origin of the disease comes from studies of neonatal blood from non-twin children with ALL[2]. Archived neonatal blood spots from three children with ALL carrying the 4;11 translocation were retrospectively analysed for the presence of the fusion transcript. Indeed, in these patients who were diagnosed at 5-24 months of life, the genetic abnormality was already present at birth, confirming prenatal initiation of the leukemia in utero. In subsequent studies, evidence was obtained that initiation of childhood ALL in utero is a frequent event and is not restricted to MLL gene rearrangements and infant leukemia. An analysis of neonatal Guthrie card blood spots from 11 children diagnosed with TEL/AML1+ leukemia during the typical ALL age peak between 2 and 5 years, including a pair of identical twins with concordant leukemia resulted in identification of the fusion transcript in neonatal blood from both of the identical twins and in six of the nine non-twin patients[3]. Large studies have now revealed that preleukemic fusion transcripts are a common finding at birth. Among 496 cord bloods, typical genetic rearrangements of ALL and AML were detected in as many as 1% of newborns[4]. Clearly, the majority of these children do not go on to develop leukemia which means that secondary events must follow postnatally in a minority of children to trigger overt leukemia.

Based on these observations, a 2-step model has now been proposed (Figure 1): A first mutation affecting a hematopoietic stem or precursor cell in utero induces a specific molecular abnormality which characterizes the resulting preleukemic clone. The preleukemic stem cell will remain silent in most cases. In a minority of children harboring preleukemic clones, further postnatal genetic hits will lead to malignant transformation and leukemia.

3.3 Role of Genetic Factors

Genes and environment have both been suspected to induce the molecular events responsible for leukemogenesis in children. Down´s syndrome, which is a consequence of constitutional trisomy 21 strongly predisposes to leukemia and has become a model disease in which the pathogenesis starts being understood[5]. In Down-associated leukemia, the constitutional chromosomal aberration in hematopoetic progenitors is thought to provide the first hit. Transformation is then induced by an acquired mutation of GATA1, resulting in „transient leukemia“ at birth in 5-10% of children. While the GATA1-mutated clone in many children is extinguished or remains silent, in others, a subclone persists and later undergoes further mutations that result in clinical myeloid leukemia. Even though a number of other genetic conditions predisposing to leukemia have now been described, the proportion of childhood leukemia with a known genetic etiology altogether is low and almost entirely accounted for by Down´s syndrome. In recent years, there has been an increasing interest in more subtle genetic alterations or variations which may determine an inherited susceptibility to childhood leukemia by affecting individual responses to specific environmental exposures.

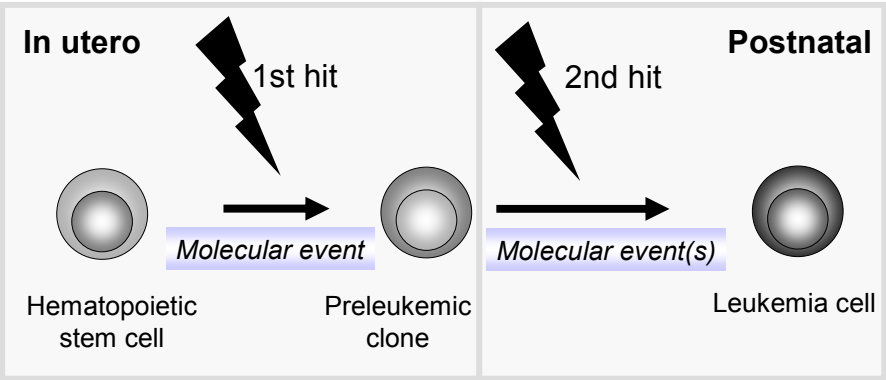

Figure 1. Model for leukemogenesis in children

3.4 Role of Environmental Factors

Despite a multitude of epidemiological studies, the extent to which environmental factors contribute to leukemogenesis in children has not been clarified, and it is now considered unlikely that the majority of chlidhood leukemia cases are attributable to a single exclusive cause. As exemplified in the following overview, a majority of candidate environmental exposures have been suggested to cause leukemia in children.

3.4.1 Factors of the prenatal environment

The prenatal origins of childhood leukemia have suggested a causative role within the prenatal environment. A high birthweight has indeed been associated with leukemia in the majority but not all of multiple epidemiological studies[6]. Leukemia and high birth weight were hypothesized to be a common consequence of elevated levels of circulating growth hormones, such as insulin-like growth factor-1 (IGF-1)[7]. IGF-1 receptor signaling is critically involved in cell growth and proliferation and provides a potent stimulus for hematopoiesis. Therefore, hematopoietic stem cells in big babies may be exposed to increased proliferative stress, resulting in a higher likelihood of transformation. Another factor associated with leukemia in some but not all epidemiological studies is advanced maternal age. Proposed explanations are an accumulation of genetic alterations of germ cells as well as environmental exposures over the longer lifetime of the mother. Due to confounding socioeconomic variables, these data are difficult to interpret. Maternal diet during pregnancy may be another factor involved in leukemia initiation in utero. Among the numerous components affecting human DNA, topoisomerase inhibitors have been found in various food items, such as beans and soy, many other vegetables, and in coffee, tea, cocoa, and wine. Topoisomerase inhibitors are potent anticancer agents and have been directly related to treatment-related secondary leukemias, demonstrating their leukemogenic potential. In a case control study from the United States, maternal consumption of dietary topoisomerase II inhibitors was found to be associated with infant AML, but not ALL[8]. A few years later, a molecular study provided direct evidence that dietary topoisomerase inhibitors available can induce cleavage of the MLL gene in human lymphoid and myeloid progenitor cells by inhibition of topoisomerase II[9]. Thus, ingestion of these compounds during pregnancy may be directly involved in causing genetic damage. However, MLL-rearranged infant leukemia is a rare subtype, and nutritional factors are not likely to contribute substantially to the general incidence of leukemia.

3.4.2 Ionizing and non-ionizing radiation

There is no doubt that exposure to ionizing radiation can induce acute leukemia. Among the survivors of the atomic bomb explosions in Japan in 1945, who were acutely exposed to up to

200 mSv, the incidence of leukemia rose rapidly and steeply in a dose-related manner[10,11]. The early peak was due mostly to leukemia in children, in which ALL remained the most common type. While the potential of ionizing radiation for causing childhood leukemia is undisputed, the question remains whether it represents a significant cause. With regard to in utero radiation exposure, conflicting results have been reported. Alice Stewart has found an increased leukemia in children who had been exposed in utero to x-rays for diagnostic purposes in the 1950s[12]. Surprisingly, however, the risk was not increased among more than 1200 children exposed to up to 200 mSv of radiation in utero during the atomic bombings in Japan: Only 2 cases of childhood cancer were found, none of which was a leukemia[13]. Unfortunately, the earliest of these studies were performed in the 1950s, and there are concerns regarding their retrospective nature. Follow-up investigations in specific regions of the former Soviet Union and in various countries affected by nuclear fallout after the Chernobyl accident have also failed to show a consistent increase in the rate of childhood leukemia, though results from ongoing large studies have not been published yet. Multiple studies have been conducted to assess the risk associated with routine emissions from nuclear power plants or the fallout from atmospheric nuclear testing. A large epidemiological case control study has just been completed in Germany and revealed an increased risk of childhood ALL for children living in the proximity (5 km) of nuclear power plants, with a population-attributable risk of 0.3% translating into 0.8 cases of childhood leukemia per year in Germany[14]. At present, there is no biophysical explanation for this observation.

Even more difficult to interpret are data regarding exposure to electromagnetic fields (reviewed in[15]). Concerns were initially raised in a report from 1979[16]. Later, contrasting large case control studies have been published from various countries. A pooled analysis adjusted for electromagnetic waves has now suggested that the risk is indeed increased in those children exposed to the highest levels, representing a minority[17].

3.4.3 Socioeconomic status

Several features of common childhood ALL suggest an association with a higher socioeconomic status. A characteristic age peak is found in 2-5 year olds which historically has emerged at the beginning of the 20th century[18]. This age peak is restricted to the B-cell precursor subtype of ALL and to affluent countries. Furthermore, the incidence of B-cell precursor ALL shows a geographic pattern with the highest incidence among white populations of Europe and the United States and a much lower incidence in developing and less affluent countries of Asia and Central Africa. In well-developed societies, the incidence of childhood leukemia has further increased over the past 30 years[19]. Importantly, this increase can not be attributed to reporting bias, since the majority of other cancer types have not shown any increase with time. Specific time trends for ALL were reported from various countries during economic transitions towards a more affluent life style. During the first 8 years after the reunification of Germany, an annual increase of around 3.3% per year was observed in Eastern Germany, resulting in a catch-up to western German ALL incidence rates[20]. Thus, it was suggested that changes in lifestyle during reunification had an impact on childhood leukemia. Another example is the ALL incidence in New Zealand, which increased 1.6 fold in children <4 years of age from the 1950s to the 1980s, years during which the country underwent substantial economic improvements. The native Maori population whose lifestyle did not change to the same extent, with higher rates of unemployment and lower incomes, maintained a lower risk of childhood ALL[21]. Furthermore, in Czech Republic, during the 1980s and 1990s, ALL incidence in the preschool age group increased 1.5 times[22]. Importantly, the increase was restricted to those within 1-4 years of age, while the incidence in other age groups remained constant, which strongly argues against reporting bias. In order to establish the proposed association between a high socioeconomic status and common childhood ALL, a multitude of epidemiological studies have been performed. As measures of socioeconomic status, mostly household income, area of residence, or parental education levels were applied.

The results of these studies are largely controversial, and many of them lack statistical significance[23].

3.4.4 Common infections in early childhood

The apparent link of affluence and modernization to changes within the infectious environment in which children are growing up has stimulated interest in a potential role of infection and immunity in childhood ALL. To date no convincing association between childhood or maternal infection with a specific virus and leukemia has been found. However, in 1988, two hypotheses were proposed how infection could still be an important causal factor in childhood leukemia. The Kinlen hypothesis emerged in the context of the observed leukemia clusters around nuclear reprocessing plants in Sellafield[24]. Since the radiation exposure levels were too low to convincingly explain the increased leukemia incidence, Leo Kinlen argued that the unusual population mixing caused by migrant workers recruited to these remote regions might be the causative factor. His hypothesis implies that childhood cancer is a rare response to a specific, yet unidentified infection, which is transmitted preferentially during periods of population mixing. In the same year, Mel Greaves has proposed an alternative but related hypothesis[25], in which he explained the observed correlation between lifestyle and the characteristic age peak among 2-5 year old children by an inadequate priming of the immune system. Children who face a narrower range of antigens in young childhood and thus remain immunologically more naive for a longer period of time, may be more susceptible to leukemogenesis in their maturing B-cell compartment. Malignant progression of a preleukemic clone is hypothesized to occur as part of an abnormal immune response to a common infectious agent during delayed exposure. The Greaves hypothesis was now substantiated by epidemiological evidence. The most conclusive epidemiological studies have been based on the day-care attendance in early life as a parameter for early and broad exposure to common infections. The majority of these studies have found a protective role for early institutional day care[26].

3.5 Conclusions

In summary, the causes of childhood leukemia remain largely unresolved. While a defined genetic predisposition and specific environmental exposures may account for individual cases, the bulk of childhood leukemia can not be explained by any of these factors. A dysregulated immune response to common infections has emerged as a plausible etiological mechanism for a subgroup of children. However, the majority of underlying data are epidemiological by nature. To expand the knowledge in this field, biological models as well as large-scale clinical studies are required, including the analysis of gene-environment interactions on biological specimens.

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4 EPIDEMIOLOGY OF CHILDHOOD LEUKAEMIA AND IONISING RADIATION

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4.1 Abstract

The particular sensitivity of childhood leukaemia to induction by ionising radiation was recognised early in the study of the Japanese survivors of the atomic bombings of Hiroshima and Nagasaki, the Excess Relative Risk (ERR, the proportional increase in risk) of leukaemia at an equivalent dose of 1 Sv to the red bone marrow (RBM) for those irradiated as young children rising to a peak of ~100 a few years after exposure and then falling away rapidly, so that the ERR is largely expressed as a wave of excess cases within two decades of exposure. The high ERR/Sv for radiation-induced childhood leukaemia has been confirmed by most (but not all) studies of therapeutic irradiation of children for a number of medical conditions, although RBM doses are often difficult to estimate in these studies. Of some interest are the case-control studies of childhood cancer and diagnostic abdominal X-ray examinations of the pregnant mother, which show a consistent positive statistical association for both childhood leukaemia and other childhood cancers, implying that a fetal dose ~10 mGy of X-rays raises the risk of childhood leukaemia. The ERR/Sv for childhood leukaemia derived from the largest of these case-control studies is compatible with that obtained from risk models based on leukaemia among the Japanese atomic bomb survivors irradiated after birth. These radiation risk models for leukaemia suggest that natural background radiation may account for 15-20% of childhood leukaemia cases in Great Britain, although the uncertainties in this estimate are considerable. A recent nationwide case-control study of childhood acute lymphoblastic leukaemia (the commonest leukaemia in children) and residential exposure to radon in Denmark found a positive statistical association, but further examination of the role of both background gamma radiation and radon in the aetiology of childhood leukaemia is required before reliable conclusions can be drawn. Extensive research as a consequence of reports of excess cases of childhood leukaemia near certain nuclear installations has not discovered any major deficiencies in the radiological risk assessments that have inferred that radiation exposure resulting from the operation of these installations is much too small (by a factor of at least 100) to account for the excess cases. This research has considered, *inter alia*, radiation exposure of fathers prior to the conception of their children (a hypothesis now effectively abandoned) and the influence of internal exposures from intakes of man-made radionuclides – this latter issue has been addressed by a study of childhood leukaemia incidence around the world following ubiquitous exposure to the radioactive debris of atmospheric nuclear weapons testing, which found no evidence of a gross underestimation of the risk of childhood leukaemia from this source of exposure. In summary, high dose and dose-rate studies have demonstrated that childhood leukaemia has a high sensitivity to induction by ionising radiation, and risk models based on the experience of the Japanese atomic bomb survivors predict risks at low dose/dose-rate conditions that are compatible with epidemiological evidence, although data are limited and more research is desirable.

4.2 Introduction

Over the past quarter century considerable attention has been paid to the risk of childhood leukaemia posed by exposure to ionising radiation. This interest has (re-)arisen primarily because of reports of increased rates of incidence of leukaemia among children living near certain nuclear installations [1], which has led to the detailed examination of the evidence for the level of the increase in the risk of childhood leukaemia following radiation exposure. In this paper the epidemiological evidence indicating the risk of childhood leukaemia consequent to radiation exposure will be reviewed.

4.3 Early studies of leukaemia in radiologists

Leukaemia (in adults) was the first malignant neoplastic disease to be positively linked to exposure to ionising radiation, the earliest persuasive evidence being published in 1944 by March who found a notable increase in leukaemia mortality among US radiologists when compared to other US physicians [2-4]. Other reports of excesses of leukaemia among medical workers had occurred before that of March, including a report by Aubertin who found a raised level of myeloid leukaemia among French radiologists when compared to other French physicians [3,5], but at the time, these were not generally taken to indicate that it was radiation exposure that had caused a raised risk of leukaemia in medical staff working with radiation [3,4].

4.4 The Japanese atomic bomb survivors

Following the explosion of atomic bombs over Hiroshima and Nagasaki in August 1945, reports of higher than expected numbers of cases of leukaemia among the Japanese survivors of the atomic bombings were starting to be made by alert clinicians in 1948 [6]. This was one of the reasons for the establishment, through the Japanese national census of October 1950, of a cohort of Japanese atomic bomb survivors for epidemiological study, which became known as the Life-Span Study (LSS). The LSS consists of ~93 500 survivors, ~86 500 of whom have been assigned estimates of doses received as a consequence of the bombings, and of these ~49 000 were non-trivially exposed to radiation (i.e. they received an assessed dose of ≥5 mSv); the LSS includes almost all of the survivors who were closest to the detonations [7]. The LSS is a study of members of the general public of both sexes and all ages who were not selected for exposure for a particular reason (e.g. a medical condition) − the survivors just happened to be in the wrong place at the wrong time. Considerable effort has been expended on ensuring that the data generated by the LSS are as complete and accurate as possible, including the assessment of organ doses received by each survivor in the cohort, the latest doses being those in the Dosimetry System 2002 (DS02), which has replaced the previous DS86 database [7]. A wide range of doses was received by the survivors: around two-thirds of the non-trivially exposed survivors received doses <100 mSv (i.e. low doses) whereas just over 2000 individuals received doses exceeding 1 Sv (i.e. high doses). Mortality among the LSS is determined through the Japanese *koseki* family registration system and death certificate information. Cancer incidence data are collected through two specialist cancer registries based in Hiroshima and Nagasaki, from 1950 (for haematopoietic and lymphatic cancers) and 1958 (for other cancers). The collection, collation and initial analysis of data relating to the Japanese atomic bomb survivors is the responsibility of the Radiation Effects Research Foundation (RERF), a joint Japanese/US organisation, and

since nearly one-half of survivors were still alive at the last analysis of mortality in the LSS database, studies continue today.

The most recent analysis of leukaemia mortality for the period 1950-2000, using DS02 red bone marrow (RBM) doses, shows a clear and pronounced dose-related excess risk [7]. For deaths at all ages and both sexes, the Excess Relative Risk (ERR, the proportional increase over background) at 1 Sv RBM equivalent dose over the entire study period was 4.02 (90% confidence interval (CI): 3.02, 5.26) [8]. The dose-response for the RBM dose range 0-2 Sv exhibits upwards curvature in the upper part of this range so that the best fit is a linear-quadratic model, with downward curvature occurring at higher doses (i.e. >2 Sv) as a result of cell killing [7]. The leukaemia mortality data are more unstable statistically in the 0-0.5 Sv dose range, but the linear-quadratic dose-response fitted to data in the 0-2 Sv range still provides a reasonable description in the low dose region, where the dose-response is essentially linear [7]. About half of the ~200 leukaemia deaths among the survivors who were non-trivially exposed are attributable to irradiation during the bombings. The most recent analysis of leukaemia incidence for the period 1950-1987 used the previous DS86 RBM doses and found, for cases at all ages and both sexes over the entire study period, an ERR at 1 Sv RBM dose of 4.84 (90% CI: 3.59, 6.44) [8]. An update of leukaemia incidence among the Japanese atomic bomb survivors is expected soon.

Richardson *et al.* [9] examined the leukaemia mortality data for the Japanese atomic bomb survivors during 1950-2000 using the DS02 RBM doses, and demonstrated the marked variation of ERR with age at exposure, the risk being notably higher at young ages at exposure. Further, the ERR fell away with increasing time since exposure, particularly for those exposed in childhood. So, for those irradiated during the atomic bombings as children, the ERR of leukaemia was manifest as a wave, peaking (for an individual aged 10 years at the time of the bombing) at an ERR of ~70 at 1 Gy RBM dose some 7 years after exposure and then attenuating such that at 25 years after exposure the ERR was still raised but at a level (~2) compatible with the ERR experienced by those exposed as adults this long after exposure – for those ≥30 years of age at the time of the bombing the ERR is essentially flat with time since exposure, illustrating the marked variation of the expression of leukaemia ERR with age at exposure. Thus, a notably raised risk of leukaemia was experienced by the Japanese atomic bomb survivors exposed as children soon after exposure, but then the risk falls away with increasing time since exposure.

After October 1950 (the start of the LSS) there were 10 cases of leukaemia diagnosed among those survivors less than 15 years of age, which compares with less than one case expected among these children. This represents a marked excess risk of leukaemia in those exposed as children. These 10 cases provide, using the DS86 RBM doses, an ERR at 1 Sv of 34.4 (95% CI: 7.1, 414) [10], confirming the notably raised risk of childhood leukaemia following the atomic bombings of Japan.

Although the studies of the Japanese atomic bomb survivors are impressive in the detailed information on radiation risks that they provide, they cannot generate direct information on all aspects of radiation-induced risks. The bomb survivors received briefly delivered doses at a high dose-rate of mainly external γ-radiation, and some of the, obviously retrospective, dose estimates remain uncertain. The exposed population was malnourished at the end of a long war (with a low proportion of men of an age to serve in the military), and to enter the Life Span Study the survivors had to have lived until October 1950 in conditions that were far from perfect (especially if they had suffered deterministic effects from high radiation doses), which raises the possibility of bias due to the “healthy survivor effect” – potentially, those entering the epidemiological studies were stronger individuals not representative of the general population in terms of radiation-induced cancer risks. Further, data for the period before October 1950 were not collected systematically, and this is especially important for childhood leukaemia, since it is clear that excess cases were occurring in the late-1940s – hence the Japanese atomic bomb survivor data cannot, by themselves, determine the minimum latent period for leukaemia, although the data are compatible with the value of two years that is usually

assumed for this period. There is also the question of how the finding derived from a Japanese population in 1945, with its particular range of cancer risks (e.g. a high risk of stomach cancer but a low risk of female breast cancer), should be applied to another population (say, from present day Western Europe) with a different range of cancer risks (e.g. a low background risk of stomach cancer but a high risk of female breast cancer) – is the excess relative risk (i.e. the proportional increase in risk) or the excess absolute risk (i.e. the additional risk) or some mixture of the two to be transported between populations? Finally, a sizable proportion of the atomic bomb survivors are still alive, so that lifetime risks for those exposed at a young age are not available, although this will not be relevant for childhood leukaemia.

4.5 Medically exposed groups

The aspects of radiation risk that cannot be directly addressed by studies of the Japanese atomic bomb survivors mean that it is important to have results from other exposed populations to complement the risk estimates derived from the Japanese survivors. Medical practice provides a number of opportunities to study groups exposed for therapeutic or diagnostic purposes, and it is from the study of medically exposed groups that a minimum latent period for radiation-induced leukaemia of two years is derived. Most of the therapeutically exposed groups – children irradiated to treat cancer or other conditions such as tinea capitis or enlarged thymus gland – have confirmed the high excess relative risk of childhood leukaemia following irradiation, although Swedish children exposed to radiation in the treatment of skin haemangioma have not experienced a detectably raised risk for reasons that are unclear [11]. Although these groups give valuable additional information on the radiation-induced risk of childhood leukaemia there are a number cautionary points that require examination. Medical exposure occurs because of a known or suspected disease and this may affect the consequent radiation-induced risk of cancer, so that generalisation to a healthy population is not straightforward. Further, radiotherapy requires high doses designed to kill abnormal cells and these doses are frequently highly localised. This means that tissues in the vicinity of the abnormal target cells may also experience high doses that are sufficient to kill substantial numbers of normal cells, leading to a reduction in the cancer risk per unit dose in these tissues when compared with that resulting from moderate doses. Also, tissue-specific doses to regions of the body away from the target of radiotherapy (largely due to radiation scattering) are difficult to calculate and accurate dose estimates are often lacking in medical studies so that the resulting risk coefficients can be unreliable. Substantial effort is presently being devoted to the reconstruction of doses in several epidemiological studies of medical exposures, using modern modelling techniques.

Of some interest, given the low doses involved (~10 mGy), are the studies of childhood cancer and prior abdominal diagnostic X-ray examinations of the pregnant mother. The first, and largest, of the case-control studies was the Oxford Survey of Childhood Cancers (OSCC), which started in Great Britain in the early-1950s and found a highly significant statistical association between the risk of mortality from childhood leukaemia and of other cancers in childhood and an antenatal X-ray examination. The initial report of the statistical association, in 1956, was greeted with some scepticism – because *inter alia* of concerns about the influence of recall bias, the early findings being based upon maternal recall of X-ray examinations during pregnancy – but the association has now been confirmed by many case-control studies carried out around the world (including studies based upon medical records of antenatal exposure) and the association is now accepted as real although some debate its interpretation (e.g. [12]). The most recent result from the OSCC for childhood leukaemia as a separate entity was published in 1975 when a relative risk (RR) of 1.47 (95% CI: 1.33, 1.67) was reported [13]. Appropriately combining the results of case-control studies other than those produced by the OSCC gives a childhood leukaemia RR of 1.28 (95% CI: 1.16, 1.40)

[14]. So, the association between childhood leukaemia and an antenatal X-ray examination found by the OSCC and all other case-control studies combined is highly statistically significant – the lower RR found by the combined other studies may be due, among other things, to later case-control studies examining periods when the fetal doses received during obstetric radiography were lower than in earlier years.

Considerable debate has surrounded the interpretation of the statistical association between leukaemia and other cancers in childhood and antenatal diagnostic radiography [10-12]. Many of the objections to a cause-and-effect explanation have now been met [11,14]. For example, cohort studies of antenatal exposure to diagnostic X-rays have not found a raised risk of childhood leukaemia, but the only such cohort study with sufficient statistical power to seriously challenge the findings of the OSCC was that of Court Brown *et al.* [15] and one of the authors of this study (Richard Doll) later questioned the accuracy of the linkage between the mothers and their children in this study, and felt that the findings of the study could not be relied upon [11,16]. However, the finding that the relative risk of childhood leukaemia and that of all the other typical cancers of childhood are raised to the same extent, unlike the pattern of risk when exposure occurs after birth, is an outstanding issue that requires a satisfactory resolution [11,12].

To obtain a risk estimate (excess risk per unit dose) from the case-control studies, estimates of fetal doses are required. The only study for which a reasonable estimate of fetal dose is available (the Adrian Committee's estimate of 6.1 mGy for 1958), and which is large enough to give an acceptably precise risk estimate, is the OSCC, and an ERR coefficient of 51 (95% CI: 28, 76) Gy^{-1} for all childhood cancers combined is obtained [10]. Since the results of the OSCC indicate that similar relative risks exist for both childhood leukaemia and other childhood cancers, this ERR coefficient is taken to apply to childhood leukaemia. Applying this ERR coefficient to the background absolute risk of childhood leukaemia in Great Britain during the period when the OSCC was conducted of 1577 cases per million live births gives an Excess Absolute Risk (EAR) coefficient of 0.08 (95% CI: 0.044, 0.12) Gy^{-1} [10]. However, the uncertainties surrounding these risk estimates are considerable, and there are reasons to believe that the data obtained from the later years of the OSCC could be unreliable and that the estimates may overestimate the risk by perhaps a factor of four [10].

These ERR and EAR coefficients obtained from the OSCC may be compared with the risk estimates derived from the Japanese atomic bomb survivors. During the bombings 807 survivors were irradiated *in utero* and received doses of at least 10 mGy, the average dose being 0.28 Gy, an order of magnitude greater than the level of fetal dose received during an obstetric radiographic examination. (The Japanese survivors irradiated *in utero* are a separate cohort than the LSS, which consists of survivors irradiated after birth.) Two incident cases of cancer occurring while less than 15 years of age were observed among the intrauterine exposed group, a fatal liver cancer and a non-fatal kidney cancer, which compares with, at most, 0.48 cases expected from contemporaneous Japanese national rates. This gives an ERR coefficient of 11 (95% CI: -1, 44) Gy^{-1} for all childhood cancers, which is compatible with the ERR coefficient obtained from the OSCC of 51 (95% CI: 28, 76) Gy^{-1}, bearing in mind the uncertainties inherent within both estimates [10]. However, the EAR coefficient derived from the Japanese survivors is 0.008 (95% CI: -0.001, 0.03) Gy^{-1} for all childhood cancers, which is not compatible with that derived from the OSCC of 0.08 (95% CI: 0.044, 0.12) Gy^{-1} [10]. This illustrates the importance of the transport of the risk between exposed populations: the background absolute risk of childhood cancer experienced by the Japanese survivors irradiated *in utero* was 660 cases per million live births whereas the risk for British children during the OSCC was 1577 cases per million live births, a factor of 2½ more. So, compatibility of the risk estimates for the OSCC and the Japanese survivors irradiated *in utero* depends on whether it is more appropriate to compare the ERR coefficients or the EAR coefficients. There is some evidence, albeit weak, that for childhood leukaemia at least, the transfer of risk is closer to ERR than EAR, but this is open to debate [17,18].

It will be noted that no case of childhood leukaemia occurred among the Japanese atomic bomb survivors irradiated *in utero*, although the number of cases expected in the absence of radiation exposure was only 0.2, giving an upper 95% confidence limit for the observed to expected (O/E) ratio of 15 [10]. This absence of cases of childhood leukaemia contrasts with the O/E ratio for childhood cancers other than leukaemia of 7.14 (95% CI: 1.20, 23.60), a statistically significant excess. Thus, the ERR coefficient for childhood leukaemia is 0 (95% CI: 0, 50) Gy^{-1} and the ERR coefficient for childhood cancers other than leukaemia is 22 (95% CI: 0.7, 81) Gy^{-1}. Although the absence of childhood leukaemia cases among the Japanese survivors irradiated *in utero* is noteworthy, the 95% confidence interval of (0, 50) Gy^{-1} for the ERR coefficient is not incompatible with that derived from the OSCC data, (28, 76) Gy^{-1}, and there may be other reasons for the absence of cases. For example, systematic follow up of the Japanese atomic bomb survivors only commenced in October 1950, so any cases occurring among the survivors before this date may have gone unrecorded, and in the difficult years following the war some cases of childhood leukaemia may not have been recognised as such against the background of other causes of death in childhood. However, recently Ohtaki *et al.* [19] have identified a potentially important factor in the risk of childhood leukaemia following irradiation *in utero*: they found that, with the exception of a small (but statistically significant) rise at doses below ~100 mGy, there was no increase with dose of the frequency of stable chromosome translocations in the blood lymphocytes of the survivors irradiated *in utero*. This contrasted with the anticipated increase in translocations with dose found for some of the mothers of the individuals exposed *in utero*. An interpretation of this finding is that the haematopoietic system *in utero* is particularly sensitive to radiation-induced cell killing, which would imply that moderate and high acute doses of radiation received *in utero* do not materially increase the subsequent risk of childhood leukaemia, a potential explanation for the absence of cases of childhood leukaemia among the Japanese atomic bomb survivors irradiated *in utero*.

The ERR coefficient of 51 (95% CI: 28, 76) Gy^{-1} for childhood leukaemia implied by the OSCC is compatible with that found in the Japanese LSS (of survivors exposed after birth) of 34.4 (95% CI: 7.1, 414) Sv^{-1} RBM dose. In contrast, no case of a childhood cancer other than leukaemia was found among the Japanese atomic bomb survivors irradiated after birth (but a significant excess of cancers other than leukaemia did develop in adult life), while a significant excess of such childhood cancers (based upon 2 observed cases) was found among the survivors irradiated *in utero* – a possible explanation is that while the cells sensitive to the induction of the typical cancers of childhood other than leukaemia remain active throughout pregnancy, they “switch off” at birth [11].

In summary, the ERR coefficient for childhood leukaemia derived from the OSCC is compatible with that obtained from the Japanese atomic bomb survivors irradiated after birth and those irradiated *in utero*, although the absence of cases of childhood leukaemia in the cohort of survivors irradiated *in utero* may indicate that the ERR coefficient derived from this group is an underestimate. As for childhood cancers other than leukaemia, the ERR coefficient derived from the OSCC is compatible with that obtained from the Japanese atomic bomb survivors irradiated *in utero*, but not with the absence of cases among the survivors irradiated after birth; a satisfactory explanation for this difference remains to be found, although suggestions have been proposed (such as the relevant active cells being present only *in utero*). The compatibility of the childhood leukaemia risk coefficients obtained from the OSCC and from the groups of Japanese atomic bomb survivors do depend, however, on the ERR coefficients being more pertinent than the EAR coefficients, which are dissimilar, and this returns to the important issue as to whether the transfer of the ERR or EAR between populations is more appropriate.

Risk models for radiation-induced leukaemia indicate that the excess relative risk of childhood leukaemia consequent to exposure during early postnatal life does not differ greatly from that derived from the OSCC for fetal exposure, suggesting that X-ray examinations of children produce a raised risk of leukaemia that, analogously to the findings of case-control studies of

antenatal X-ray exposures, should be detected by suitably designed case-control studies. Unfortunately, such studies that have been conducted have generated conflicting findings, probably due to deficiencies in the studies, and the results cannot be considered reliable [14]. This is especially disappointing given the rise in frequency of relatively high dose diagnostic procedures such as paediatric CT scans, which if conventional predictions of radiation-induced leukaemia risk are accurate, give rise to a risk of leukaemia that should be taken into account when assessing the need for such examinations.

4.6 Occupational exposures

Clearly, leukaemia cannot be the direct consequence of the occupational exposure to radiation of children, but a statistical association between the incidence of childhood leukaemia and the recorded dose of external radiation received by men while working at the Sellafield nuclear installation in Cumbria, England, before the conception of their children suggested a heritable genetic link [20]. The authors of this study proposed that the association could explain statistically the cluster of childhood leukaemia cases in the village of Seascale, adjacent to Sellafield; but the association was based upon just four cases of childhood leukaemia with cumulative paternal preconceptional doses in excess of 100 mSv, and the association had not been found by previous epidemiological studies [20]. The association received considerable attention, and a large programme of research was initiated. After substantial study, little or no support for a cause-and-effect interpretation of the original statistical association between childhood leukaemia and paternal preconceptional irradiation has been found, and the hypothesis has now been effectively abandoned [21,22].

4.7 Environmental exposures

Risk models for leukaemia suggest that natural background radiation in Great Britain, where the average RBM equivalent dose for children is ~1.3 mSv per annum, may account for 15-20% of cases of childhood leukaemia, although the uncertainties associated with this estimate are considerable [17,18]. Epidemiological studies have been unable, in general, to detect the influence of natural background radiation upon the risk of childhood leukaemia, but this may be due to a lack of statistical power resulting from insufficient geographical variation in the RBM dose. However, a recent nationwide case-control study conducted in Denmark reported a statistically significant association between childhood acute lymphoblastic leukaemia (ALL) and residential exposure to radon [23], and the authors suggested that, on the basis of the study results, 9% of Danish childhood ALL cases could be attributable to radon; although not explicitly reported, the 95% CI for this attributable fraction can be calculated from data presented in the paper to be (1%, 21%), a lower 95% confidence limit that is not incompatible with what would be predicted by conventional assessments. The study used predicted residential radon concentrations calculated from a model based on a previous measurement programme and a number of explanatory variables such as house type and geology. These model predictions of radon concentrations in homes avoid the bias potentially associated with limited participation in a measurement programme conducted as an integral part of the case-control study, which has been a major problem in some studies (such as the UK Childhood Cancer Study [24]); but the model estimates inevitably introduce uncertainties that require further investigation in relation to their influence upon risk estimates. However, the case-control study approach using individually assessed doses and measures of background factors that could influence the risk of childhood leukaemia avoids the shortcomings of

geographical correlation studies using group-averaged doses, which often lead to difficulties in the interpretation of the findings of such studies.

Harley and Robbins [25] have suggested that the results of the Danish study might be explained by the dose received from radon and its decay products by circulating lymphocytes while present in the tracheobronchial epithelium. However, while the dose to individual lymphocytes in the tracheobronchial epithelium can be substantial and higher than the dose to the RBM, circulating lymphocytes spend only a limited time within the tracheobronchial epithelium and the average dose received by the whole population of lymphocytes is likely to be of most relevance to the consequent risk of childhood ALL. Further, the dose received by haematopoietic stem cells within the RBM is understood to be the most pertinent with respect to the risk of radiation-induced childhood leukaemia, and radon delivers a relatively small component of the dose from natural background radiation to these cells. That radon rather than the dose from background gamma-radiation can detectably influence the risk of childhood leukaemia seems rather unlikely, and large, probably international, studies will be required to satisfactorily investigate the issue of the influence of natural background radiation upon the risk of childhood leukaemia, of which radon is a relatively small component.

Reports of excesses of childhood leukaemia near certain nuclear installations have led to suggestions that the risk of childhood leukaemia resulting from the intake of man-made radionuclides has been grossly underestimated (by a factor in excess of 100) [26]. Atmospheric nuclear weapons testing in the late-1950s and early 1960s led to ubiquitous exposure to the radioactive debris of these test explosions [27] and the intake of a range of radionuclides similar to that released from nuclear reactors and nuclear fuel reprocessing plants, providing the possibility of an investigation of the influence of weapons testing fallout upon childhood leukaemia incidence around the world. Such a study is not, however, straightforward because large-scale accurate registration of childhood leukaemia in the early-1960s and before was not commonplace, and the use of childhood leukaemia mortality data is not an acceptable alternative since treatment, and hence survival, was becoming increasingly successful after 1960. Nonetheless, the childhood leukaemia incidence data from eleven large-scale registries from three continents has been collated and examined by Wakeford *et al.* [28] who found no evidence that the marked peak of intake of man-made radionuclides present in nuclear weapons testing fallout has detectably influenced the subsequent risk of childhood leukaemia beyond the predictions of conventional leukaemia risk models, providing strong evidence against the suggestion that the risk arising from the intake of anthropogenic radionuclides can account for the reports of raised levels of childhood leukaemia near some nuclear facilities.

Studies of childhood leukaemia and exposure to radioactive contamination resulting from material released from nuclear installations have provided mixed findings. Following the Chernobyl nuclear reactor accident in 1986 investigations have failed to find unequivocal evidence for a raised risk of childhood leukaemia – although an earlier study found a notably raised risk of childhood leukaemia in the heavily contaminated districts of the Ukraine (but not in Belarus or Russia) [29], a later study in the Ukraine obtained a risk estimate that was substantially lower, raising questions about the accuracy of the data used in these studies [30]. Although studies of leukaemia in the riverside communities of the Techa River, which was heavily contaminated by early radioactive releases from the Mayak nuclear installation in the Southern Urals of Russia, have found raised risks related to the level of exposure [31], childhood leukaemia has not been considered separately and RBM dose estimates are being revised.

4.8 Conclusions

There is a broad consistency of results from the epidemiological study of childhood leukaemia and exposure to ionising radiation, and low dose/dose-rate risks appear to be compatible with the predictions of radiation-induced leukaemia risk models based upon the experience of the Japanese atomic bomb survivors, although certain aspects (e.g. the risk from natural background radiation, including radon) require further investigation.

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5 CHILDHOOD LEUKAEMIA AROUND NUCLEAR INSTALLATIONS

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5.1 Introduction

Nuclear installations can be found worldwide. At the end of 2008, in 31 countries 438 nuclear power plants were in operation and 44 under construction (IAEA, 2009). In addition to the nuclear power plants there are quite a number of reprocessing plants and research reactors (according to IAEA 274 research reactors in 56 countries at the end of 2004). Since in 1983, triggered by the television documentary "Windscale – the nuclear laundry", an impressing number of epidemiological studies has been carried out in order to look for childhood leukaemia in the vicinity of nuclear installations.

The results of those studies are not consistent. The problems involved in epidemiological studies around nuclear installations will be outlined in the following and a summary of the results will be given.

5.2 Methodological problems

A lot of different approaches have been chosen by epidemiologists to estimate childhood leukaemia risk in the vicinity of nuclear installations. It is always a little surprising for the non-epidemiologist to realize that after the publication of an epidemiological study, there is frequently an outcry in the community of epidemiologists, because of all the mistakes that have been made in the design of the study and the interpretation of the results. One must keep in mind, however, that there are a lot of methodological problems, so that the "ideal" study is usually impossible to carry out and compromises are inevitable. Opinions can be different, of course, to which extent such compromises are justified or indeed mis-leading.

Some of the methodological problems are the following:

- How to calculate the **expected** number of cases?

  Irrespective of the design of the study (ecological, cohort or case/control), the choice of the control group in order to estimate the expected number of cases is always a serious problem. Ideally, the only difference among the "nuclear installation" population and the controls should be the radiation exposure due to the nuclear installation. There are always other differences. This is no problem as long as these differences have no influence on the outcome of the comparison or if they can be taken into consideration properly. Sometimes, however, the differences are not known or have been considered in an inadequate way.

- How to define **„around"** nuclear installations?

  In a sense, the definition of "around" a nuclear installation is arbitrary. If, however, one wants to compare results of various studies, identical definitions are essential. Problems start already, when one group bases distance on miles and another on

kilometers. Sometimes the major direction of the wind is taken into consideration, sometimes not.

- Which **age group** should be chosen?

  The age group is of considerable importance. Meanwhile, it looks that if there is a conspicuous result at all, then the youngest (0 to 4 years) are affected. The observation, that when one looks at the entire childhood age group (0-14 years, for example) mostly no effect is observed, points to the possibility that, whatever the factor might be that causes the increase in risk for the youngest, some sort of a time-shift occurs: the leukaemia is shifted forward in time.

- Which **types of leukaemia** should be studied?

  During childhood, the acute forms of leukaemia are observed almost exclusively; chronic leukaemias are very rare. Some studies include all leukaemia types, some also lymphomas, whereas others restrict the analysis to the acute forms only. Problems might arise due to mis-classifications or to modified classifications of specific leukaemia types over the years.

- Should the conclusions be based on **incidence** or **mortality**?

  In the past, the choice of whether to base a study on incidence or mortality was almost irrelevant, because both endpoints were more or less identical. This has changed markedly, because about 80% of childhood leukaemias are curable nowadays. Provided that a well functioning registry is available, incidences are preferable, because the power of the study will be higher due to the higher number of cases compared to mortality studies.

- Is the study based on a **chance observation** or has a **hypothesis** been formulated before the study started?

  “Chance observations” of increased risk are similar to the famous “Texas sharp shooter” problem: shooting first and then drawing the target around the hit. Proceeding that way means that you will come up with an increased risk all the time. One should, of course, look for possible explanations of the observed unexpectedly high risk, but it is not the proper way to decide whether nuclear installations in general are responsible for the induction of childhood leukaemia. In order to answer that question, it is necessary to formulate a hypothesis before the epidemiological study is started and then to look whether the hypothesis is supported by the data or not. Chance observations are closely related to the problem of clusters.

- The cluster issue

  Usually, a chapter entitled “The cluster issue” should start with a definition of what is meant by a cluster. The problem: epidemiologists are very reluctant to come up with a definition of a cluster. And it is hard to analyse a phenomenon that is not defined precisely. In order to give an impression on the difficulties a definition is cited out of the “Principles of Epidemiology in Public Health Practice, 3rd Edition (U.S. Department of Health)”:

  “An aggregation of cases of a disease or other health-related condition, particularly cancer and birth defects, which are closely grouped in time and place.” This sounds quite reasonable and simple. The next sentence, however, points to the first difficulty*:* “*The number of cases may or may not exceed the expected number;”* and the final

statement almost kills the usefulness of the entire definition*: "frequently the expected number is not known".*

There seems to be, at least, some agreement that worldwide there are three clusters of childhood leukaemia in the vicinity of nuclear installations: Dounreay (Scotland), Krümmel (Germany), and Sellafield (England). This does not necessarily mean that the radiation exposure due to these installations is the causative factor of the observed leukaemia cases. One aspect that always has to be taken into account is related to childhood leukaemia as a rare event. On the average, about 5 leukaemia cases per 100,000 children below the age of 15 per year are observed. Rare events follow a Poissonian distribution which is characterized by a non-negligible fraction of "unexpectedly" high frequencies of an event.

5.3 Results obtained around nuclear installations

A huge number of studies has been carried out with the aim to look for childhood leukaemia around nuclear installations. Comprehensive recent reviews are available and will be referred to in the following. A somewhat surprising trend in the results, however, can be observed: compared to the publications in the eighties and early nineties with relative risks exceeding the 1, the more recent ones are close to a relative risk of 1 (Figure). This could mean, that the risk factor was more prominent in the past, but it could also mean that there is a publication bias in the sense that originally only studies were published that showed a "spectacular" effect, something well-known from publications in the non-scientific press, but, unfortunately, not uncommon for publications in the scientific field, too.

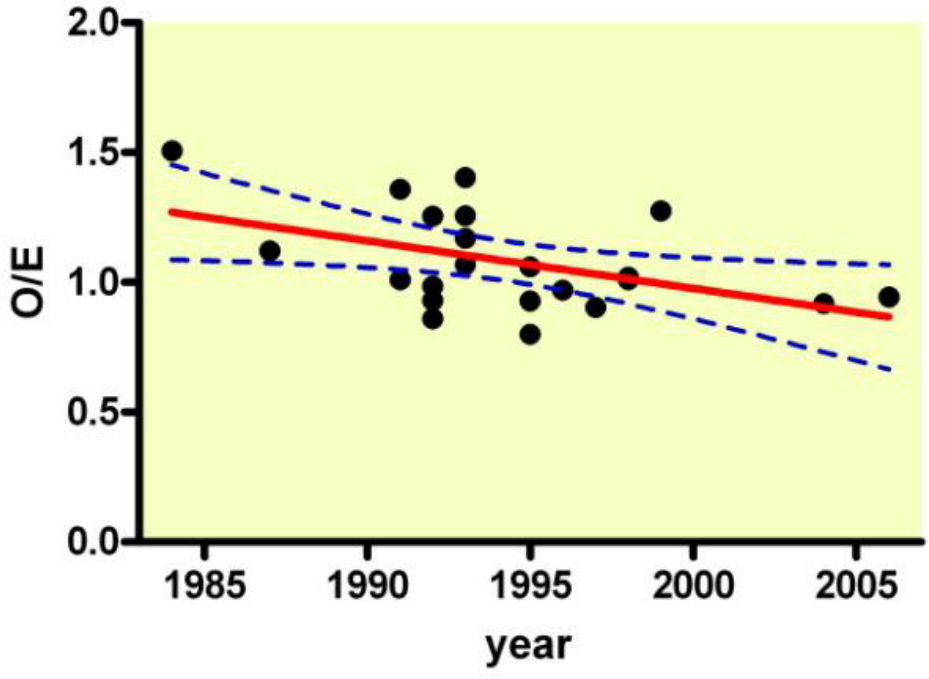

Figure 1: Dependence of the observed to expected effect (O/E) on the year of publication (slope significantly non-zero at p<0.05; source of data: Laurier et al. (2008))

5.3.1 IRSN Report (IRSN, 2008; Laurier et al., 2008)

A working group of the Institut de Radioprotection et de Sûreté Nucléaire (IRSN) looked critically at all the publications worldwide (Table 1). With the exception of three sites (Dounreay, Sellafield, Krümmel), there was either no indication of an excess of leukaemia cases, or an originally suspected excess was not confirmed, or, at three sites there is still some suspicion (La Hague, Aldermaston, Burghfield; the latter two, however, are so close together that they can be looked at as one site).

The overall conclusion of the IRSN report was: “Néanmoins, l’ensemble des études multisites actuellement disponibles, y compris en France, ne montre pas d’augmentation de la fréquence des leucémies globalement chez les 0-14 ans ou 0-24 ans à proximité des sites nucléaires.” (Anyhow, taken together all the available multisite studies, France included, essentially, they do not show an increase in the frequency of leukaemia in the vicinity of nuclear installations for ages 0 to 14 or 0 to 24.)

Risk	Number of installations
Confirmed	3
Possible	3
No confirmation of original suspicion	12
No increase in risk observed	177

Table 1: Results of risk analyses in the vicinity of nuclear installations (IRSN, 2008)

5.3.2 COMARE Report (COMARE, 2005)

The Committee on Medical Aspects of Radiation in the Environment (COMARE) published several reports on childhood leukaemia in the vicinity of nuclear installations in Great Britain. In its 10th report, COMARE examined the incidence of cancer at ages 0-14 years during 1969-1993. No excess of leukaemia and non-Hodgkin lymphoma was found within 25 km of any nuclear power plant, nor any increasing trend in incidence with proximity to any plant. COMARE concluded: “The results for nuclear power stations are unambiguous”. (Please, note: this conclusion refers to nuclear power plants, not to reprocessing plants or plants for weapon production. In the latter cases, COMARE 10 states that “the situation with the other nuclear sites is more complicated” and gives detailed explanations.).

5.3.3 Meta-analyses

One could hope that meta-analyses might help to get more insight, because, at least in theory, meta-analyses should increase the power considerably. Unfortunately, the individual studies which are intended to be summarized in a meta-analysis are frequently so heterogeneous that it is impossible to summarize them. Thus, a lot of studies cannot be included in a meta-analysis (the Baker and Hoel analysis (Baker and Hoel, 2007), for example, includes only 50 of the known 194 nuclear installation analyses). This opens such summarizing studies to quite some criticism (IRSN, 2008; Spix and Blettner, 2009).

5.3.4 Potential sites of nuclear installations

In 1989 an intriguing observation was published for the first time: an increased risk in childhood leukaemia was also detected at potential sites of nuclear installations, that is at sites where nuclear installations were planned but never built (Cook-Mozaffari et al., 1989). This observation was supported by another observation: there are nuclear installation sites where childhood leukaemia risk was elevated already before the installation started operating (Jablon

et al., 1991). This points to a factor common to sites of nuclear installations different from radiation.

5.4 Some examples of factors that are suspected to induce childhood leukaemia

Besides ionizing radiation quite a number of factors are, at least, suspected to induce childhood leukaemia (for a review see (Belson et al., 2007)); some of these are:

- Infections (McNally and Eden, 2004)
- Low frequency magnetic fields (Kleinerman et al., 2000)
- Various chemicals
    - Pesticides ((Infante-Rivard and Weichenthal, 2007; Zahm and Ward, 1998)
    - Asbestos (Kishimoto et al., 1988)
    - Benzene (Steensel-Moll et al., 1985)
    - Chemotherapeutics (Hawkins et al., 1992)
- Birth weight (Okcu et al., 2002)
- Genetic predisposition (Trevino et al., 2009)
- … … …

Several researchers have stressed the possibility that infections may play a major role in the causation of childhood leukaemia (Greaves, 2006; Kinlen et al., 1993; Smith, 1997). Smith favours an in-utero infection during pregnancy. Greaves argues that a pre-leukaemic cell clone is formed during pregnancy. After birth, one of the ordinary childhood infections results in an increase of cell numbers of this clone, thus increasing the probability of the final hit that is necessary for the manifestation of leukaemia. Kinlen observed that migration of many individuals into a previously isolated area is able to increase the number of leukaemia cases. He explains this observation by the import of a specific leukaemia inducing microorganism for which there is no defense mechanism available in the native population.

5.5 Conclusions

- In most studies, no increase in childhood leukaemia cases has been found around nuclear installations.
- In the case of positive results, it is mostly the youngest age group (0-4 years) that is affected; this strongly points to an induction of leukaemia during pregnancy.
- In those cases, in which an increase in childhood leukaemia was actually observed around nuclear installations, the calculated/measured radiation doses never reached a level that could explain the increase.
- Thus, even if the nuclear installation is responsible for the increase, there is no indication that it is radiation that causes this effect.
- The previous conclusion is supported by the observation that in some studies an increase in leukaemia risk is also observed around potential sites of nuclear installations.

5.6 What can be done to solve the riddle?

Unfortunately, our knowledge of the biological mechanisms that are involved in the induction and development of childhood leukaemia are rudimentary at best. Thus, it is very difficult to detect the factor/the factors that cause childhood leukaemia. There are a lot of indications that point to a multifactorial background of this disease with each factor being too weak to be easily identified in epidemiological studies. Identification of the biological mechanisms is urgently required that form the chain of events ending up in childhood leukaemia.

Due to the multifactorial character of childhood leukaemia it cannot be a single scientific discipline that will be successful. An interdisciplinary approach is necessary with, at least, the following disciplines being involved: epidemiology, (molecular) genetics, haematology, immunology, and radiobiology.

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6 CONTRIBUTIONS TO THE ROUND TABLE DISCUSSION

6.1 Viruses and common acute lymphoblastic leukaemia

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Leukaemia in childhood includes several entities with different cancer cell phenotypes and age-specific incidence curves, which are likely to be aetiologically distinct (Greaves 2006). Common acute lymphoblastic leukaemia (cALL), in which the leukaemic cell is a B-cell precursor, accounts for the peak in incidence rate seen between the ages of 2 and 5 years in areas of high socio-economic status (Greaves 2006;Greaves et al. 1993). There is now compelling molecular evidence that childhood leukaemia is frequently initiated *in utero;* however, further events are required for development of overt leukaemia. Both inherited and environmental factors have been implicated as secondary events (Greaves 2006). There is a body of evidence suggesting that infectious agents may play some role in the development of childhood leukaemia. Two models have been proposed based on time trends, geographic variation in incidence and clustering of cases (Bhatia et al. 1999;Greaves 1988;Kinlen 1988;Greaves 2006;Greaves et al. 1993). The association between cALL and socio-economic development (Greaves et al. 1993) has led to the suggestion that cALL may occur as a consequence of delayed exposure in infancy to an infectious agent or agents (Greaves 2006;Bhatia et al. 1999;Greaves 1988). An abnormal or dysregulated immunological response to post-natal infection, promoting secondary genetic events and leukaemia development, is the critical feature of this model. The putative agent(s) may be viral or bacterial, but the mechanism of action is indirect or non-transforming. The second model predicts that childhood leukaemia occurs as a rare response to a common transforming virus under certain demographic conditions (Kinlen 1988). It has been shown that influxes of new populations into previously isolated communities, such as occurred during the development of British New Towns during the 1950s, are associated with transient increased incidences of childhood leukaemia. Kinlen postulated that this was due to unrecognized epidemics of infection with an unidentified virus within such communities, and that these epidemics led to a transient increase in the incidence of leukaemia (Kinlen 1988;Kinlen 1995). Similar studies outside the UK support this hypothesis (Alexander et al. 1997), as does space-time clustering (Birch et al. 2000;Gustafsson and Carstensen 1999).

Over the last 15 years our group has examined cALL samples for the presence of a directly, transforming virus, i.e., a virus that is present in all the leukaemic cells and is necessary for leukaemia development. We have focused on virus families with known transforming potential that are widespread in the human population, namely herpesviruses and polyomaviruses. Samples were initially screened using highly sensitive PCR assays for known members of these virus families. We found no consistent evidence for involvement of the herpesviruses: varicella zoster virus; cytomegalovirus; human herpesvirus 6; human herpesvirus 7; and human herpesvirus 8. Similarly, we found no evidence for involvement of the polyomaviruses: JC virus; BK virus; the recently identified Merkel cell polyomavirus; and the primate polyomavirus SV40. In addition we looked for evidence of novel members of these virus families using degenerate PCR assays. These utilise primers derived from peptide sequences of viral proteins that are conserved across all, or multiple, members of a virus family (Jarrett et al. 2006). No novel viral sequences were detected using herpesvirus or polyomavirus degenerate PCR assays; however, the polyomavirus assay could miss some family members, such as the Merkel cell polyomavirus.

We also analyzed a series of cALL cases using the genomic subtractive hybridization technique, representational difference analysis (RDA) (MacKenzie et al. 2006). RDA has been used successfully in the identification of novel viruses, notably the Kaposi's sarcoma-

associated herpes virus (KSHV or HHV-8) (Chang et al. 1994) and TTV (Nishizawa et al. 1997). RDA requires no *a priori* knowledge of the putative viral agent and, under the conditions we adopted, should be capable of detecting infectious agents with a DNA genome or DNA intermediate of ≥ 9kb present at single copy level. No known or novel agents were detected in this study. This lends weight to the idea that no single, transforming, viral agent is involved in the aetiology of cALL; however, it is possible that viruses with small genomes (such as polyomaviruses, anelloviruses and parvoviruses) escaped detection by RDA because their genomes did not contain restriction fragments included in the genomic representations we investigated.

In summary, we have not detected viral sequences in leukaemic cells from cALL samples. Although we have used complimentary techniques to search for viruses, it remains possible that a viral genome, or a remnant of a viral genome, could have escaped detection. Recently, complete transcriptome sequencing of samples followed by digital subtraction (sequencing all the mRNA in a sample and discarding sequences that match the reference human genome, leaving potential viral sequences) has been successfully used in virus discovery (Feng et al. 2008). It is likely that complete genome sequencing will be performed in the near future. It is important that these techniques are applied to the study of cALL as it is only using these techniques that we will be able to show, or exclude, involvement of a single transforming agent. If we can exclude involvement of a single transforming agent, then efforts can be directed to the study of indirect effects of infectious agents.

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6.2 Investigation of virus in Guthrie cards from children who develop acute lymphoblastic leukaemia (ALL)

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6.2.1 Abstract

Background. In search of a proposed viral aetiology of childhood acute lymphoblastic leukaemia (ALL) specific viruses were analysed in achieved neonatal blood spots (Guthrie cards). **Methods and Results:** Guthrie cards from children who later developed ALL and matched controls were collected and analysed by nested PCR for the presence of different viruses including polyoma- parvo., EBV-CMV and HHV6. The results could not show any presence of human polyomaviruses, parvovirus, HHV-6, EBV or CMV investigated by nested PCR. All tested samples had amplifiable DNA, confirmed by HLA PCR. From the same material we later detected adenovirus DNA in 13/51 Guthrie cards from the ALL patients but in only 6/47 healthy controls, indicating that adenovirus may be such a causative agent. However, an expanded study including Guthrie cards from 243 children who later developed ALL and 486 matched healthy controls were analysed by nested PCR for the presence of adenovirus DNA. Adenovirus DNA was reliably detected from only two of these subjects, both of whom developed ALL. **Conclusion.** *In utero,* infections with these viruses are too low a frequency to reveal an association between these viruses and the development of leukaemia ALL.

6.2.2 Epidemiological studies

The etiology of most childhood acute lymphoblast leukaemia (ALL) cases is unknown, except of those <5% that depend on inherited conditions, immunodeficiency and exposure to ionizing radiation or chemotherapeutic agents [1].

The epidemiological characteristics of the disease suggest that it may have an infectious etiology [2]. One hypothesis concerning infectious mechanisms is that exposure *in utero* may promote the onset of leukemia [3]. If this transmission occurs in the beginning of foetal haematopoiesis in the liver at 2-6 months or later when active haematopoiesis is established in the bone marrow, different stages of foetal haematopoiesis can be affected. A secondary postnatal molecular event is then required for the preleukaemic clone to expand. The second event, leading to clinical leukaemia, can occur at a time of maximum stress on lymphocyte precursor proliferation and may be promoted by exposure to an infectious agent [3-6].

Suspicions of an infectious agent have to a great extent relied on reports of clusters, a high number of cases occurring in a small area over a limited period. Most reports have emphasized space-time clustering at diagnosis, but new studies have found space-time clustering at time around birth, which implicates a prenatal exposure to leukemia [7-11].

6.2.3 Specific viral infections

About 20% of malignancies can be etiologically associated with viruses such as human T-lymphotropic virus type 1 (HTLV-1), human papillomaviruses (HPV), hepatitis B virus, human herpes virus 8 (HHV-8) and EBV. Papillomaviruses, causing cervical cancer are in special focus, since vaccine can prevent HPV-induced neoplasia. The only known infectious cause of leukaemia is HTLV-1, causing adult T-cell leukaemia, being rare outside the Caribbean, equatorial Africa and southern Japan [12-13].

Smith (1997) has suggested that one possible causative infectious agent in childhood ALL could be a human polyomavirus. The virus could induce genomic instability, with specific effects on B lymphocytes, have a limited general oncogenic potential could cross the placenta and infect the fetus without causing severe fetal abnormalities [3].

Another possible agent is a human parvovirus B19, etiologically related to human diseases including erythema infectiosum and aplastic crisis in hemolytic diseases. An acute infection, associated with a cytokine cascade release, can lead to a disturbed hematopoiesis and induce proliferation of a malignant clone [2, 14].

Certain human herpes viruses such as HHV-8 and EBV are also causally associated with malignancies. Causative link has been discovered between a HHV-8 encoded chemokine receptor ORF74 and Kaposi's sarcoma. EBV which causes infectious mononucleosis during adolescence has a B-cell transforming activity and is close related to B-cell malignancy, e.g. Burkitt's lymphoma and post-transplant lymphoproliferative disease [15-16]. HHV-6, etiologically related to exanthema subitum play a role in the development of complications after stem cell transplantation [17]. CMV is a leading agent of intrauterine infections, causing congenital malformations. The virus can also induce growth transformation in rodent cells which can explain a possible role in e.g. cancers [18]. Species C adenovirus oncoproteins can disable the cellular DNA repair machinery and have the power to transform cells by a "hit and run" mechanism, making species C adenovirus a candidate for promoting the initial genetic lesion leading to leukaemia [19-20].

The aim of our studies was to investigate if specific prenatal virus infections could be indirectly correlated to the development of childhood ALL, by studying Guthrie cards from children who later developed ALL and healthy controls. The presence of human polyomaviruses, parvovirus B19, EBV, HHV-6 and CMV were investigated. We could not detect any viral DNA from the ALL cases or from the healthy controls, although all tested samples had amplifiable DNA as confirmed by an HLA DQ PCR [21-25]. We than analyzed adenovirus and reported that adenovirus DNA was detected in 13/51 Guthrie cards from ALL patients and in 6/47 healthy controls, indicating that adenovirus may be such a causative agent ($p = 0.0122$) [26]. An expanded study including Guthrie cards from 243 children who later developed ALL and 486 matched controls were collected and analysed by nested PCR. The presence of adenovirus DNA was reliably from only two subjects, both of whom developed ALL (in press).

6.2.4 Guthrie cards

The use of stored Guthrie cards as a genomic archive has considerable benefit since they are simple to store, DNA is relatively stable and contains 2-3 fold higher levels of DNA than DNA collected from adults. Guthrie cards have also been used for diagnosis of neonatal infections as detection of CMV and HSV in neonates [27-28].

We collected Guthrie cards obtained at birth from 54 respectively 243 children who had developed ALL. The majority of these children had been diagnosed as pre-B ALL and some were diagnosed as T-ALLs. The median age in both groups was 5 years. These children had been admitted for treatment at different Paediatric Oncology Swedish Centres. As a control group we obtained 47 respectively 486 healthy controls that were matched for age and birthplace. The capillary blood from both groups is collected at 3-5 days of age.

For DNA extraction from Guthrie cards the Minimal Essential Medium™ (MEM) extraction method was used. Three uniform discs, three millimetres in diameter, were punched from the Guthrie cards and 100 μl of MEM was added to each tube to elude the blood. Presence of a viral DNA was analyzed by sensitive and reproducible PCR-methods specific for respective virus. In addition, a set of HLA DQ primers were used to exclude the possibility of false negative results due to the failure of DNA extraction or the presence of inhibitory factors [29].

6.2.5 Discussion

Guthrie cards have been used for many purposes, including the backtracking paediatric leukaemias to birth. Many studies support the hypothesis that some childhood ALL may arise *in utero*. If these genetic events are acquired prenatally during foetal haematopoiesis, they can be initiated by a virus infection. In several of these cases it is also a long latency period from the initial ALL clone to the diagnosis of leukaemia. Greaves hypothesized that whatever cause the gene rearrangements *in utero,* postnatal events or hits including infection are required to promote the development of leukemia [30-31].

The presumable factor for demonstration of viral DNA in Guthrie cards is the presence of viremia at birth, when samples were collected. The reason why we did not detect any viral DNA, except in a few cases, may be due to the fact that infection occurred early during the pregnancy and viremia may have disappeared at the time of birth. However, during infection with CMV and HSV in pregnant mothers, Guthrie cards were positive for virus DNA even if the maternal infection took place early in pregnancy, [27].

After primary infection certain viruses became latent in different cell types and difficult to detect. Since peripheral blood cells can harbour latent viruses such as polyoma and herpes viruses it should be possible to detect them in Guthrie cards.

For discovering low grade viremia, the methods used for amplification of DNA of specific viruses were highly sensitive and reproducible. In addition, we also included positive controls that were tested positive in Guthrie cards. In summary, the possibility that our results could be false negative must be considered very small.

Space-time clustering data, based on time and place of birth indicates events *in utero.* Since there still is considerable interest in a potential relationship between childhood ALL and infection *in utero,* the search for a viral etiology will continue even if no specific virus yet has been defined.

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6.3 Infection, population mixing, and childhood leukaemia

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In childhood leukaemia, dominated by acute lymphoblastic leukaemia, infection has long been a suspicion, encouraged by reports of apparent 'clusters' (although here chance must often be involved), and by the discovery of the viruses underlying the leukaemias in several animal species. As the disease is not contagious (marked space–time clustering being absent), it must, if infective in origin, belong to that large category of disorders that are rare responses to the relevant (but here unidentified) infection. Infective dose is considered an important determinant of whether illness will occur. Such infections are mainly subclinical, and their effects largely immunising, and examples include poliomyelitis, infectious mononucleosis and hepatitis A, besides the virus infections that are causally linked to more than ten specific cancers (e.g. of cervix and liver).

The excesses of childhood leukaemia near the Sellafield and Dounreay nuclear installations (in north-west England, and northern Scotland respectively), have been the subject of much public concern and scientific investigation; they cannot, however, be explained in terms of radiation exposure. To build and operate these plants in such isolated areas involved major population influxes. From the well-established premises that epidemics are fuelled by the availability of sufficient susceptible individuals and that these are more prevalent in rural

areas (reflecting the reduced opportunities for contacts with a wide infective pool), the population mixing hypothesis was proposed. This holds that a localized epidemic of a relevant underlying infection would be promoted by the large-scale mixing of rural and urban people (implying appreciable mixing of susceptible and infected individuals), tending thereby to produce an excess of its uncommon complication, childhood leukaemia (Kinlen, 1988; 1995; 2000).

In fact, transient excesses of childhood leukaemia have been found in all the major examples of rural–urban mixing in Britain in the past 60 years, including rural new towns (Kinlen et al., 1990), wartime evacuation of children to rural areas (Kinlen and John, 1994), post-war increases of national servicemen in rural areas (Kinlen and Hudson, 1991), areas around large rural (non-nuclear) construction sites (Kinlen et al, 1995), rural Scottish communities where a large proportion of men worked away from home in the North Sea oil industry (Kinlen et al., 1993) and in wartime Orkney and Shetland where large numbers of servicemen were stationed (Kinlen and Balkwill, 2001). The incomers in the last four of these rural situations did not include children, pointing to the importance of adults in the chain of transmission.

Observations outside Britain have produced similar findings – in Greece and Italy (Kinlen and Petridou, 1995), the New Territories, Hong Kong (Alexander et al., 1997), Ontario, Canada (Koushik et al., 2001), La Hague, France (Boutou et al., 2002), and USA (Wartenberg 2004). Recently, in the small desert town of Fallon in Nevada, US a large excess of childhood leukaemia occurred in 2000 when the intake of trainees stationed temporarily at the nearby naval air-base peaked at 55,000, having risen from 20,000 per year in the early 1990s (Steinmaus et al, 2004; Kinlen and Doll, 2004).

The findings in the extreme high exposure category of rural population mixing studies are summarised in the pooled analysis shown in Figure 1; the overall excess is highly significant (Kinlen 2010). Corresponding findings after urban population mixing are shown in Figure 2, which include areas subject to the same type and extent of mixing as in Figure 1. The contrast could not be more striking, and the absence of any overall urban excess points to immunity attributable to early exposure to some agent ubiquitous in urban areas.

Evidence for an infective basis in childhood leukaemia has come not only from population-based, but from individual-based (case-control), studies. In the excesses associated with rural population mixing, a disproportionate number was noticed of pre-school children with fathers in ‘high contact’ occupations (such as teachers), plausibly widening their indirect community contacts (Kinlen 1997); similar observations have been made in cytomegalovirus infection and in certain poliovirus epidemics. In the national case-control studies in Scotland and Sweden that followed, significant positive trends were present across the categories of increasing levels of paternal occupational contacts (Kinlen and Bramald 2001; Kinlen et al 2002); as in the earlier studies, these effects were seen in rural, but not in urban, areas. Again, the role of adults in transmission is highlighted.

Turning again to the source of the hypothesis, it is noteworthy that Dickinson and Parker (1999) were able to account statistically for the excess in Seascale, near Sellafield, in a model of population mixing and childhood leukaemia risk using information on births in Cumbrian wards other than Seascale. This, in conjunction with the other work, led Doll (1999) to conclude that the epidemiological evidence for an infective basis in this disease was compelling.

Virtually all elucidated infective disorders are caused by specific agents, and in childhood leukaemia a virus seems most likely. Most viral studies have consisted in excluding specific viruses in the aetiology of the disease, and there is a dearth of searches for a previously unidentified, ubiquitous urban virus having a lower prevalence in rural areas. Thus, the study of affected urban children using as controls either the same children in remission or their

parents (MacKenzie et al 2006), however novel in other respects, was not directed towards a virus having the characteristics indicated by rural population mixing studies. Virus studies are needed of the disease in isolated areas with appropriate control children.

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6.3.2 Figures

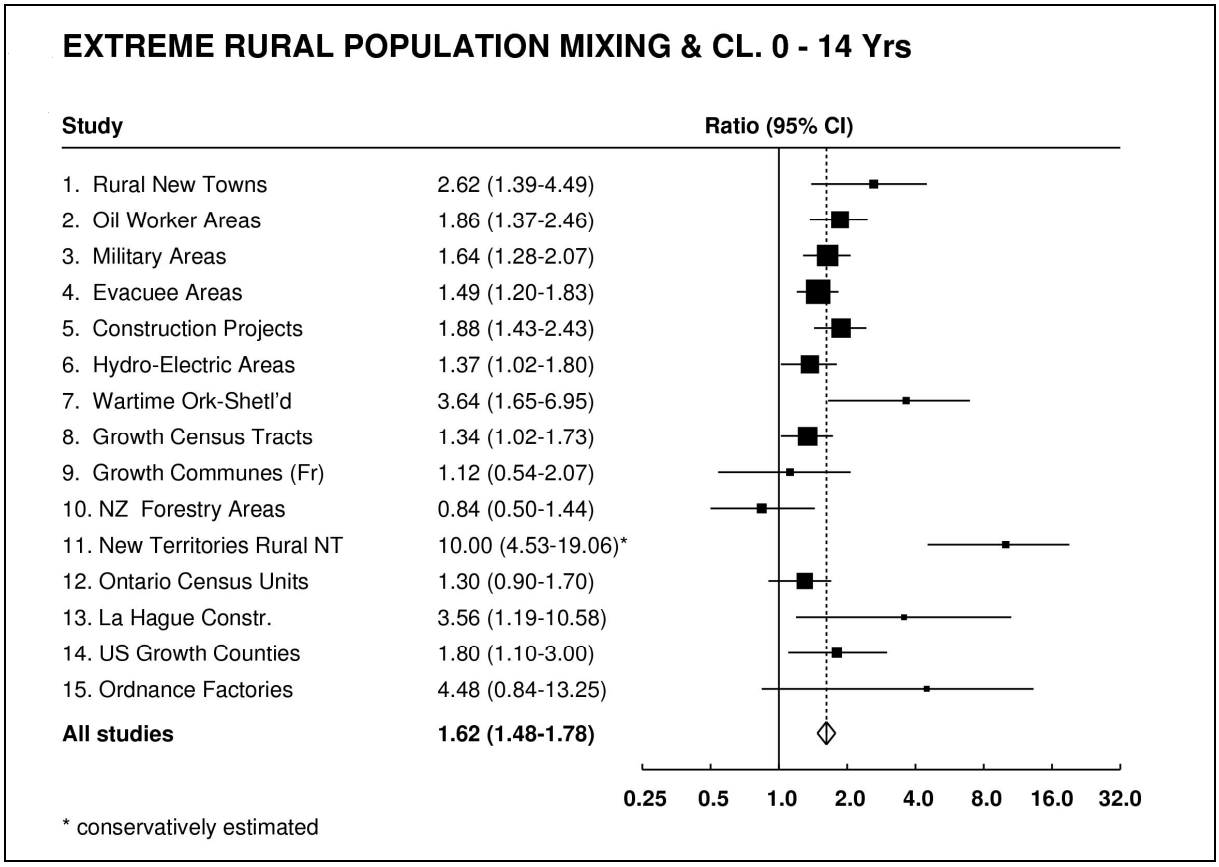

Figure 1: Childhood leukaemia (or all) & extreme rural pm*

Kinlen et al 1990 and unpublished; 2. Kinlen et al 1993; 3. Kinlen & Hudson 1991; 4. Kinlen & Hudson 1991; 5. Kinlen & Hudson 1991; 6. Kinlen & John 1994; 7. Kinlen et al 1995; 8. Kinlen et al 1995; 9. Kinlen & Balkwill 2001; 10. Rodrigues et al 1991; 11. Laplanche & de Vathaire 1994; 12. Dockerty et al 1996; 13. Alexander et al 1997 and personal communication.; 14. Koushik et al 2001; 15. Boutou et al 2002; 16. Wartenberg et al 2004; 17. Kinlen 2006.

* Population mixing.

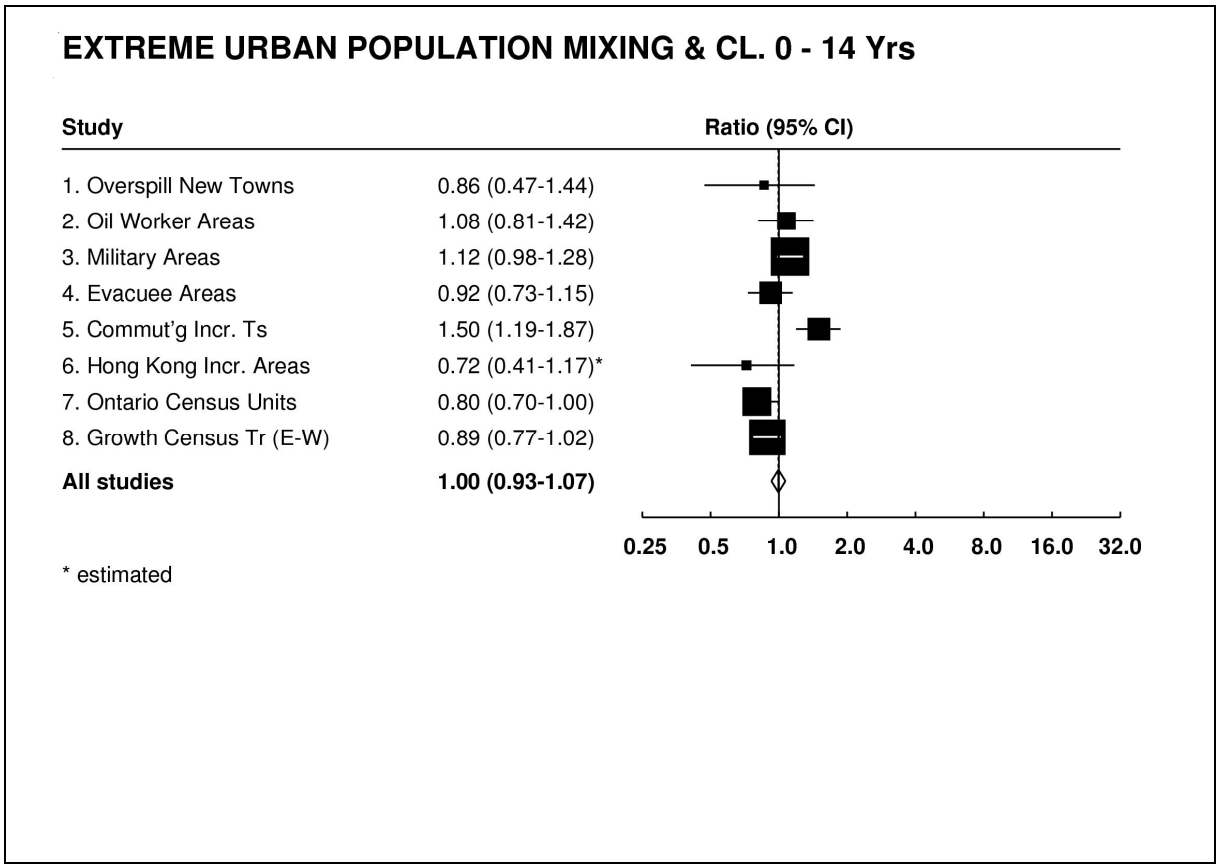

Figure 2: Childhood leukaemia (or all) & extreme urban pm*

Kinlen et al 1990 and unpublished.; 2. Kinlen et al 1993; 3. Kinlen & Hudson 1991; 4. Kinlen & John 1994; 5. Kinlen et al 1995; 6. Alexander et al 1997 and personal communication.; 7. Koushik et al 2001; 8. Rodrigues et al 1991.

* Population mixing.

7 SUMMARY

Prepared by Dr Jean Piechowski, CEA, France
On behalf of the Working Party "Research Implications on Health and Safety Standards" of the Article 31 Group of Experts[§]

7.1 Introduction

This document provides the background, summarizes the presentations and tries to emphasize the potential implications of the Scientific Seminar on “Childhood Leukaemia - mechanisms and causes”, held in Luxembourg on 3 November 2009. It takes into account the discussions that took place during the Seminar and during the subsequent meeting of the Article 31 Group of experts on 5 November 2009, although it is not intended to report in an exhaustive manner all the opinions that were expressed. The document has been submitted for comments to the lecturers, as far as their contributions were concerned.

7.2 The Article 31 Group of experts and the rationale of the RIHSS Seminars

The Article 31 Group of experts is a group of independent scientific experts referred to in Article 31 of the Euratom Treaty, which assists the European Commission in the preparation of the EU Basic Safety Standards for the protection of the health of workers and the general public against the dangers arising from ionizing radiation. According to the Euratom Treaty and to their Code of Ethics, this group of experts has to give priority to the protection of health, to the safety and to the development of the best available operational radiation protection. For doing so, they have to follow carefully the scientific and technological developments and the new data coming from the world of research, particularly when these could affect the health of the exposed persons.

In this context, a Scientific Seminar is devoted every year to emerging issues in Radiation Protection – generally addressing new research findings with potential policy and/or regulatory implications. On the basis of input from the Directorate General Research of the European Commission and of information provided by individual members of the Article 31 Group of experts, the RIHSS Working Party proposes relevant themes to the Article 31 Group that could be discussed during a subsequent seminar. After selection of the theme and approval of a draft programme by the Article 31 Group, the RIHSS Working Party deals with the preparation and the follow up of the seminar. Leading scientists are invited to present the status of scientific knowledge in the selected topic. Additional experts, identified by members of the Article 31 Group from their own country, take part in the seminars and act as peer reviewers. The Commission convenes the seminars on the day before a meeting of the Article 31 Group, in order that members of the Group can discuss the potential implications of the combined scientific results. Based on the outcome of the Scientific Seminar, the Group of Experts referred to in Article 31 of the Euratom Treaty may recommend research, regulatory or legislative initiatives. The European Commission takes into account the conclusions of the Experts when setting up its radiation protection

§ Besides J. Piechowski (who was acting as rapporteur for the seminar), the following members of the Working Party on Research Implications on Health and Safety Standards of the Article 31 Group of Experts contributed to the preparation of this overview: L. Lebaron-Jacobs, W-U Müller, P. Olko, S. Risica, P. Smeesters (Chairman of the WP), R. Wakeford. They were assisted by the following official of the European Commission: S. Mundigl.

programme. The Experts' conclusions are also valuable input to the process of reviewing and potentially revising European radiation protection legislation.

7.3 Key Highlights of Presentations Seminar

Danièle Sommelet - *Advances in childhood acute leukaemias: general overview.*

Acute leukaemias are the most common (30%) cancers in children. Intrinsic genomic factors are combined with environmental factors. Therapeutic case management has improved considerably: the survival rate has gone from 1% in 1960 to 85% for lymphoblastic leukaemias and 60% for myeloblastic leukaemias. The prognosis is determined by different factors: age, blood count, cytology, immunophenotype, caryotype and molecular genetics. The outcome of the disease and therapeutic efficacy are associated with the expression of groups of genes involved in cell functions linked with the proliferative potential of the cell. On the other hand, no link with the cause of the disease has been established. There are many different childhood leukaemias, resulting from various chromosomal translocations, mutations, aneuploidies and deletions, associated with epigenetic modifications. Leukaemia is a clonal disease that begins *in utero*. Preleukaemic clones have been detected in umbilical cord blood in less than 1% of a sample of births and around 1% of these clones lead to an established case of leukaemia. An abnormal immune response, in the course of a commonplace infection, could be the factor that triggers this switch over to leukaemia. In the present state of our knowledge, leukaemias appear as genetically heterogeneous and complex. Various research fields (molecular biology, animal models, cytopathology, pharmacogenetics, epidemiology, etc.) have opened up simultaneously to further our knowledge of the disease, particularly with regard to its characterisation, response to therapeutics and aetiology.

Jacqueline Clavel - *Risk factors of childhood leukaemia: the French research program.*

Various causes are suspected in the aetiology of childhood leukaemias: environment, infections and immune system, genetic factors, etc. Elaborate means are currently available to epidemiology to delve more deeply into the environmental approach to the problem. For instance, in the GEOCAP project, the role of various cases of environmental exposure located by geocoding is being studied in a systematic manner, by linking them to the addresses of cases of paediatric leukaemias recorded in the national register of malignant haematological diseases in children. In particular, the project is focusing on road traffic, high voltage lines, service stations, Seveso classified industrial sites, radon and nuclear installations. Concerning the possible causes of leukaemia, there is now certainty as regards the unfavourable role of late exposure to infection, whereas early infection may instead play a protective role. There exists a possible suspicion concerning radon, regarding a possible cluster of leukaemias in Massif Central in France, while in all likelihood nuclear installations do not seem to be involved. Chemical factors, concerning for example the exposure of pregnant women to insecticides, certain compounds in automobile fuels and cigarette smoke, are strongly suspected.

Heribert Jürgens - *Aetiology of childhood leukaemia.*

The currently proposed model is based on two-step genomic events: creation of a preleukaemic clone *in utero* in around 1% of cases, then the occurrence of an event during childhood that transforms this preleukaemic clone into a leukaemic clone with a probability of

around 1%. Some hereditary diseases involving chromosomal anomalies, with Down's syndrome at the forefront, are very susceptible to leukaemia. Several factors conducive to leukaemias have been alluded to, such as overweight or pregnant woman's diet rich in topoisomerase II inhibitors. The relation with ionising radiation is well established on the other hand, at least as regards atomic bombs. The Chernobyl accident does not seem to have caused an obvious jump in childhood leukaemia. Exposure to electromagnetic fields does not appear to be leukomogenic either. Although a relation with high socio-economic status appears to be established, it does not for the moment have a very convincing explanation although various speculative hypotheses have been suggested. Finally, the role of infections has been underlined with ambivalence widely described (unfavourable effect of late exposure to infections, whereas early exposure protects), highlighting the involvement of the immune system in the emergence of the disease, even though a convincing mechanism has yet to be put forward.

Richard Wakeford - *Ionising radiation epidemiology of childhood leukaemia.*

From a historical viewpoint, epidemiology has for a long time demonstrated the role of ionising radiation in the induction of leukaemias in children as well as in adults: radiologists, victims of atomic bombs, radiotherapy effects, *in utero* radiography, etc. The slope of the dose-response increases as the dose increases. Excess relative risk (ERR) is clearly greater at a younger age-at-exposure. It falls away with time-since-exposure. The latency period is shorter than for solid cancers. All in all, the risk factors that can be determined from most studies tie in with each other, leading to a representative ERR coefficient of around $50.Sv^{-1}$, for either ante-or-postnatal irradiation. However, the set of epidemiological data may be truncated, namely records for the exposed bomb survivors have only begun after October 1950. This data gap possibly explains why neither leukaemia nor chromosomal translocations were observed in the *in utero*-exposed bomb survivors included in the study: if these malignant conditions occurred in *in utero*-exposed children who died before October 1950, they went *de facto* undetected, whilst translocations were actually present and increase with the dose, in their mothers. Radon in dwellings has been alluded to as a possible cause, but more work needs to be done before any firm conclusions can be drawn. The evolution in the worldwide incidence of childhood leukaemia does not appear to have been significantly altered by fall-out from atomic tests in the atmosphere.

Wolfgang-U. Müller - *Childhood leukaemia around nuclear installations*

A key problem in leukaemia studies, with the appearance of clusters, is precisely the definition of what a cluster actually is. In the definition given by the US Department of Health, the number of cases observed in a cluster is not necessarily greater than the number of expected cases. The definition is instead based on the qualitative aspect of the grouping of cases and thus is not very precise. The distribution of childhood leukaemia cases in Germany is variable and heterogeneous, without any clearly identifiable trend. Overall, in global terms, only 3 clusters (Sellafield, Dounreay and Krümmel) have been confirmed in the world, out of around 200 possible associations between a cluster and a nuclear installation that have been subjected to statistical analysis. Does it need to be pointed out once again that they concern a geographic relation, without the nuclear aspect of the installations concerned necessarily being involved in any way? Meta-analyses are almost impossible because the studies that have been carried out are far too heterogeneous. We did not have the means to perform the necessary data consolidations, so that a lot of analyses could not have been included in a meta-analysis, resulting in a selection bias that would have really mattered. In any case, the level of dose that would be needed to explain the cases where an excess of leukaemias grouped within a cluster are observed is at least 1000 times higher

than the actual level corresponding to radioactive releases from nuclear installations. We therefore need to look for other causes, particularly since clusters have been observed in future potential nuclear installation sites. The diversity of possible causes means the aetiology of the childhood leukaemias observed in the vicinity of a few nuclear installations is a question that remains completely open for the moment.

7.4 Summary of the round table discussion

Ruth Jarrett, Britt Gustafsson, Danièle Sommelet, Heribert Jürgens, Richard Wakeford (also representative of Leo Kinlen), Wolfgang-Ulrich Müller.

Infectious aetiology is strongly suspected in childhood leukaemia. The search for leukomogenic viruses is thus entirely vindicated. Various possibilities of contamination of the child may be conceived, either direct or via their mother. For the moment, tests carried out on children suffering from leukaemia to detect the presence of viruses have turned out to be negative, particularly as regards polyomavirus, parvovirus, adenovirus or herpes viruses. Nevertheless, we need to continue to explore the viral hypothesis, especially since the analysis of the complete transcriptome, subtracting the human RNA spectrum, has recently proved to be highly effective in other situations by revealing the hidden presence of viral RNA spectra. The advantage of this technique is that it is not necessary to have prior knowledge of the sought-after virus. In this way, new viruses can be discovered. The infectious cause is again underlined by epidemiological studies on the risk of leukaemia as a function of the mixing of certain populations: a significant risk in the case of rural populations where children are generally less exposed and are exposed at a later stage to infections, compared to no risk in the case of urban populations where children are much more prone to early and varied exposure to infections.

The question of clusters has been discussed. We know very well how to identify a cluster statistically. However, a cluster is a small number and there is no aetiological signature. The clusters may therefore just be due to coincidence but we need to continue to look for an explanation in the most rational way possible. Until now, it has been the infectious cause that seems to tie in best with the observed facts. The difficulty is that there is no incontestable determining biological element to confirm this hypothesis. As for other possible causes, for the moment they are simply suspected, without there being any direct proof of their involvement in such or such a particular case. Obviously, several potentially leukomogenic factors interacting to trigger the disease may also be envisaged.

Concerning the particular case of clusters around nuclear installations, the notion of likelihood goes completely against the grain of a cause and effect relation between the radioactivity induced in the environment and the appearance of leukaemias. In fact, on the basis of extensive epidemiological studies, the risk concerning radiologically induced leukaemias is well known but the level of assessed releases from nuclear installations is significantly incompatible with the number of leukaemias observed. It is too low by a factor of at least 1000. The question therefore remains why these clusters exist, especially since they also exist on potential nuclear installation sites. The subject is far from being closed.

The difficulty in the aetiological approach, coupled with the extreme genetic polymorphism of childhood leukaemias, suggests that different and complementary research means need to be employed in this field, including for example epidemiology, molecular genetics, haematology, immunology, virology and radiobiology. One attractive avenue of research concerns environmental genetics, with convergent geo-localisation of potential nuisances and leukaemia diagnoses. This would however involve a considerable body of work and, at the outset, without any known “toxic-genomic impact” type of reference link.

It may thus be concluded that childhood leukaemia remains a completely open research topic, where there is a willingness to combine the use of state of the art biological methods with epidemiological management based on environmental data, in the hope of demonstrating more substantial relations between some of the suspected toxic sources and the onset of the disease. Childhood leukaemia remains a complicated subject. Moreover, viral research needs to continue to explore the hypothesis of the involvement of still unknown viruses or the particular expression of certain known viruses. The involvement of radioactive releases from nuclear installations, on the basis of the known facts, is highly improbable.

8 CONCLUSIONS

Working Party "Research Implications on Health and Safety Standards" of the Article 31 Group of Experts[**]

Although they constitute relatively rare diseases with a currently better prognosis than in the past, childhood leukaemias affect young children, mainly of 2 to 4 years of age, imposing upon them heavy and painful treatments and remain lethal in a significant proportion of the cases (survival rate of 85% for ALL and 60% for AML). The incidence rate of the ALL is increasing slowly but continuously in Western countries over decades. In addition, childhood leukaemias have a strong symbolic character and are often perceived as the sign of an environmental threat. For all these reasons, they merit a specific attention.

Childhood leukaemias are characterized by their complexity and heterogeneity, and represent in fact a variety of diseases, with possibly a variety of causes, as is the case for many other cancers.

This being said, ionizing radiation is one cause for childhood leukaemia for which we have solid scientific evidence, besides some genetic diseases (like trisomia 21 or Fanconi's disease) and chemotherapeutic agents that account only for less than a few % of childhood leukaemias. Associations were also found with maternal use of insecticides during pregnancy, pesticides, proximity of gas stations (benzene?), mixing of populations (infections?), high birth weight, high level electro-magnetic fields… and radon (to be further investigated). On the other hand, breastfeeding seems to be protecting. Neither virus nor viral fragment has been identified up to now in humans. A frequently advocated hypothesis is the link with the delay of primary infections during the first months of life. This hypothesis could explain the observed association with high socio-economic status (Greave's hypothesis).

From a mechanistic point of view, there seems to be currently a large agreement on the fact that ALL-type childhood leukaemia require for two chronologically separate oncogenic events, one occurring during pregnancy (first hit), giving rise to preleukaemic cells and clones that are more susceptible, after birth, to additional oncogenic events (the "second hit").

As indicated, ionizing radiation is a good documented cause of childhood leukaemia. The Excess Relative Risk observed in the Life Span Study (survivors in Hiroshima and Nagasaki) has been confirmed in medical therapeutic studies and in various medical diagnostic studies (among which the famous Oxford Survey of Childhood Cancers). Childhood leukaemias are induced after *in utero* irradiation even at low doses (of the order of may be some mGy).

Clusters of childhood leukaemias in the vicinity of nuclear installations have been described but they are very rare (only three well documented associations could be found in over 195 investigations) and no relation could be found with doses attributable to radioactive discharges. It has to be underlined that most epidemiological studies were very poor with regard to their detectability power. The reason for these three clusters remains unknown. Thus, even if the nuclear installation is responsible for the increase, there is no indication that it is radiation that causes this effect. The previous conclusion is supported by the observation

[**] The following members of the Working Party on Research Implications on Health and Safety Standards of the Article 31 Group of Experts contributed to the preparation of these conclusions: L. Lebaron-Jacobs, W-U Müller, P. Olko, S. Risica, P. Smeesters (Chairman of the WP), R. Wakeford.

that in some studies an increase in leukaemia risk is also observed around potential sites of nuclear installations.

There was an agreement amongst the experts present at the Seminar on the need for multidisciplinary studies on childhood leukaemia and for a revaluation of the epidemiological approaches. Large scale epidemiological studies should be preferred and gene-environment interactions should be explored.

The weight of evidence for radiation-induced childhood leukaemia after *in utero* irradiation requires a precautionary approach with respect to the protection of pregnant women, in all types of exposure situations. With regard to medical exposures, the need for well-informed medical doctors is emphasised in order to guarantee the necessary risk awareness. In this respect, the frequent spreading of opinion that there is no risk below a threshold of 100 mSv, in particular for *in utero* exposures, should be firmly refuted.

A prudent approach is also advised with regard to medical exposures of children. The use of CT in children has to be carefully justified and the exposures adequately optimised. This again asks for more effort in education and information.