A QUEST TO FIND CURES FOR PAEDIATRIC CANCERS

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The cancer mortality rate – the number of deaths due to cancer per 100,000 people per year – among children ages 0-19 has declined dramatically in the last four decades thanks to improved treatment regimens. Some cancers, such as acute lymphoblastic leukaemia go into remission in 98% of cases and have a 90% cure rate, defined as more than 10 years of remission.

Because of major treatment advances in recent decades, more than 80% of children with cancer now survive 5 years or more (Figure 1). Overall this is a dramatic increase since the mide-70s, when the 5-year survival rate was about 58%, but nevertheless survival rates vary depending on the type of cancer and other factors. Thus, whereas mortality from leukaemia and lymphomas has decreased by more than half, many solid tumours, including sarcomas still have unacceptable mortality rates (Figure 1). Although the odds of a child developing cancer by the age of 19 are only 1 in 330, cancer is second only to accidents as a cause of death in children.

Most childhood cancers are not inherited – they are primarily the result of DNA changes that occur early in the child's life, sometimes even before birth. Cancers that develop in children include leukaemia, brain and spinal cord tumours, neuroblastoma, Wilms tumour, lymphoma, including Hodgkin and non-Hodgkin lymphomas, rhabdomyosarcoma, desmoplastic small round cell tumour (DSRCT), retinoblastoma, and bone cancer, including osteosarcoma and Ewing sarcoma.

Much of paediatric cancer therapy was initially based on that of adult cancers. However, most paediatric malignancies develop and behave in ways that differ from those of adult cancers, largely due to genetic and epigenetic differences. In contrast to the majority of adult cancers, which develop years to decades following transformation of a single cell, paediatric cancers, particularly those that occur in the first few years following birth, emerge far more rapidly. Genetic analyses have shown that whereas most adult cancers accumulate numerous genetic mutations, paediatric cancers are for the most part genetically "quiescent", meaning that they harbour few and sometimes even only single mutations. This notion focuses the search toward understanding paediatric sarcoma pathogenesis on a single defined event and its consequences and heightens the likelihood of finding relevant new therapeutic options. However, the experimental models used to elucidate the pathogenesis of any cancer are critical. We have learned a great deal from mouse models but they can approximate human malignancies only up to a point. Furthermore, many human sarcomas, and notably paediatric sarcomas, have been difficult to model in mice, and reliable mouse equivalents are not available. The use of established human sarcoma cell lines also has its limitations as cells that have been propagated in in vitro culture for years lose key biological features and acquire new ones such that they may no longer represent the tumour from which they originated with high fidelity.

We begin with the premise that no child should die of cancer. With that in mind, we set out to build a paediatric cancer research program that should overcome many of the obstacles that have limited progress in paediatric cancer treatment in the past. Our approach is to work on primary, patient-derived paediatric cancer cells and primary normal tissue-derived cells from which these cancers originate. This is a significantly more challenging approach

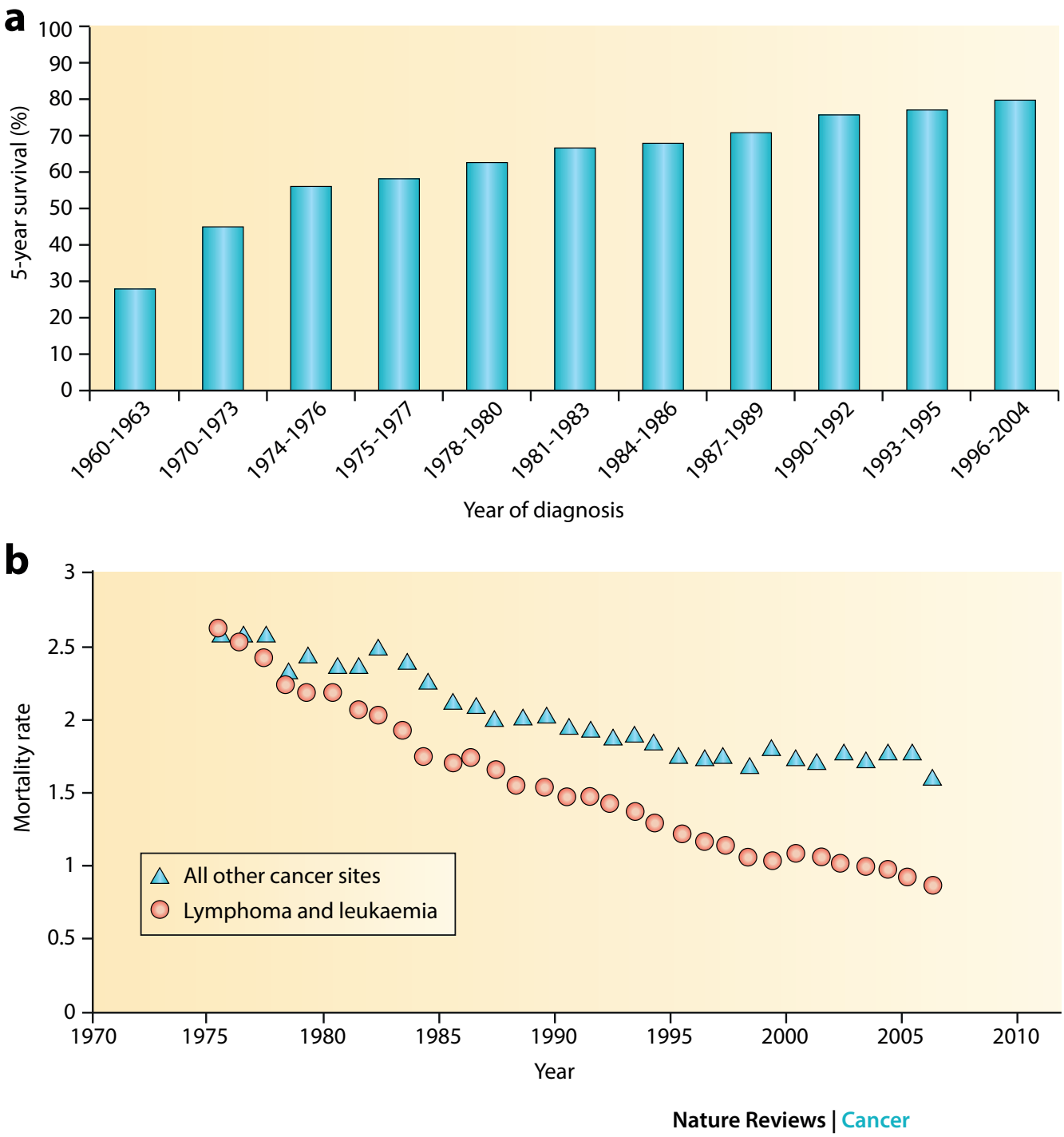

Figure 1. (a) Evolution of 5-year survival rates of all paediatric cancers; b) Decrease in mortality rates of children with leukaemia ad lymphoma versus all other paediatric malignancies. Source: Norris, R.E. & Adamson P.C. Nature Reviews Cancer 12:776-782, 2012.

than working with mouse models and cell lines but it goes to the heart of the matter by relying on patient-derived tissue, which is precisely the material whose behaviour we want to understand and ultimately target. In the process of establishing primary patient derived tumour cell cultures, we have acquired a unique expertise in handling rare paediatric cancers and in exploring their biological properties. This has led us to uncover a number of properties of these malignancies that we would not have identified using mouse models or established cancer cell lines.

Our focus is on solid paediatric malignancies. We want to understand how primary cells undergo transformation in response to oncogenic events, some of which are specific to paediatric cancers. Thus, several unique chromosomal translocations are associated almost exclusively with paediatric

malignancies, including Ewing sarcoma, rhabdomyosarcoma and DSRCT, to mention just a few. Each of these translocations gives rise to fusion genes that encode fusion proteins, which may partially recapitulate the functions of the wild type proteins of which they are composed, but which also, and more importantly, display aberrant functions of their own that have oncogenic properties. Does this mean that these oncogenic events occur exclusively in children? The answer is probably no, but rather that the cells, which are permissive for the expression and function of the aberrant proteins, may be uniquely present or more abundant in children. Key toward understanding paediatric tumour development is the identification of cells from which these tumours originate. Most normal cells are resistant to transformation. If a mutation that activates an oncogene occurs in a differentiated cell, the cell undergoes what is referred to as oncogenic stress, which induces genes that cause the cells to stop proliferating and guide them toward a state of permanent growth arrest known as senescence. The state of senescence constitutes a powerful tumour suppressive mechanism and a safeguard against transformation. However, some cells, particularly stem cells, are constantly engaged in the cell cycle, which requires suppression of some of the key growth inhibitory genes. These cells thereby already display some of the properties that transformed cells recapitulate. Various types of stem cells, from embryonic stem cells to more lineage-committed variants, are more susceptible to transformation than differentiated cells and may constitute the origin of a variety of cancer types. Although transformation can occur in a differentiated cell, it requires reprogramming that many potentially oncogenic events may not be able to fulfil. A specific oncogenic event would first need to reprogram the cell to acquire stem cell features, including the suppression of growth inhibitory genes, to provide permissiveness for transformation. In contrast an oncogenic event that occurs in a stem cell does not need to induce reprogramming, as permissiveness for transformation may be a by-product of the pluripotency with which the cell is endowed. Tissues in young individuals have many more stem and progenitor cells than adult and ageing tissues. It is therefore likely that at least part of the explanation as to why certain tumour types arise almost exclusively in children is the presence of more abundant populations of stem and progenitor cells that are susceptible to the oncogenic properties of the underlying genetic events.

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We have shown that mesenchymal stem cells (MSCs), which are a heterogeneous population of stromal cells that can differentiate into a variety of lineages, are the most likely cells of origin of several childhood bone and soft tissue malignancies, known as sarcomas. However, MSCs are also present in adult tissues, suggesting that there may be MSC subpopulations in children that become depleted in adults and that are particularly permissive for the expression and function of aberrant oncogenic fusion proteins. One of our quests is to characterize these subpopulations and determine their biological properties, which should lead to an understanding as to why they are uniquely permissive for oncogenic events to which other MSCs are resistant.

Although genetic mutations have long been thought to play the key role in the pathogenesis of cancer, it is becoming increasingly clear that in many malignancies, posttranslational and epigenetic modifications may play an equally important role, sometimes even dominating that of the genetic mutations. “Epigenetics” was originally coined to describe heritable changes in a cellular phenotype that were not due to alterations in DNA sequence. It is most commonly used to describe chromatin-based

events that regulate DNA-templated processes such as gene expression. Chemical modifications, such as methylation, of DNA within gene promoters and of histones (acetylation and methylation), which are proteins intimately associated with DNA provide mechanisms that control gene expression. Histone modifications determine whether DNA segments assume a compact or relaxed structure. Activating histone marks are associated with relaxed DNA that is accessible to transcription factors, allowing expression of genes within the corresponding DNA segments. Repressive histone marks induce DNA compaction rendering it inaccessible to the transcriptional machinery and resulting in the silencing of genes located in the corresponding DNA segments. As the genes in question may control cell division, growth and survival, regulation of their expression by epigenetic modifications may be a key determinant of cancer development, behaviour and response to treatment.

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Epigenetic changes are thought to play a central role in cancer heterogeneity. Understanding paediatric cancer heterogeneity is one of our key goals, as it is heterogeneity of tumour cell properties that constitutes the most formidable challenge toward developing effective therapies. Several mechanisms underlie cancer heterogeneity, including clonal evolution, where different cancer cell clones arise as a result of the accumulation of novel mutations and epigenetic changes. Some of the mutations and epigenetic modifications confer growth advantage whereas others may inhibit growth. The emergence of different clones shapes the identity of the tumour, which is determined by the clones with the greatest survival and proliferative advantage. These clones also display different behaviour in response to therapy and some of them may be responsible for resistance to cytotoxic drugs. However, the relative genetic quiescence of paediatric cancers suggests that clonal evolution through accumulation of genetic mutations may not be the primary determinant of heterogeneity. Another mechanism of heterogeneity is the establishment of a cellular hierarchy, which mimics, albeit in aberrant fashion, hierarchies that are required for normal tissue development. At the apex of the tumour cell hierarchy are poorly differentiated cells that display pluripotency and give rise to heterogeneous progeny, most of which is not tumorigenic. These cells have the ability to self renew and to initiate tumour growth and are commonly referred to as cancer stem cells (CSCs). Their plasticity and tumour initiating properties are largely determined by epigenetic changes and CSCs most likely underlie tumour heterogeneity in paediatric sarcomas. We have shown that Ewing sarcoma behaves according to the CSC model and have identified and characterized the corresponding CSCs. We are now addressing tumour cell heterogeneity in a series of other solid paediatric tumours. These cells constitute the driving force of the tumours and are thought to hold the chief responsibility for relapse. They usually divide slowly and may evade conventional cytotoxic drugs, which typically eliminate rapidly dividing cells. Clearly, these are among the key cells that need to be targeted therapeutically.

Increasing evidence suggests that epigenetic changes play a critical role in the development of paediatric cancers. The fusion proteins associated with several solid paediatric malignancies that arise as a result of unique reciprocal chromosomal translocations, not only provide a diagnostic signature for these particular types of cancer, but

also and more importantly, behave as aberrant transcription factors or transcriptional regulators responsible for their pathogenesis. They alter the gene expression repertoire of the cells, augmenting the expression of genes that promote cell survival and growth and silencing those that induce differentiation and quiescence. To execute their functions, these aberrant transcription factors may form complexes with chromatin-modifying enzymes and instruct them to reconfigure DNA structure, opening domains that in normal cells remain compact and therefore inaccessible to transcription, while condensing domains that are open in normal differentiated cells. Chromatin modification may thus contribute to critical changes in the gene expression profile of the cells, rendering them more susceptible to uncontrolled division, inhibiting their differentiation and maintaining them in an undifferentiated, pluripotent, state, which resembles that of stem cells. Aberrant transcription factor-driven chromatin modification may therefore recapitulate a developmental state and establish a cellular hierarchy within the tumour with CSCs at its apex. Elucidating the biological properties of CSC, including the mechanisms that underlie their plasticity will be key toward developing therapeutic strategies to target tumour heterogeneity in general and paediatric cancer heterogeneity in particular. We are adopting n approach that consists of single cell analysis at the mRNA levels in diverse paediatric cancers to determine exactly what subpopulations compose any given malignancy, identify CSCs or their equivalents that drive and maintain tumour growth and determine uncover their vulnerabilities that can then be targeted by appropriately designed therapeutic strategies.

An underexplored area of sarcoma biology is the interaction of sarcoma cells with the normal host tissue microenvironment or stroma. In adult epithelial malignancies, or carcinomas, this is an intensely investigated field, which has shown that the stromal, “wound healing” response to cancer growth conditions both the local growth, helping sustain cancer cell survival, as well as dissemination. In sarcomas, the nature and effects of the interactions with the host tissue stroma are largely unknown. Given that sarcomas are, in a sense, a malignant stroma, the obvious question is to what extent the normal stroma contributes to sarcoma growth, invasion and dissemination. This is an area of investigation in our lab, focusing on the role of normal MSCs and other stromal cells including activated fibroblasts as well as hematopoietic cells in promoting sarcoma progression. Histological analysis shows that, similar to carcinomas, paediatric sarcomas display variable degrees of infiltration by a variety of leukocyte subtypes. However the mutual influence between sarcoma cells and the infiltrating leukocytes remains obscure and requires elucidation.

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Sarcomas typically display high metastatic proclivity, which renders their prognosis particularly poor, as metastases are for the most part unresponsive to conventional chemotherapy, even if the primary tumour displays sensitivity. Most of our understanding of the multistep process, which constitutes metastasis, comes from studies on carcinomas. Interestingly, carcinomas mimic a sarcoma phenotype in order to metastasize. Most disseminating carcinoma cells undergo epithelial-to-mesenchymal transition (EMT), a reversible process by which they transiently adopt a variable mesenchymal phenotype. EMT appears to be critical for carcinoma cell motility, as epithelial cells are typically non-motile, invasion, and possibly other steps leading up to secondary colony formation. Once they have reached their final destination, disseminated

carcinoma cells revert to their epithelial phenotype in order to grow and form metastatic tumours. Being mesenchymal cells, sarcoma cells are naturally motile and probably possess all of the properties necessary for dissemination. They therefore do not need to undergo any particular phenotypic changes and provide ideal cells to study metastasis and determine how the metastatic lesions may differ from the primary tumours. One of our objectives is to study patient-derived metastatic paediatric cancer lesions, which when unique, can be surgically removed. Key questions to address are the intrinsic properties of metastatic cells, particularly epigenetic modifications that provide them with the ability to disseminate and form secondary colonies; and the effect that disseminated cancer cell growth may have on it host tissue, which may differ from that exerted by the primary tumour on its microenvironment. It is possible, and in fact likely, that metastatic tumour resistance to therapy is due to a combination of tumour cell-intrinsic properties and elements within the host tissue response.

Optimal investigation of any human disease requires the appropriate research staff, which should invariably include, researchers, physicians and physician-scientists. We are therefore establishing a paediatric cancer-training program to which we recruit MD-PhD candidates and provide them with in-depth research training prior to their clinical residency. The MD-PhD candidates are part of a highly competitive MD-PhD program, which selects the most motivated students who have a genuine desire and drive to become physician scientists. The students engage in a 4-year research program at the end of which they obtain their MD-PhD. They are then hired in the paediatric residency program, which provides them with 20% protected time for research during their clinical training. They can thus continue to participate in lab meetings and to be involved in the follow-up of their research projects. They can even conduct specific, appropriately designed experiments that take their time constraints into account. At the end of their clinical training in paediatric oncology, they can select their career path: 80% research and 20% clinical activity; or 80% clinical activity and 20% research. The training of MD-PhDs will provide a unique staff in paediatric oncology composed primarily of physician scientists who bring essential questions from the clinic into the laboratory and conversely, bring into the clinic an analytical approach provided by a scientific mind. The end result should be a natural fusion between clinical and experimental medicine that will provide a powerful foundation for translational paediatric oncology at its best.

Our quest is to understand the mechanisms that underlie paediatric cancer development and to identify those that may be targeted from a therapeutic standpoint. A strong, although not unique, focus is on paediatric sarcomas whose pathogenesis is driven by unique fusion proteins described above. Having identified MSCs, which give rise to fatty tissue, bone, cartilage and other connective tissues, as the most likely cells of origin of several sarcomas, we will determine what makes these cells permissive for transformation by paediatric sarcoma fusion proteins. We have begun to understand how the fusion proteins associated with defined paediatric sarcomas transform these particular cells and to unravel the mechanisms whereby they drive tumour development and progression. We will identify key cellular regulators of the fusion genes whose presence may be crucial to fusion protein expression and function and whose targeting may provide additional therapeutic options. In essence, we have built a multipronged and multidisciplinary approach, based on a team composed of paediatric oncologists, basic researchers, MD-PhDs, chemists and bioinformaticians to address the key issues regarding paediatric sarcoma development, maintenance and progression with the goal of opening new therapeutic avenues and making strides to fulfilling the notion that no child should die of cancer.

CHILDHOOD CANCER TRENDS: HOW TO INTERPRET NEW FINDINGS

Dr Eva Steliarova, Scientist at IARC's Section of Cancer Surveillance explains how research results can be used to reduce the burden of cancer in children…

IARC's international study on the incidence of cancer in childhood found a global increase of 13% in childhood cancer between 2001-2010 compared to the 1980s. The percentage increase compares the incidence rates of 124 per million for the earlier period and 141 per million for the more recent one. With the exception of sub-Saharan Africa, the increase affected all the world regions we studied, and ranged between 3% (Central America and Caribbean) and 30% (Southeast Asia). What could this increase be attributed to?

The challenge of data collection

Although the 2 studies used comparable methods and provided the best estimates of incidence for their respective periods, the covered populations differed. Data were not available for some areas included in the earlier study, while new areas were included in the more recent statistics.

It was important to ensure that data collected in different countries are comparable, so that we can draw conclusions from the observed differences in incidence rates. The established data flow system, motivated staff in cancer registries around the world, a set of international standards and extensive communication between the contributors were required for conducting this study. Our main aim was to define the level of cancer occurrence in the young populations, the most important determinant for cancer control planning and for further research into the causes of cancer in this young population. The produced information does not suffice, however, to explain the causes of the observed patterns. Further focused investigations are required in this direction.

The role of improved diagnosis

Future detailed examination of the incidence trends for specific cancer types could indicate the role of improved diagnosis. For example, a rise in the CNS tumours could be attributed to the implementation of magnetic resonance imaging (MRI) in the 1980s in high-resource countries, and their gradual uptake in less privileged areas. The lack of CNS tumours as well as sophisticated imaging technology on the African continent supports indirectly the role of diagnosis in the temporal trends. Over the time, diagnoses are increasingly based on molecular and genetic analyses, new entities are being recognised and changes in tumours classification proposed. More tumours may be counted as malignant. The novel imaging techniques may detect tumours earlier in life, which would contribute to higher rates within the childhood age-range. On the other hand, the increase in incidence rates was seen in the areas with advanced, as well as with developing diagnostic facilities, which may suggest that the improved diagnosis does not explain the observed increase entirely.

Improved awareness and referral system

More cancers may be detected also because of improved awareness among primary healthcare providers and more frequent referral of suspected cancers for correct diagnosis and treatment. The role of professional associations such as the International Society of Paediatric Oncology (SIOP), as well as charitable actions of patients' families may drive a better or faster access to diagnostic facilities.

Registration of diagnosed cases might have improved with the accumulation of local expertise and maturation of international cooperation. A legal requirement to register (childhood) cancer cases, instituted in numerous registration areas during the 3 decades would definitely enhance the registration completeness.

A role of exposure to risk factors?

The data assembled in our study cannot confirm or refute an increased exposure to various risk factors of childhood cancer identified in other studies. The early life onset and the association of some childhood cancers with a number of inherited syndromes may explain some 5% of cancers in children. Multiple external risk factors have also been examined. While ionising radiation from atomic bombs, industrial accidents or medical interventions may cause leukaemia, thyroid and possibly other childhood cancers, the levels and opportunities of these exposures are relatively limited, as is the proportion of cases due to radiation. Some wide-spread viruses (Epstein-Barr or Human immunodeficiency virus, HIV) together with other co-factors may also lead to cancer development, such as Burkitt lymphoma or Kaposi sarcoma, both highly prevalent in sub-Saharan Africa. Environmental pollution, exposure to pesticides or other carcinogens and some dietary constituents of children or their parents were also associated with childhood cancer in some studies, but not in others. The barriers to a better knowledge of the causes lie in the low frequency of cancer in children, difficulty of accurate exposure assessment, as well as in isolation of potential risk factors from the multitude of simultaneous exposures.

Geographical and ethnic differences in childhood cancer occurrence

Our large international study characterises the geographical and ethnic differences in childhood cancer occurrence and suggests possible associations worthy of further study. One example may be the high relative incidence of childhood leukaemia in South-East Asia, the area which is also known for the wide-spread use of pesticides in agriculture. Another illustration is the drop in the incidence rates in sub-Saharan Africa, at least in part attributed to a reduction of HIV infection load through antiretroviral therapy in the exposed childhood population. This observation implies that external factors may be involved in childhood cancer development and also that preventive measures may result in a reduction of incidence.

Cancer prevention and control is a recognised priority by the WHO[1] and integrates continued surveillance to help with planning childhood cancer care. Our study also serves as a springboard for detection of associations which, if confirmed, may lead to taking preventive actions and possibly reducing childhood cancer incidence in the future.

The International Agency for Research on Cancer (IARC) the specialised agency of the WHO, is coordinating the International Incidence of Childhood Cancer study in collaboration with International Association of Cancer Registries (IACR) and with a financial support of The Union for International Cancer Control (UICC). The first bulk of results of the study were released online on the occasion of the International Childhood Cancer Day on 15 February 2017 and at http://iicc.iarc.fr/ and they will be followed by a printed publication later in 2017 with a complete background on data sources and methods. An overview paper summarising and interpreting the main findings by world regions for period 2001-2010 was published in June by The Lancet Oncology (http://www.sciencedirect.com/science/article/pii/S1470204517301869).

About the author

Dr Eva Steliarova is a senior scientist, at IARC's Section of Cancer Surveillance. She coordinates the work on the third volume of the International Incidence of Childhood Cancer (IICC-3), presented at http://iicc.iarc.fr/ and other international studies of cancer in children. She relies on active collaboration of hundreds of data contributors, the international boards of advisors and editors, as well as the support by her IARC colleagues.

1 http://apps.who.int/gb/ebwha/pdf_files/WHA70/A70_R12-en.pdf

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