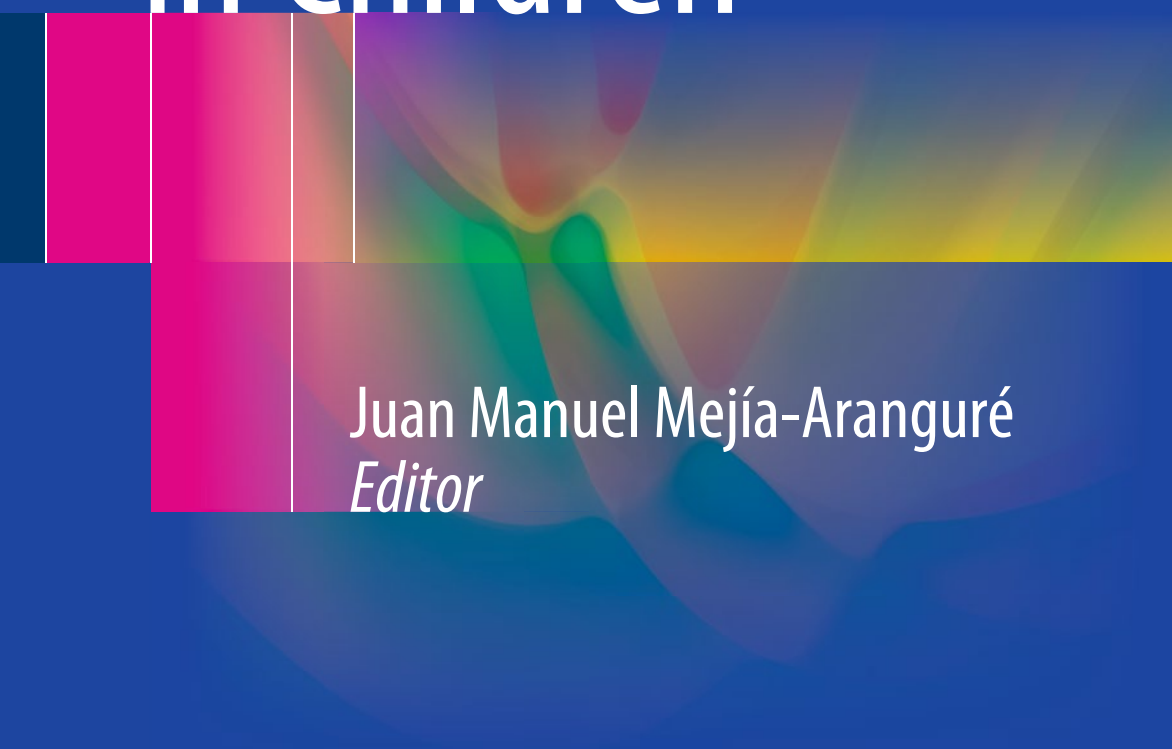
Etiology of Acute Leukemias in Children



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Preface

Leukemias cause the greatest number of deaths of children in the developed world and some developing countries. Advances in the treatment of this disease laid the foundation for much of what is known today about the treatment of cancer. In some places in the world, survival rates of nearly 90 % have been attained for children with leukemia. Despite these enormous achievements, not all countries have been able to reach such survival rates for children with this disease. Even in countries where cure of this illness is most probable, the anxiety that accompanies a diagnosis of leukemia in the family and the suffering provoked by this disease underscore the great necessity of seeking measures to prevent this disease. However, it is difficult to prevent something when little is known about how and why it occurs. Nevertheless, in the history of medicine there have been examples showing that, despite not knowing with precision the cause of a disease, the fact of having a "theory" or a theoretical model that supposes the causes leading to the occurrence of that disease can lead to prevention of the illness. Such was the case of cholera in London in the time of John Snow (1813–1858). In the mid-nineteenth century the cause of cholera was not known, but Snow's theoretical model of the pattern of outbreak of the disease permitted him to postulate measures to end the outbreak.

The objective of this book is to expand a little more on what is known about the origin of childhood leukemias. Although this volume contains some theoretical aspects concerning the origin of childhood leukemia, the major portion of the content focuses on the different aspects that permit us to understand how leukemia originates in children. First, a series of definitions is presented, followed by a discussion of the different environmental considerations that have been proposed and studied as contributing factors in the development of leukemia. In addition to the factors associated with the development of leukemia, another aspect, time, is considered as a variable—a window of vulnerability in a child's life when environmental factors may more readily affect the child, thereby enabling the development of the disease. Thereafter, an analysis of the possible role of some viruses in the development of leukemia, most notably lymphocytic T-cell leukemia, is presented.

A later chapter describes one of the most interesting models aimed at understanding childhood leukemia: the origin of leukemia in children with Down

syndrome. Myeloid and lymphoid leukemias in the child with Down syndrome have presented the medical and research community with a great opportunity for understanding how this disease develops. Further chapters deal with other interesting topics, such as the molecular origin of lymphoid leukemias in children, and how a niche in the bone marrow can contribute to the development of leukemia. Lastly a theoretical model is presented, which attempts to integrate all the aspects described in the preceding chapters, thereby allowing researchers to understand more about how acute leukemia begins in children and, as a result, to begin to visualize possible strategies for the prevention of this type of cancer.

This book is the commencement of a vast project—trying to understand the etiology of acute leukemias in children. The authors do not pretend that the ideas contained this book describe the manner in which leukemia originates; rather, the authors present data, ideas, and theories that may serve as starting points from which investigators will be able to further the understanding of how the factors involved in this disease interact.

In the continuing study of the causes and origin of leukemias in children, I think it imperative that the research be conducted in a more integrated manner, such that advances made in the various disciplines are woven together to achieve a better understanding of, and possibly the development of an integrated theory for, leukemias in children. For the epidemiologist, molecular biologist, cellular biologist, and those pediatricians and hematologists interested in the origins of childhood leukemia, this book will provide a general vision of this disease from the point of view of researchers who have been working in this field. The readers will be the best critics and a major stimulus for a reinvigoration of these research efforts, such that more theories on leukemias in children may be combined, thus producing a broader panorama of the origin of this illness in children.

México, D.F., Mexico Juan Manuel Mejía-Aranguré

Acknowledgments

To each one of my collaborators who gave of their time and expertise, I extend my sincere and profound thanks and gratefully acknowledge the enormous effort that each one, despite facing full agendas, expended on this book. This volume is the result of the commitment of researchers who realize that their principal function is to divulge new findings, instead of solely concentrating on their findings, or meditating on the current state of research and where it is heading. I feel very fortunate to be the editor of a book that has such distinguished researchers as contributors. Some of these collaborators are close friends. For others, I have had the opportunity to play a role in their formation as researchers, and it is an honor to see them so involved in a theme that, for many, has represented a life's work. Likewise, I thank Veronica Yakoleff who plays an important role in my work, in that she not only translates the original Spanish text into English, but also provides counsel and suggestions that are vital to each manuscript. I also thank the effort of Gabriel Pires for his continual reminders for the completion of this work. Twenty-six years ago, I began my career of carrying out research on leukemias in children, when my great friend and mentor Arturo Fajardo presented me with the opportunity of working side by side with him in a study of leukemias. His passion for this field of research inspired me; his formation as a pediatrician helped me to feel for the children who were ill and to understand the pain they suffered. I thank him profoundly for all his support throughout the 26 years we have worked together.

I dedicate this book to my friends and colleagues at the Hospital de Pediatría del Centro Médico Nacional Siglo XXI del Instituto Mexicano del Seguro Social in Mexico City and, most especially, to my close friend Roberto Bernáldez. Roberto and I had planned to be coeditors for this book; unfortunately, an illness impeded his ability to collaborate with me, and, regrettably, he died last year. Roberto taught me how to involve myself in the clinics; he always made me feel a part of his group of hematologists in my beloved hospital. For that I will be forever thankful.

Space does not permit a complete list of all individuals whose collaboration helped to produce this book. Ours is an ongoing collaboration, as this is not a completed work. I offer my sincere thanks to each of my colleagues and to each of the hematologists and oncologists that participated in the Mexican Inter-institutional

Group for Identifying the Causes in Children with Leukemia. Over the years, several sponsors have supported us to develop these studies on leukemias. Special thanks are due to each of these sponsors, most especially the Instituto Mexicano del Seguro Social (IMSS) and the Consejo Nacional de Ciencia y Technología of Mexico (CONACYT), to whom we promised that this book would be completed; to the Agrupación Mexicana para el Estudio de la Hematología, the agency that provided me with the opportunity to travel to gather information to complete some aspects of this work; and to the Coordinación de Investigación en Salud del IMSS, which has always supported me in developing my administrative functions, but, more importantly, has always given me the opportunity to continue advancing my activities as a researcher. I am enormously grateful to all these individuals and agencies; without their support, this work would not have been possible.

Undoubtedly, every success brings with it someone who must make sacrifices. In this case, I thank my wife Norma and my son Yurián, as they are the loved ones who pay for each achievement I attain through afternoons being occupied and weekends spent reviewing material. However, when I tell them what we have achieved, I see that they share the pleasure with me; they, too, feel proud of each goal, of each achievement. I am happy that they are proud of me: at least in my little world, I am a hero.

As I have said, this book is only a beginning, the first in a series concerning all aspects of childhood leukemia. I hope that in the future, more researchers will be able to join in a common effort to integrate what is known to date about, or what are thought to be, the causes of leukemia in children. Perhaps none of us will be able to demonstrate or discover all that we researchers would like to know about leukemia in children, but the sharing and discussion of our "theories" may serve as a springboard to both present and future investigators, thus leading to a better understanding of the etiology of childhood leukemia. I am sure many researchers share the hope that our legacy to others will be that, by passing on our knowledge and discoveries to the next generation of researchers, the day when childhood leukemia can be prevented will no longer be a dream.

I continue to pray to God that He guide us in this career path to prevent leukemia in children. Although today we understand much concerning leukemias and our findings are published in the best peer-reviewed journals in the world, I caution researchers that, while even one child still dies from this disease, we should consider our efforts incomplete. Only God can give us the insight, the wisdom, and the understanding. Only He has the keys to life and death and, if He wishes, He will give us the keys to open the path to greater understanding of the origin of leukemias and the key that will close this chapter on death by childhood leukemia. With God's help, we can hope for a world without this disease.

Contents

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Chapter 1
Introduction: Childhood Leukemia

Aurora Medina-Sanson

Abstract Childhood leukemia is universal, with the same molecular mechanisms at play in children of different genetic and environmental backgrounds. Leukemia is regional as well, and the factors that influence its occurrence and outcome can be affected by ethnic, environmental, geographic, and social circumstances. Childhood leukemia is also unique for every person, such that two individuals with apparently the same disease can respond in a different way to the same treatment and exhibit a different toxicity pattern.

As an introduction to this book, this chapter contains an overview of childhood acute leukemias, covering the relevant aspects of the two main subtypes that occur in pediatric patients, with the aim of providing a basis for the understanding of this heterogeneous group of malignancies.

Keywords Childhood leukemia • Overview

General Issues

Leukemias comprise a heterogeneous group of neoplastic disorders resulting from a multistep process through the interaction of several acquired genetic alterations in a specific stem/progenitor hematopoietic cell population. The progeny of the transformed cell form a clone of leukemic cells that is capable of indefinite self-renewal.

The cells of the leukemic clone proliferate without maturing to end cells and dying; uncontrolled expansion of these malignant hematopoietic cells interferes with normal hematopoiesis, and leukemic cells eventually spread through the circulation and invade tissues and organs outside the bone morrow. The cell in

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which the leukemic transformation occurs may be a lymphoid precursor, a myeloid precursor, or a pluripotent hematopoietic stem cell, giving rise to a number of leukemia subtypes.

The causes of childhood leukemia are not well understood, and the pursuit of causative factors has spanned more than half a century, focusing on infectious, genetic, physical, and chemical theories. Although several epidemiological risk factors have been identified, most of them are unproven or controversial and only a few have so far been conclusive; these include some inherited syndromes, inherited immune conditions, a brother or sister with leukemia, immune system suppression, history of exposure to high levels of radiation, or exposure to antineoplastic agents and other chemicals such as benzene (Buffler et al. 2005).

In virtually all countries, leukemia is the most commonly diagnosed form of cancer in childhood, accounting for about 25–35 % of all cancers occurring before the age of 15 years.

Classification of Leukemia

Leukemias are broadly classified into acute and chronic and further subdivided into lymphoid and myeloid according to their cellular origin. In children, approximately 80 % are acute lymphoblastic (also called lymphocytic or lymphoid) leukemia (ALL), around 17 % are acute myeloid (also termed myelocytic, myelogenous, or non-lymphoblastic) leukemia (AML), and the remaining 2–3 % are essentially Philadelphia chromosome-positive chronic myelogenous leukemia and juvenile myelomonocytic leukemia (Gloeckler et al. 1999). Each leukemia subtype represents a heterogeneous group of disorders that exhibit differences in pathophysiology, morphology, immunophenotype, cytogenetic/molecular characteristics, clinical behavior, response to treatment, and prognosis.

The study of several detectable features in leukemic cells at diagnosis has enabled the classification of childhood leukemias from different perspectives: morphological, immunobiological, and cytogenetic. However, at present the classification of acute leukemia is evolving into increasingly complex entities, since important biological differences are becoming recognized. Therefore, the new classification systems integrate the key morphological features of leukemia cell types, immunophenotype, and cytogenetic/molecular characteristics.

The first internationally accepted system was a morphological classification proposed by the French-American-British (FAB) Cooperative Group in 1976 (Bennett et al. 1976) and reviewed in 1985 (Bennet et al. 1985). This system requires examination of peripheral blood and bone marrow smears and performance of differential counts. For the diagnosis of acute leukemia, the FAB scheme arbitrarily set the percentage of bone marrow blast cells at 30 % or more.

This classification included three subtypes of ALL (L1, L2, and L3) and eight subtypes of AML (M0 to M7), which were differentiated based on morphological characteristics and immunophenotypic profile or electron microscopic characteristics for those cases that could not be accurately identified by morphology.

For AML diagnosis, the FAB classification established that at least 30 % of non-erythroid cells must be blast cells, with lymphocytes, plasma cells, and macrophages also being excluded from the differential count of non-erythroid cells.

According to the 1982 reviewed-FAB system, M0 designates AML with minimal morphological or cytochemical differentiation. M1 and M2 AMLs have minimal or moderate granulocytic differentiation, and the myeloperoxidase (MPO) or Sudan black B (SBB) stains are positive in more than 3 % of the blasts; M2, unlike M1, exhibits maturation at or beyond the promyelocyte stage. M3 is the acute promyelocytic leukemia (APL), which has a variant form (M3v), MPO and SBB reactions are strongly positive, and the presence of the characteristic morphological features of APL is diagnostic despite blast cell percentage. M4 refers to AML with mixed myelomonocytic differentiation; it has positivity for SBB or MPO and both specific and nonspecific esterase. M5 is the monoblastic leukemia, distinguished from the others because 80 % or more of all non-erythroid cells in the bone marrow are monocytic cells; M5a has a maturation index <4 % and M5b >4 %; the monoblasts and promonocytes in acute myelomonocytic (M4) and monoblastic/monocytic (M5) leukemia are considered as "blast equivalents" when the percentage of blasts is calculated; α-naphthyl butyrate esterase is specific and exhibits a strong reaction, whereas MPO and SBB show weak diffuse reactivity. In case of erythroid predominance, a diagnosis of AML M6 can be made when ≥50 % erythroblasts of total nucleated cells and at least 30 % of non-erythroid cells are blast cells; M6a is a myeloid leukemia with dysplastic background erythropoiesis and M6b, acute erythroblastic leukemia; unlike normal erythroblasts, M6 AML erythroblasts, especially pronormoblasts, present coarse positivity of periodic acid-Schiff (PAS). M7 designates acute megakaryoblastic leukemia, whose diagnosis is usually made by immunophenotyping using platelet antigens such as CD41, CD42, and CD61 or ultrastructural examination; megakaryoblasts exhibit positivity for acid phosphatase and α-naphthyl acetate esterase reaction and a negative reaction with α-naphthyl butyrate esterase; they are negative for MPO, SBB, and chloroacetyl esterase, whereas PAS is only positive in the more mature cells.

For ALL, the distinction between L1 and L2 is no longer relevant because morphology does not predict immunophenotype, genetic abnormalities, or clinical behavior. The L3 subtype of ALL represents the leukemic phase of high-grade Burkitt, non-Hodgkin lymphoma with a mature B-cell immunophenotype, and its identification has therapeutic implications. ALL is currently characterized only according to immunophenotype and cytogenetic/molecular features.

The FAB classification allowed uniform diagnosis and classification of leukemias over three decades but has been largely abandoned in practice.1994 Saw the

publication of the Revised European-American Lymphoma (REAL) Classification system, which included all lymphoid leukemias and lymphomas recognized until that time. This system classified lymphoid malignancies as B-cell neoplasms, T-cell and putative natural killer cell neoplasms, and Hodgkin's disease. In its approach to leukemias, the REAL system used the FAB group model but also considered clinical features and genetic characteristics. The REAL Classification is still used by some pathologists and clinicians (Harris et al. 1994).

Leukemia has also been extensively classified from an immunological point of view. According to the primary lineage, acute leukemias can be either myeloid or lymphoid precursor neoplasms, which are subdivided into B-cell precursor (BCP) or T lineage. Uncommon cases that cannot be assigned to one lineage are diagnosed as having ambiguous lineage leukemia, including both acute undifferentiated leukemia and mixed phenotype acute leukemia (also known as acute biphenotypic or hybrid leukemia).

The EGIL group (European Group for the Immunological Characterization of Leukemias) proposed in 1995 a system to establish a guideline for the characterization of acute leukemias based on the expression of individual cluster of differentiation (CD) markers to provide a uniform basis for the diagnosis of the various development subgroups. It classified acute leukemia as B- or T-lineage ALL, AML, or biphenotypic. The consensus established a 20 % minimum threshold to define a positive reaction of blast cells to a given monoclonal antibody (Bene et al. 1995). However, the EGIL system has several limitations and has not been widely used for childhood leukemia.

The World Health Organization classification for hematopoietic and lymphoid neoplasms, developed in 2001 and revised in 2008, was superior to the previously proposed schemes and is currently the most widely used classification system for acute leukemias (Swerdlow et al. 2008; Vardiman et al. 2009). For both lymphoid and myeloid leukemias, this classification contributed toward improving the entities previously defined. It essentially follows the FAB morphological, cytochemical, and immunophenotypic criteria but requires cytogenetic/molecular analysis of leukemic blasts.

The threshold for the diagnosis of AML was decreased from 30 % to 20 % in bone marrow, and those patients with the recurrent karyotypic abnormalities t(8;21)(q22;q22), inv(16)(p13q22), or t(16;16)(p13;q22), and t(15;17)(q22;q12) are considered to have AML despite blast percentage. With respect to ALL, there is no agreed-upon lower limit for the percentage of blasts needed to definitively diagnose lymphoblastic leukemia, but it is advised that diagnosis be avoided when there are fewer than 20 % blasts.

This group also outlined the criteria for ambiguous lineage, where most cases are classified as mixed phenotype acute leukemia, although acute undifferentiated leukemias and natural killer lymphoblastic leukemias are also included. Table 1.1 describes the World Health Organization (WHO) classification of the lymphoid and myeloid neoplasms and the criteria to define ambiguous lineage leukemia.

Table 1.1 WHO classification of acute leukemias

Subtype	Description
Precursor lymphoid neoplasms	
B lymphoblastic leukemia/lymphoma not otherwise specified (NOS)	B lymphoblasts are almost always positive for the B-cell markers CD19, cytoplasmic CD79a and cytoplasmic CD22, CD10, surface CD22, CD24, PAX5, and TdT, whereas CD20 and CD34 are variable; CD13 and CD33 may be expressed
B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities	Includes
	B lymphoblastic leukemia/lymphoma with t(9; 22)(q34; q11.2); *BCR-ABL1*
	B lymphoblastic leukemia/lymphoma with t(v;11q23); *MLL* rearranged
	B lymphoblastic leukemia/lymphoma with t(12;21)(p13; q22);*TEL-AML1* (*ETV6-RUNX1*)
	B lymphoblastic leukemia/lymphoma with hyperdiploidy
	B lymphoblastic leukemia/lymphoma with hypodiploidy
	B lymphoblastic leukemia/lymphoma with t(5; 14)(q31; q32); *IL3-IGH*
	B lymphoblastic leukemia/lymphoma with t(1; 19)(q23; q13.3); *E2A-PBX1*(*TCF3-PBX1*)
T lymphoblastic leukemia/lymphoma	T lymphoblasts are usually TdT positive and variably express CD1a, CD2, CD3, CD4, CD5, CD7, and CD8; CD10 may be positive
Acute myeloid leukemia	
AML with characteristic genetic abnormalities	Includes
	AML with translocations between chromosomes 8 and 21 [t(8;21)]
	AML with inversions in chromosome 16
	AML with translocations between chromosomes 15 and 17 [t(15;17)]
	In general, these patients have a higher rate of remission and a better prognosis compared with other types of AML
AML with multilineage dysplasia	Includes patients who have had a prior myelodysplastic syndrome (MDS) or myeloproliferative disease that transforms into AML. This occurs most often in elderly patients and often has a worse prognosis
AML and MDS, therapy related	Includes patients who have had prior chemotherapy and/or radiation and subsequently develop AML or MDS. These may have specific chromosomal abnormalities and often carry a worse prognosis
AML NOS	Includes other subtypes of AML that do not fit into the above categories (AML with minimal differentiation, AML without and with maturation, acute myelomonocytic leukemia, acute monoblastic and monocytic leukemia, acute erythroid leukemia, acute megakaryoblastic leukemia, acute basophilic leukemia, and acute panmyelosis with myelofibrosis)

(continued)

Table 1.1 (continued)

Subtype	Description
Acute leukemia of ambiguous lineage	
Acute undifferentiated leukemia (AUL)	Blasts lack T or myeloid lineage-specific markers MPO and cCD3 and do not express B-cell-specific markers such as cCD22, cCD79a, or strong CD19
Mixed phenotype acute leukemia (MPAL) with t(9;22)(q34;q11.2); BCR-ABL1	The great majority of cases have blasts meeting criteria for B and myeloid lineage, though some cases have T and myeloid blasts. Triphenotypic leukemia has also been reported. All cases have either the t(9;22) translocation or the BCR-ABL1 rearrangement
Mixed phenotype acute leukemia with t(v;11q23); MLL rearranged	Presence of lymphoblast population CD19 positive, CD-10 negative, B-precursor (pro B) immunophenotype, frequently positive for CD15; CD22 and CD79a are often weak. Cases also fulfill criteria for myeloid lineage and a separate population of myeloid, usually monoblastic leukemic cells, is commonly found. All cases have *MLL* gene rearrangements
Mixed phenotype acute leukemia, B/myeloid, NOS	Blasts meet criteria for both B-lymphoid and myeloid lineage assignment. Myeloperoxidase (MPO)-positive myeloblasts or monoblasts commonly express myeloid-associated markers including CD13, CD33, or CD117. Expression of more mature B-cell markers, such as CD20, may occur
Mixed phenotype acute leukemia, T/myeloid, NOS	Blasts meet criteria for both T-lymphoid and myeloid lineage assignment. MPO-positive myeloblasts or monoblasts commonly express myeloid-associated markers including CD13, CD33, or CD117. In addition to CD3, the T-cell component commonly expresses other T-cell markers including CD7, CD5, and CD2
Mixed phenotype acute leukemia, NOS-rare types	In some cases, leukemic blasts show clear-cut evidence of both B- and T-lineage commitment. There are also few cases with trilineage assignment
Other ambiguous lineage leukemias	Leukemias express combinations of markers that do not allow classification as either AUL or MPAL. Examples may include cases with T-cell associated, but not T-cell--specific markers such as CD7 and CD5 without cytoplasmic CD3 along with myeloid-associated antigens (CD13 or CD33) without MPO

Acute Lymphoblastic Leukemia

ALL incidence is around 41 cases per 1,000,000 individuals younger than 15 years (Surveillance Epidemiology and End Results (SEER) 1975–2010; Shah and Coleman 2007), but its global incidence has important variations, with the highest rates in Costa Rica and the lowest in Mali and other African countries (Stiller and Parkin 1996). Occurrence rate is higher in Caucasian populations of Europe and North America (Stiller and Parkin 1996). Childhood ALL peaks between 1 and 7 years of age, although age variations have also been noted between individuals of different geographical regions and socioeconomic backgrounds. In developed countries, the age distribution of ALL shows a major peak between 1 and 5 years of age, with a slow decline toward adolescence (Gurney et al. 1995; Mc Nally et al. 2000; Parkin 1988a), whereas in some developing countries, the diagnosis of ALL may be

infrequent below the age of 5 years (Parkin et al. 2003; Williams 1984; Babatunde et al. 2008).

In low-income countries or in poorer communities, a decreased leukemia incidence has been reported internationally (Kroll et al. 2012), and higher socioeconomic status has been associated with a higher risk of childhood ALL, although the evidence for socioeconomic status contribution in ALL incidence is not conclusive (Poole et al. 2006; Raaschou-Nielsen et al. 2004).

Explanations for this progress-related difference in leukemia incidence have focused on the relationship between delayed exposure to infectious agents and leukemogenesis. Development accompanies improvement in hygiene practices and better health access and is expected to lead to a reduction in exposition to infections. In addition, reduction in infant mortality might have also contributed to the higher ALL incidence, since children survive and reach an age when they can develop a malignant neoplasia. Affluence, industrialization, and urbanization, and an increase to some degree in the number of people working in industry, may also be factors in the exposition of children to leukemogens through parental occupation.

Variation in the global incidence of childhood leukemia may also result from the hidden real epidemiology of the developing world. Many countries lack national data registries and data sources, or the quality of the information is often inadequate because of underdiagnosis, registration bias, and rate calculation artifacts. In this context, comparisons between countries and epidemiological conclusions are unreliable.

With respect to ethnicity, rates of ALL are higher in Hispanic children than in any other ethnic/racial groups. Hispanics have 1.3 times of the risk for ALL compared with non-Hispanic white children (Matasar et al. 2006; Perez-Saldivar et al. 2011; Surveillance Epidemiology and End Results (SEER) 1975–2010; Greaves et al. 1993; Linabery and Ross 2008). These differences between ethnic population subgroups could suggest genetic predisposition, higher exposure to leukemogens, or the interaction of both factors.

Genetics and Biology

Clonal chromosome abnormalities can be detected in 70–75 % of ALL children at the time of diagnosis (Mrózek et al. 2009). These genetic changes commonly affect cellular processes that control B- and T-cell differentiation and proliferation. The molecular mechanisms by which various oncogenic proteins exert their function and their respective effects are being extensively investigated.

Numerous genetic and biological features that influence leukemogenesis and leukemia behavior have been identified as a result of the application of standard diagnostic methods such as a conventional karyotype and polymerase chain reaction (PCR) and through intensive research using the new genomic technologies. These technologies include gene expression profiling, transcriptome profiling, whole-genome sequencing studies, genome-wide analyses, genome-wide association

studies (GWAS), mutation analysis, microsatellite analyses, and microarray and non-microarray-based DNA methylation assays, among others.

Genetic alterations range from point mutations to gross gains and losses of chromosomal material, other structural rearrangements, loss of heterozygosity, and uniparental disomy. Epigenetic changes comprise silencing of gene expression via DNA hypermethylation, aberrant methylation of CpG islands, histone modifications, microRNA alterations, and dysregulation of DNA binding proteins. All these changes can influence clinical behavior and drug effects (Paulsson et al. 2010; Mullighan et al. 2007; Burke and Bhatla 2014). In addition to their diagnostic importance, there is a growing body of evidence indicating the relevance of cytogenetic aberrations in the identification of genes that play a central role in leukemogenesis and in the understanding of the processes implicated in biological and clinical behavior of childhood leukemia.

Chromosomal rearrangements (e.g., translocations, deletions, inversions, duplications) are considered as the cytogenetic traits of acute leukemia and result in the production of genetic mutations that play a direct role in the transformation of hematopoietic stem cells. These recurrent chromosomal aberrations can be either structural (balanced or unbalanced) or numerical. The balanced aberrations are primarily reciprocal translocations, with rearrangement but without visible gain or loss of chromosome material.

Translocations are produced by double-strand breaks in different chromosomes or different regions of one chromosome, which are then recombined through non-homologous end-joining mechanisms (Greaves and Wiemels 2003; Pfeiffer et al. 2000). They frequently result in fusion genes coding for chimeric proteins that have a key role in leukemogenesis.

These well-established genetic alterations have been associated with specific biological and clinical subtypes.

Genetic Subgroups

Genetically different ALL subtypes with individual gene expression profiling, biology, and response to therapy include a number of BCP subgroups and T-cell leukemias (Pui and Evans 1998; Yeoh et al. 2002).

The cytogenetic subgroups of precursor BCP-ALL comprise high hyperdiploidy, hypodiploidy, the chromosomal translocations *ETV6-RUNX1*, *BCR-ABL*, and *E2A-PBX1*, and the *MLL* gene rearrangements.

High hyperdiploidy is one of the largest cytogenetic subsets of childhood ALL. Occurring in 25–30 % of the patients with BCP-ALL, it has been associated with young age at diagnosis and low white blood cell (WBC) counts and is uncommon in T-cell ALL (Paulsson and Johanson 2009). High hyperdiploidy is characterized by specific nonrandom gains of extra chromosomes. Karyotypes contain a chromosome number of 51–67 and the DNA index is >1.16. The most common gained chromosomes are +21, +X, +14, +6, +18, +4, +17, and +10, each

of which is gained in more than 50 % of hyperdiploid ALL patients, followed by chromosomes 8, 5, 11, and 12, gains that occur more often in patients with 57 or more chromosomes (Heerema et al. 2007).

Extreme hypodiploidy is defined as fewer than 44 chromosomes or a DNA index below 0.81. It is estimated to occur in around 7 % of childhood ALL cases and has been considered a separate subtype of childhood ALL, because international analyses of large groups of hypodiploid ALL patients have found that those with 43 or fewer chromosomes have a very poor prognosis (Gadner et al. 2006; Heerema et al. 1999; Raimondi et al. 2003).

The cytogenetically cryptic recurrent translocation t(12;21)(p13;q22)/*ETV6-RUNX1*(*TEL-AML1*) represents the most common chromosomal rearrangement in childhood ALL, occurring in approximately 25 % of children. Patients are typically young and have B-cell precursor ALL (Shurtleff et al. 1995). The *ETV6-RUNX1* translocation results in the fusion of the dimerization domain of *ETV6* to almost the entire DNA binding and activating regions of the *RUNX1* gene, generating an aberrant transcription factor (Golub et al. 1995; Zelent et al. 2004; Romana et al. 1995).

It is acquired prenatally during fetal hematopoiesis but requires additional somatic mutations for overt leukemia, and through a slow mutational process *ETV6-RUNX1*-positive lymphoblasts are transformed, targeting the promoters, enhancers, and first exons of genes that normally regulate B-cell differentiation (Papaemmanuil et al. 2014).

The translocation t(9;22)(q34;q11.2)/*BCR-ABL1*, also known as the Philadelphia chromosome, occurs in 1–3 % of children ALL, is almost exclusively correlated to precursor B-cell ALL, and is more frequent in older patients with high WBC counts at diagnosis (Forestier et al. 2000). This translocation leads to the production of a chimeric protein with augmented tyrosine kinase activity that causes activation of numerous cell-signaling pathways, contributing to transformation of hematopoietic cells.

The translocation t(1;19)(q23;p13)/*TCF3-PBX1*(*E2A-PBX1*) is associated with pre-B ALL and results in the fusion of the *TCF3* gene on chromosome locus 19p13 with the *PBX1* gene on chromosome locus 1q23, generating a chimeric transcription factor that contains the N-terminal transactivation domain of *TCF3* fused to the C-terminal DNA binding homeodomain of *PBX1*. The chimeric oncoprotein is generally a transcriptional activator that seems to interfere with key regulatory pathways and functions of leukemia biology, including the WNT and apoptosis/cell cycle control pathways, and thus may comprise an essential component for the propagation and maintenance of the leukemic process (Diakos et al. 2014). African-American children have a higher frequency of pre-B ALL with the t(1;19) translocation (Pui et al. 2003b).

Rearrangements in the mixed-lineage leukemia (*MLL*) gene localized at 11q23 occur in approximately 5 % of childhood ALL patients. The most common of them is the t(4;11)/*ALL1-AF4* translocation, which results in the ALL1-AF4 fusion protein (Raimondi et al. 1989; Harrison et al. 2005). This translocation is mainly correlated with pro-B immunophenotype and is almost exclusive of infants, who

typically present with high WBC counts and have a higher incidence of central nervous system (CNS) leukemia. Translocations lead to a breakage in the *MLL* gene where the 5′ part of this gene is retained on the derivative chromosome 11 and fused with the 3′ part of the partner gene (De Braekeleer et al. 2010). Blasts from infants with *MLL* rearrangements are typically CD10 negative and express high levels of FLT3 (Armstrong et al. 2002).

Approximately one-quarter of B-ALL patients lack the characteristic chromosomal rearrangements (Pui et al. 2004), but recent studies including the new genome technologies have identified other recurring molecular genetic abnormalities with prognostic significance in ALL patients. These include *IKZF1* deletions, *CRLF2* overexpression, and *JAK2* mutations.

Ikaros (IKZF1) was the first identified member of a family of zinc finger transcription factors. The function of IKZF1 during early hematopoiesis is required for differentiation into the three major hematopoietic lineages (Yoshida et al. 2006; Georgopoulos 2009; Georgopoulos et al. 1994; Dumortier et al. 2003, Dijon et al. 2008). Deletions of *IKZF* have been identified in about 30 % of high-risk B-cell precursor (BCP)-ALL and in 83.7 % of *BCR-ABL1* ALL (Sun et al. 1999; Mullighan et al. 2007, 2008).

There is also a subgroup of patients with high-risk *BCR-ABL1*-negative ALL that is characterized by *IKZF1* deletion and a genetic profile similar to that of cases with *BCR-ABL1* fusion; they have been referred to as Philadelphia-like ALL (Den Boer et al. 2009).

Some studies have reported an association of IKZF1 deletions with *CRLF2* (cytokine receptor-like factor 2) overexpression and JAK2 (Janus kinase 2) mutations (Harvey et al. 2010).

The *CRLF2* gene encodes for a type I cytokine receptor that is overexpressed in approximately 15 % of high-risk pediatric B-ALL that lack *MLL*, *TCF3*, *ETV6*, and *BCR/ABL* rearrangements. Its ligand (the thymic stromal lymphopoietin or TSLP) mediates B-cell precursor proliferation and survival (Levin et al. 1999; Yoda et al. 2010). *CRLF2* overexpression occurs at a high incidence in Down syndrome (DS)-ALL patients (Russell et al. 2009). The majority of DS-ALL patients who overexpress *CRLF2* also have *JAK2* mutations, but the association of *CRLF2* deregulation with mutations of the *JAK2* gene has also been found in non-DS patients, suggesting an oncogenic cooperation between these two events in the pathogenesis of BCP-ALL (Russell et al. 2009).

The frequency of activating mutations of the Janus kinases in children with high-risk non-DS ALL has been reported to be about 10 %, and *JAK2* mutations represent 80 % of them (Mullighan et al. 2009b). The JAK2 protein, one of the four members of the JAK family, is a non-receptor tyrosine kinase that mediates signals from a variety of cytokines and growth factors. JAK proteins and their downstream transcription factors, termed STATs (signal transducers and activators of transcription), conform the JAK-STAT signaling pathway. Activating *JAK* mutations and translocations lead to constitutive activation of their tyrosine kinase activity with oncogenic properties. *JAK2* mutations occur in about 20 % of pediatric DS-ALL cases and have been described as a particularly important event in the development of BCP-ALL in these patients (Malinge et al. 2007; Bercovich et al. 2008).

Approximately one-third of hyperdiploid (>50 chromosomes) cases harbors a mutation involving the *FLT3*, *NRAS*, *KRAS*, or *PTPN11* genes. These mutations seem to be mutually exclusive in this group of BCP-ALL patients and are very rare in T-ALL (Paulsson et al. 2008).

Recurrent cytogenetic aberrations can be detected in about half of the pediatric T-ALL patients (Raimondi 1993), and some translocations and molecular markers have been correlated to T-ALL subtypes.

T-ALL chromosomal abnormalities often include reciprocal translocations that disrupt developmentally important transcription factor genes, as a result of rearrangements to loci for the T-cell receptor (*TCR*) genes, most commonly *TCR*a (14q11.2) and *TCR*b (7q35). TCR genes are frequently translocated to basic helix-loop-helix (bHLH) genes (*MYC*, *TAL1*, *TAL2*, *LYL1*, *bHLHB1*), cysteine-rich genes (*LMO1*, *LMO2*), or homeodomain genes (*HOX11*/*TLX1*, *HOX11L2*/ *TLX3*, members of the *HOXA* cluster) (Aifantis et al. 2008). Some of them are not translocation breakpoints but are defined as mutations or overexpression of distinct genes.

TAL1 (T-cell acute lymphocytic leukemia 1; also known as SCL) is the most common bHLH gene, with aberrant expression observed in T-ALL cases. This transcriptional regulator was first identified in T-ALL patients with the t(1;14)(p32;q11) translocation, which is observed in 3 % of cases (Chen et al. 1990; Carroll et al. 1990). Other translocations involving homeodomain genes have also been described. About 20 % of pediatric T-ALL patients have the HOX11L2 (TLX3)-BCL11B fusion (Bernard et al. 2001).

The most commonly mutated genes in T-cell ALL are not cytogenetically detectable and include *NOTCH1*, *FBXW7*, *PTEN*, *CDKN2A*/*B*, *CDKN1B*, *6q15-16.1*, *PHF6*, *WT1*, *LEF1*, *JAK1*, *IL7R*, *FLT3*, *NRAS*, *BCL11B*, and *PTPN2*.

NOTCH1 is one of the most important genes in T-cell leukemogenesis and is one of the most extensively studied; it is involved in the regulation of several cellular processes including differentiation, proliferation, apoptosis, adhesion, and spatial development. NOTCH1 mutations are observed in 34–71 % of T-ALL (Larson Gedman et al. 2009).

Genetic Polymorphisms

The risk of childhood leukemia, as in other complex diseases, is likely to be influenced by independent and interactive effects of genes and environmental exposures. Genetic and biological features can influence the pathogenesis of ALL and the risk of treatment failure. Individual differences in drug responses are an important cause of resistance to treatment and adverse drug reactions.

Recently published GWAS have identified several single-nucleotide polymorphisms associated with an increased risk of ALL development. Most of these polymorphic sites are localized in genes that encode transcription factors taking part in hematopoiesis (Enciso-Mora et al. 2012; Georgopoulos 2009).

Some of these genetic polymorphisms may also contribute to racial disparities in the incidence and treatment outcome of childhood leukemia (Xu et al. 2012; Chen et al. 1997; Pollock et al. 2000; Evans and Relling 2004; Kishi et al. 2007; Pui et al. 1993).

A higher proportion and different distribution of some TPMT variants has been reported in Hispanic patients with ALL (Moreno-Guerrero et al. 2013; Garrido et al. 2013; Taja-Chayeb et al. 2008).

Immunophenotype

Hematopoietic cells express different antigens at various stages of development. These antigens can be identified by monoclonal or polyclonal antibodies tagged with a fluorescent label. Immunophenotype represents the individual expression pattern of nuclear, cytoplasmic, and cell surface antigens.

Immunological markers that identify BCP blasts include HLA-DR, TdT, CD19, cytoplasmic CD22, and CD79a (CD79b is less useful, since it is expressed later in development) and/or CD34. CD10 is expressed in both T and B lineage, but more commonly in B-lineage blast cells. Precursor B-lineage ALL has been subdivided into four groups: *pro-B ALL* (*also called early pre-B*), which expresses HLA-DR, TdT, CD19, and CD10 (with negative cytoplasmic immunoglobulin); *pre-B ALL*, characterized by the expression of cytoplasmic immunoglobulin and CD10; *transitional pre-B ALL*, characterized by surface expression of heavy-chain membrane immunoglobulin; and *mature B-ALL*, in which the blast cells express surface light and heavy-chain membrane immunoglobulin and lack TdT and CD34. However, this subtype distinction of BCP-ALL does not appear to be clinically relevant.

For T-lineage blasts, the most specific antibody is CD3, whereas CD2, CD4, CD5, and CD7 are less specific. Recently a new category of T-cell ALL to distinguish early T-cell precursor from other T-cell blasts was described. This was named early T-cell precursor ALL and comprises up to 15 % of T-ALL (Coustan-Smith et al. 2009). This subtype is characterized by lack of expression of the T-lineage cell surface markers CD1a and CD8, weak or absent expression of CD5, aberrant expression of myeloid and hematopoietic stem cell markers (CD13, CD33, CD34, and CD117), and a gene expression profile similar to that of the murine early T-cell precursor.

In high-income countries, BCP-ALL represents about 85 % of the cases, and 15 % are of T-cell lineage (Greaves et al. 1993); however, some studies have reported a higher frequency of T-ALL subtype in low- and middle-income countries or in economically deprived communities (Bachir et al. 2009; Bhargava et al. 1988; Taskov et al. 1995; Kamel et al. 1990; Rajalakshmy et al. 1997; Kamat et al. 1985).

Clinical Presentation

Acute leukemia patients present with signs and symptoms related to the decreased production of normal marrow elements, with manifestations resulting from direct infiltration of extramedullary sites by leukemic blasts or with signs caused by complications such as cell tumor lysis, leukostasis, or coagulopathy.

Many of these symptoms are nonspecific complaints such as weakness, lethargy, fatigue, fever, and bleeding, which can be misdiagnosed as some common diseases of childhood. Fever with or without evidence of infection is one of the most common symptoms of acute leukemia, particularly ALL.

Signs and Symptoms of Bone Marrow Failure

Proliferation of leukemic cells and the subsequent decreased production of normal blood cells may result in anemia, thrombocytopenia, and neutropenia, which cause most of the clinical manifestations of acute leukemia.

The classical symptoms of anemia include pallor, fatigue, intolerance to physical exercise, palpitations, dyspnea on physical exertion, and dizziness. Thrombocytopenia typically produces cutaneous and mucosal bleeding, with petechiae, purpura, and ecchymosis, predominantly on the lower extremities, and especially when platelet count falls below 20,000/μL. Hemorrhage in other sites is less frequent, but potentially life-threatening hemorrhage may occur, particularly in patients with hyperleukocytosis, and involves the lung, CNS, and gastrointestinal tract. Patients with absolute neutrophil counts of <500 cells/μL have a higher risk of infection.

Signs and Symptoms Related to Organ and Tissue Infiltration

The most common sites of infiltration include the lymph nodes, spleen, and liver. Mediastinal mass is more common in patients with T-cell ALL; these children can present without respiratory symptoms or exhibit signs of tracheobronchial compression such as cough or dyspnea. CNS infiltration can be asymptomatic, but some cases may present vomiting and headaches. As many as 25–30 % of infants with congenital leukemia present single or multiple skin lesions attributable to cutaneous infiltration by leukemic cells (leukemia cutis). Only one-third of childhood leukemia cutis cases are seen in ALL; skin is rarely affected in older children and must be distinguished from other non-leukemic cutaneous lesions. Some patients may experience gingival swelling that may result from infiltration of leukemic cells into the gum tissue or from neutropenia-associated gingivitis. Massive bone marrow infiltration frequently manifests as bone pain, which can be severe and often atypical in distribution.

Signs and Symptoms Caused by Life-Threatening Complications

Most complications represent medical emergencies that demand immediate intervention.

High tumor burden and high rates of leukemia cell proliferation can result in tumor lysis syndrome, which is asymptomatic in most cases, but patients with severe hyperuricemia or hyperphosphatemia can develop renal failure with oliguria or anuria.

Leukostasis is another potentially fatal complication that occurs in cases of hyperleukocytosis (>100,000 cells/μL); the CNS and lungs are the most commonly affected sites. Symptoms of leukostasis include respiratory distress, confusion, dizziness, headache, tinnitus, blurred vision, somnolence, stupor, delirium, and coma, resulting from tissue hypoxia secondary to vascular obstruction by a large number of leukemic blasts in the peripheral circulation. It is less common in patients with ALL than in those with AML.

Signs and symptoms of infection, generally caused by an impaired immunological response, are frequently present at diagnosis. Fever can be the first sign, and respiratory symptoms with or without lung findings of pneumonia are the most common manifestations of a life-threatening infection in ALL patients at diagnosis.

Diagnosis

An accurate diagnosis is critical for appropriate treatment assignment and is crucial in the probability of survival of leukemia patients.

The minimal standards to diagnose acute lymphoblastic leukemia in children include a good morphological evaluation with cytochemical assessment, examination of the cerebrospinal fluid (CSF) by cytospin for the presence of leukemic blasts, and a chest radiograph to detect mediastinal involvement. In addition, immunological features and recurrent genetic abnormalities should be assessed to attain an appropriate stratification and treatment assignment.

Initial Approach

Childhood leukemia diagnosis is based primarily on clinical features. The suspicion of acute leukemia should lead to the performance of a hemogram, with differential leukocyte count.

Up to 50 % of the patients present with leukopenia, and a low neutrophil count is a common finding, regardless of the total leukocyte number; leukocytosis is usually due to the presence of leukemic and other immature cells in the circulation. Anemia might be present in up to 80 % of the cases, which is characteristically normocytic/normochromic. In 75 % of cases, the platelet count is below 100,000/μL.

These findings, along with examination of the peripheral blood smear for blast, lead to the decision to perform a bone marrow aspiration or a marrow biopsy in select cases, which are the key procedures in establishing the definitive diagnosis of acute leukemia.

Once the bone marrow aspiration is performed, diagnosis starts with morphological analysis of Romanowsky, Wright Giemsa or May-Grünwald Giemsa stained bone morrow smears, followed by cytochemical studies including myeloperoxidase MPO, SBB, PAS, and esterase stains.

Morphological diagnosis is based on cell characteristics such as size, presence of nucleoli, nuclear chromatin, nuclear shape, nucleus/cytoplasm ratio, and the amount and appearance of cytoplasm (vacuolation, granules, basophilia). It represents the first approach to leukemia demonstration and lineage definition.

In addition to the blood cell counts, other routine laboratory studies must be carried out to assess pretreatment organ function and to detect a preexisting medical condition, emergencies, or complications such as tumor lysis syndrome, hypercalcemia, and renal failure.

Some specific studies must also be performed to assess extramedullary disease, including examination of the CSF by cytospin for the presence of blast cells and chest radiography to detect mediastinal enlargement.

Mediastinal mass is defined as a mass of greater than one-third the thoracic diameter at the level of the fifth thoracic vertebra.

CNS status includes three categories: CNS-1, no detectable blast cells in a sample of cerebrospinal fluid; CNS-2, <5 WBC/mm^3 with blast cells in a sample with <10 erythrocytes/mm^3; and CNS-3, ≥5 WBC/mm^3 with blast cells in a sample with <10 erythrocytes/mm^3 or traumatic lumbar puncture (≥10 erythrocytes/mm^3) with blast cells or the presence of affected cranial nerves or a cerebral mass detected by cranial computed tomography or magnetic resonance imaging (MRI) (Pui and Howard 2008).

Immunophenotyping

Immunophenotyping can be performed on cell suspensions of peripheral blood or bone marrow cells through measuring the percentage of positivity for specific antigens by flow cytometry or on histological sections using immunohistochemistry, when considered necessary. It enables reliable definition of leukemia lineage, provides relevant information to identify prognostic differences within a subtype, and allows the detection of one or more leukemia-associated immunophenotype that can be used for monitoring minimal residual disease.

Multiple combinations of antibodies have been proposed for diagnostic immunophenotyping, and most consensuses recommend the use of an initial panel of antibodies for lineage orientation and a second panel for the characterization of sublineages (van Dongen et al. 2012; Bene et al. 1995, 2011; Bain et al. 2002; Stewart et al. 1997).

Cytogenetic and Molecular Analysis

Cytogenetic analysis is essential for a more accurate diagnosis and classification; it provides specific tumor markers that are essential in prognostic assessment and as an additional tool for monitoring residual disease.

Successful cytogenetic analysis is more often possible on a bone marrow aspirate than on peripheral blood cells. Routine techniques used to detect leukemia-specific chromosome abnormalities or their molecular equivalents include conventional G-banding cytogenetic analysis, standard and multicolor fluorescence in situ hybridization (FISH), PCR-based assays, and standard sequencing for clinical screening of mutations.

Conventional cytogenetics requires cell culture and quality metaphases for analysis, and this method depends on the availability of analyzable metaphases; therefore, FISH and reverse transcriptase-PCR are necessary as screening tools.

Flow cytometry is used for quantitating the relative DNA content in leukemic cells using a fluorochrome that binds to DNA. The DNA index is the quantity of DNA content in the test cell population in relation to that in the nuclei of normal matched diploid cells (lymphocytes). A DNA index of 1.0 indicates no detectable change in the DNA content on leukemic cells with respect to normal cells and corresponds to diploid cells in the G0/G1 phase. Abnormal changes in the DNA index (aneuploidy) may represent gain or loss of genetic material. A DNA index <1 represents hypodiploidy, whereas >1 indicates hyperdiploidy. A DNA index of 1.16 or higher indicates a modal chromosome number of 54 or more.

Specific markers of each leukemia subtype were explained in detail in a previous section.

Thiopurine Methyltransferase Genotype

Polymorphisms of drug-metabolizing enzymes have been associated with increased toxicity on the one hand and with increased or decreased efficacy of the treatment on the other hand (Kishi et al. 2007). Thiopurine S-methyltransferase (TPMT) is a key enzyme in the inactivation of thiopurine drugs such as 6-mercaptopurine. TPMT genotyping represents one of the best examples of the clinical application of pharmacogenetic studies. Polymorphisms of this enzyme are associated with increased toxicity of 6-mercaptopurine but also with increased efficacy indicated by improved relapse-free survival. In many centers, this analysis is performed routinely as a part of the diagnostic workup to individualize the doses of thiopurine drugs.

Minimal Residual Disease

It is estimated that the total number of leukemic blasts at diagnosis is around 10^{12}. The majority of patients achieve complete remission after induction chemotherapy, however at this time, up to 10^{10} malignant cells still remain in the patient, but their level is beyond the sensitivity level of cytomorphological methods.

The minimal residual disease (MRD) represents the leukemic cells that remain in the body after remission at a level beyond the sensitivity of classical cytomorphological methods, which can be imprecise and insensitive. MRD identifies leukemia-specific features that distinguish leukemia cells from normal hematopoietic precursors and provides specific and sensitive measurements of low levels of leukemic cells.

For clinical purposes, MRD detection methods can only include flow cytometric detection of aberrant leukemia-associated immunophenotypes, PCR amplification of immunoglobulin and TCR genes, or PCR amplification of oncogenic fusion transcripts when present (Bruggemann et al. 2012).

The most feasible and reliable technique to detect MRD is flow cytometry, which has been the most commonly used method in the BFM, St Jude, and Children's Oncology Group (COG) protocols (Chauvenet et al. 2007; Sievers et al. 2003; Coustan-Smith et al. 2003).

Prognostic Factors and Risk Stratification

The recognition of different factors that influence leukemia behavior and response to treatment has allowed stratification of patients into risk groups to help decide whether a child with leukemia should receive a standard or a more intensive treatment; this includes the identification of those patients who would benefit from a stem cell transplant.

Prognostic indicators include variables related to the patient, leukemic cells, or treatment response. However, more intensive therapy has changed their prognostic significance over the years.

Clinical and Laboratory Features

Age at diagnosis is one of the most significant predictors of clinical outcome (Hossain et al. 2014; Webb et al. 2001; Möricke et al. 2005). Children younger than 1 and older than 10 years have a less favorable prognosis than those between 1 and 9 years, and it seems that patients aged between 1 and 4 have an even better outcome than those in the age group 5–9. This may be explained in part by the age-dependent distribution of genetic alterations that strongly influence prognosis, such as *BCR-ABL1* fusion, which is more frequent in older children, or the *MLL* rearrangements characteristic of infants who have a unique biology (Kang et al. 2012).

Most studies have identified that boys have a worse prognosis than girls. This has been partially attributable to the risk of testicular relapse, the higher incidence of T-immunophenotype, unfavorable DNA index, and a higher rate of bone marrow and CNS relapse among boys, but other genetic and endocrine effects may be present as well (Pui et al. 1999).

Some reports describe poorer outcomes in black and Hispanic children when compared with white and Asian/Pacific Islander children (Kadan-Lottick et al. 2003; Macharia 1996; Parkin 1988b; Goggins and Fiona 2012). However, the effect of race on prognosis has been controversial, since these differences might depend on social factors and treatment differences between populations.

The extent of extramedullary disease at diagnosis (hepatosplenomegaly, massive lymphadenopathy, presence of mediastinal mass, or testicular disease) was in the past a useful prognostic indicator. However, with the current therapeutic protocols, these features, including testicular involvement, no longer have prognostic significance (Sirvent et al. 2007).

CNS leukemia has an impact on outcome; CNS3 patients have a higher risk of CNS and bone marrow relapse than children classified as CNS1 or CNS2 (Bürger et al. 2003).

Most studies have found a lower survival in DS patients. A recent COG study reported that DS patients have a lower rate of the favorable cytogenetic lesions and inferior event-free survival (EFS) and overall survival (OS), but when children with *MLL* translocations, *BCR-ABL1*, *ETV6-RUNX1*, and trisomies 4 and 10 were excluded, the EFS and OS were similar for children with and without DS (Maloney et al. 2010).

There is a linear relation between initial WBC count and outcome in children with ALL. The threshold to delimit prognosis has been established as 50,000/μL, and those patients with WBC counts greater than 50,000/μL are accepted as having a poorer prognosis.

In addition to age and gender, some other host characteristics such as nutritional status, low socioeconomic status, and concomitant diseases have been correlated with patient outcome (Mejia-Arangure et al. 1999; Sala et al. 2004; Lobato-Mendizábal et al. 2003; Viana et al. 1994; Smith et al. 2006; Gupta et al. 2014; Kadan-Lottick et al. 2003).

Prognostic Factors Related to Blast Features

Immunophenotype Specific immunophenotypic profiles have been associated with prognosis.

Traditionally, T-cell leukemia has been considered as an adverse feature, particularly when accompanied by a WBC count >50,000/μL. The prognostic significance of high WBC counts at diagnosis in T-cell ALL has also been contradictory, since most patients with T-cell ALL have the same outcome as those with BCP-ALL (Pullen et al. 1999). Patients with the early T-cell leukemia subtype have the highest risk of remission failure or hematological relapse among T-cell ALL patients, with a survival that ranges from 10 to 14 % at 10 years compared with patients with typical T-ALL, whose 10-year survival is between 57 and 72 %. With the exception of the early T-cell leukemia subtype, subdivision into T-cell

developmental subgroups is not predictive for outcome, and immunophenotype is no longer considered an independent prognostic factor (Coustan-Smith et al. 2009; van Grotel et al. 2008a).

Among BCP-ALL cases, CD34 expression has been significantly associated with favorable presenting features and a better outcome with respect to CD34-negative cases. In contrast, CD34 expression in pediatric T-cell ALL has been associated with initial CNS leukemia and adverse prognostic features (Pui et al. 1993) or with poor survival (van Grotel et al. 2008b).

Cytogenetics The underlying genetic lesions in ALL leukemia subtypes affect significantly the response to chemotherapy and prognosis.

High hyperdiploidy is an independent prognostic factor, with complete remission rates approaching 100 % in some studies and 5-year EFS rates between 71 and 83 % (Moorman et al. 2003; Trueworthy et al. 1992).

Patients with the concurrent presence of +4, +10, and +17 have been reported to have especially favorable prognosis (Heerema et al. 2000; Sutcliffe et al. 2005).

The prognosis of children with the t(12;21)/*ETV6L-RUNX1* translocation, often correlated with favorable features, is generally associated with good clinical outcome, and 94 % of these patients experience rapid early responses to therapy (Kanerva et al. 2004; Rubnitz et al. 1997, 2008; Forestier et al. 2008).

Hypodiploid (<44 chromosomes) has a very bad prognosis, and outcome seems to worsen with decreasing chromosome modal number. In the current COG risk stratification system, patients with leukemic cells containing <44 chromosomes or with DNA index <0.81 are considered very high risk (Schultz et al. 2007).

The t(1;19)(q23;p13)(*TCF3-PBX1*) translocation was formerly associated with a less favorable outcome when conventional antimetabolite-based treatment was used, but its adverse effect on survival was annulled by the use of more intensive chemotherapy regimens; the current 5-year EFS rate is >80 % in Western countries, which is similar to that of *ETV6-RUNX1* positive or high-hyperdiploid BCP-ALL (Raimondi et al. 1990; Borowitz et al. 1993).

In the past, the (9;22)(q34;q11.2)/*BCR-ABL1* translocation was associated with a dismal outcome; however, with the addition of imatinib to the existing intensive chemotherapy regimens, it has been possible to increase the 3-year EFS to 80.5 % without stem cell transplantation (Schultz et al. 2009).

Translocations involving the *MLL* gene (11q23) are associated with a worse prognosis in infants under 12 months compared with older children who carry these rearrangements, since infants often exhibit poor early response to prednisone and a high rate of failure when conventional chemotherapy is used (Pui et al. 2003a; Pieters et al. 2007).

IKZF1, *CRLF2*, and *JAK2* gene alterations are independent prognostic factors in patients with pediatric BCP-ALL (Mullighan et al. 2009a, b; Yung et al. 2011; Harvey et al. 2010; Palmi et al. 2012; Ensor et al. 2010; Cario et al. 2010; Den Boer et al. 2009; Kuiper et al. 2010; Chen et al. 2012; Mi et al. 2012).

Furthermore, *IKZF1* deletions of the entire gene or of specific exons have been identified as predictor of outcome in pediatric acute lymphoblastic leukemia,

independently of *BCR-ABL1* status (Dörge et al. 2013). Since *BCR-ABL1* ALL has a poor prognosis, these findings suggest that the mutation of *IKZF1* is a key determinant of the poor outcome of both *BCR-ABL1*-positive and *BCR-ABL1*-negative patients.

In T-cell leukemia, TAL1 or HOX11L2 rearrangements have been associated with good and poor outcomes, respectively. Cases with high versus low TAL1 expression levels demonstrated a trend toward good outcome. Most cases with lower TAL1 levels were HOX11L2- or CALM-AF10-positive. Thus, overexpression of HOX11, which is associated with thymic T-ALL, may confer a favorable prognosis. Other groups observed inferior outcomes in HOX11L2- and SIL-TAL-positive T-ALL. NOTCH1-activating mutations, identified in up to 50 % of T-ALL cases, have unclear prognostic relevance (van Grotel et al. 2008a).

Response to Initial Therapy

The rapidity of response to initial therapy and the level of MRD at the end of induction are associated with long-term outcome.

The prednisone response, initially used by the BFM protocols, has been extensively accepted as an important prognostic factor. It is evaluated on day 8 after 7 days of monotherapy with prednisone by counting the absolute number of blasts in the peripheral blood. A good prednisone response is defined as <1,000 blasts/mm^3, and patients with ≥1,000 blasts/mm^3 are poor responders (Dördelmann et al. 1999).

The response in the bone marrow after 1 or 2 weeks of induction therapy has also been widely used as a prognostic indicator. Rapid early responders (those who exhibit <5 % blasts in a bone marrow at day 7 of induction) have the best EFS. Lack of response in bone marrow on day 14 is an independent predictor of inferior outcome. Children who do not achieve morphological and clinical remission (defined as <5 % blasts in a bone marrow with normal cellularity and the absence of other evidence of leukemia), after the standard 4- to 6-week induction period, have the highest rate of relapse and the poorest survival (Pui et al. 2010; Bhojwani et al. 2009). Overall long-term survival for children who fail induction chemotherapy is only about 32 %, and these patients are considered to be at very high risk.

In recent years, it has been clearly demonstrated that the level of MRD at the end of induction represents one of the most powerful prognostic factors. It allows a more precise definition of remission, responsiveness to therapy, and expected long-term survival; therefore, measurement of MRD has been incorporated in many trials.

The COG identified that measurement of MRD in the peripheral blood at day 8 provides information that helps to detect patients with an exceptionally good outcome who can reach a 97 ± 1 % 5-year EFS. This subset of patients is defined by meeting National Cancer Institute Standard Risk (NCI SR) criteria, namely, absence of CNS3 or testicular disease, having either double trisomies or *TEL-AML1*, and

absence of MRD in both the day-8 peripheral blood and day-29 bone marrow samples, and achieves an excellent outcome with a therapy that does not need the use of anthracyclines or alkylating agents (Borowitz et al. 2008).

Risk-Group Assignment

The original NCI/Rome criteria distinguished two risk groups based exclusively on age and initial WBC: *Standard risk* (about 60 % of the patients with 4-year EFS rate, approximately 80 %) included those cases with age between 1.00 and 9.99 years and initial WBC at diagnosis <50,000/μL (the current 5-year EFS for these patients is higher than 85 %), and *high risk* (about 40 % of the patients with 4-year EFS rate, approximately 65 %) comprised all others (Smith et al. 1996).

Over time many prognostic factors have been identified, but even when some of them have been demonstrated to affect outcome, not all have been used for risk stratification.

Risk stratification criteria and nomenclature have considerable variation among the major pediatric ALL study groups. Most of them use a combination of clinical, laboratory, cytogenetic, and response to therapy features to stratify patients into risk groups.

Since 2000, BFM protocols categorize ALL mainly on the basis of prednisone prophase response and MRD measurements at two time points, end of induction (week 5) and end of consolidation (week 12) (Conter et al. 2010). Three risk groups are included: (1) *standard risk*, patients who are MRD-negative ($<10^{-4}$) at both time points; (2) *intermediate risk*, patients who have positive MRD at week 5 and low MRD ($<10^{-3}$) at week 12; and (3) *high risk*, patients with high MRD ($\geq 10^{-3}$) at week 12. Patients with a poor response to the prednisone prophase are also considered in the high-risk group, regardless of subsequent MRD measurements, and patients with the t(9;22) or the t(4;11) translocations are also classified as high risk, despite early response measures.

The COG end induction risk stratification algorithm for B-precursor ALL considers the NCI/Rome criteria, immunophenotype and cytogenetic/molecular features, as well as early response data, which must be available by day 35 of induction. This risk stratification system includes four categories: (1) *low risk*, NCI/Rome standard risk, with trisomy of chromosomes 4, 10, and 17 ("triple trisomies") or TEL-AML1, which comprises about 40 % of NCI SR with a projected 5-year EFS of at least 85 %; (2) *standard risk*, NCI/Rome standard risk non-TEL, non-triple trisomies, which includes around 60 % of NCI SR with EFS of 80 %; (3) *high risk*, NCI/Rome high risk, with an EFS of 70–75 %; and (4) *very high risk*, Ph+, hypodiploid (fewer than 45 chromosomes), failure to achieve remission at the end of induction therapy, which corresponds to approximately 5 % of all ALL cases, with a projected 5-year EFS of 45 % or below (Schultz et al. 2007). It is noteworthy that in nearly 2,000 children with ALL who entered on the COG classification and treatment study P9900, about half of all events occurred among patients who were MRD negative at the end of induction.

Cytogenetic/molecular features and MRD have become the leading criteria to stratify ALL patients in most industrialized countries; however, expensive laboratory techniques are not affordable in many low- and middle-income countries where these stratification systems are not a reality.

For more than 20 years, NCI/Rome risk criteria in combination with cytomorphological response were used for risk definition, and they continue to be the basis to stratify ALL patients in many countries of the developing world. NCI criteria together with prednisone and early marrow response (days 7 and 14), in addition to immunophenotype and cytogenetics when available, allow stratification into two or three risk groups (Fronkova et al. 2008).

Treatment and Outcome

Between the late 1960s and the present, the outcome of pediatric ALL has evolved from an overall survival of less than 10 % to approximately 75–80 % (Pui 2006), partly because of the current risk-oriented treatment strategies (Pui et al. 2009; Salzer et al. 2010; Mitchell et al. 2010; Schmiegelow et al. 2010).

At diagnosis, the prompt recognition and treatment of life-threatening complications, such as leukostasis, tumor lysis, coagulopathy/hemorrhage, or sepsis, is a key aspect in reducing early mortality.

The treatment is given in several phases: induction/intensification, consolidation, maintenance, and CNS-directed therapy, each of which has a specific goal.

Steroid prophase In the first week of treatment and before starting the induction chemotherapy, the BFM protocols utilize a prophase with oral prednisone as a single agent at a dose of up to 60 mg/m^2 to avoid metabolic complications and assess prednisone response.

Induction The induction phase involves an intensive treatment aimed to achieve remission and restore normal hematopoiesis. This intensive therapy lasts 4–6 weeks; it reduces 99.9 % of the total number of leukemic cells and allows attaining remission in approximately 98 % of the cases.

Drug combinations typically include a glucocorticoid (prednisone or dexamethasone), vincristine, and L-asparaginase. The addition of an anthracycline continues to be controversial; it has been shown that anthracyclines are effective against bone marrow relapse but do not seem to increase significantly the EFS, and since they appear to have a valuable antileukemic effect but involve increased toxicity, especially cardiac, they should probably be reserved for higher-risk patients (Childhood Acute Lymphoblastic Leukaemia Collaborative Group 2009). Another point of discussion is whether to use dexamethasone or prednisone. When dexamethasone was compared with prednisone at a ratio of 1:6–7, an advantage in EFS was found with dexamethasone (Bostrom et al. 2003; Mitchell et al. 2005). However, at a ratio of 1:10, this benefit was not found (Igarashi et al. 2005).

Three forms of L-asparaginase have been used in the treatment of children with ALL, namely, native *Escherichia coli* L-asparaginase, *Erwinia* L-asparaginase, and pegylated (PEG)-L-asparaginase, each with different pharmacological and pharmacokinetic profiles. PEG-L-asparaginase has several advantages, including a longer half-life; it is less immunogenic and has a lower probability of developing neutralizing antibodies, so many study groups have incorporated PEG-asparaginase in their treatment protocols. A single dose of PEG-L-asparaginase given in conjunction with vincristine and prednisone during induction therapy appeared to have similar activity and toxicity as nine doses of intramuscular *E. coli* L-asparaginase given three times a week for 3 weeks (Avramis et al. 2002). Three intramuscular doses of PEG-L-asparaginase can safely replace 21 intramuscular doses of native asparaginase (Gaynon et al. 2010). While only PEG-L-asparaginase and *Erwinia* L-asparaginase are available in the United States, native *E. coli* L-asparaginase remains the main form in many centers, particularly in developing countries. Some protocols intensify the induction phase with two more drugs that may include cyclophosphamide, L-asparaginase, cytarabine, or epipodophyllotoxins.

A bone marrow aspirate must be taken at different points of induction treatment to confirm that the child has achieved remission.

About 2–3 % of children diagnosed with ALL respond poorly to initial chemotherapy (Silverman et al. 1999; Oudot et al. 2008). The overall survival rate for these children who fail to go into remission following induction therapy is 32 %, because they often receive high-dose chemotherapy followed by a stem cell transplant. This approach has been questioned in recent years, and it has been shown that some children may not need a stem cell transplant.

In addition, another 1 % of the patients will fail induction therapy because of early death (most often caused by infection or bleeding), although a significantly higher frequency of early deaths has been described in developing countries (Gupta et al. 2011; Advani et al. 1999; Asim et al. 2011).

Consolidation This phase starts when the remission/intensification phase is concluded, and its goal is to reinforce the remission in the bone marrow and to provide CNS prophylaxis. Many current protocols use high-dose systemic methotrexate (four doses given biweekly) together with intrathecal chemotherapy (methotrexate, cytarabine, and hydrocortisone).

Maintenance This phase is aimed to ensure continuation of remission and eradicate residual leukemic cells. The drugs used in this phase depend on the assigned risk.

For low-risk patients, there is a tendency to reduce the use of drugs associated with higher risk of severe late toxic effects, such as anthracyclines and alkylating agents. These patients are generally treated with monthly pulses of vincristine plus steroid with daily oral 6-metcaptopurine and weekly parenteral (preferably intravenous) methotrexate (Childhood ALL Collaborative Group 1996).

For high-risk patients most current protocols intensify post-remission therapy by using combinations of drugs (Möricke et al. 2008; Reiter et al. 1994; Pui et al. 2000). Epipodophyllotoxins have been eliminated from most current protocols to reduce the risk of second malignancies.

Maintenance treatment lasts for about 2 years from complete remission for girls and up to 3 years for boys (Möricke et al. 2008; Gaynon et al. 2010, Pui et al. 2010).

CNS-directed therapy The CNS is a sanctuary site for leukemic cells, which are protected from systemic chemotherapy by the blood-brain barrier. Approaches to CNS prophylaxis include radiation (cranial or craniospinal), intrathecal chemotherapy, high-dose systemic chemotherapy, or combinations of these. However, the long-term neurological and neuroendocrine sequelae and the risk of secondary CNS neoplasms, in addition to the proven effectiveness of intrathecal chemotherapy alone, have led to the abandonment of cranial irradiation or to limit it to selected patients with a high risk of CNS relapse (Pui et al. 2009). The doses of intrathecally administered drugs are based on age. Intrathecal chemotherapy for CNS therapy is given during induction and must be continued throughout maintenance therapy. Effective CNS prophylactic regimens have reduced the incidence of isolated CNS relapse to less than 5 %.

Hematopoietic stem cell transplant (HSCT) Owing to the associated morbidity and mortality and long-term effects of this procedure, it is reserved for selected children with very high-risk ALL and for some patients who relapse after standard treatment.

For induction failure, several leukemia groups recommend allogeneic HSCT; however, it has recently been suggested that some of these children may do well if they receive additional chemotherapy rather than a stem cell transplant. Patients who fail induction and have T-cell leukemia appear to have a superior outcome with allogeneic stem cell transplantation than with chemotherapy, whereas patients who have BCP leukemia without other adverse features appear to do better with chemotherapy (Schrappe et al. 2012).

Radiotherapy In the past, radiation was routinely used for patients with proven CNS or testicular leukemia at diagnosis, but this approach is controversial. For prophylaxis and primary CNS infiltration, it tends to be abandoned even in cases with T-cell leukemia, and radiation is now reserved for patients who relapse (Kelly et al. 2014; Pui et al. 2009; Kamps et al. 2002, Gustafsson et al. 2000; Gaynon et al. 2010). In the case of testicular infiltration at diagnosis, it has been reported that these patients may be treated with chemotherapy alone (Hijiya et al. 2005), although there is not enough evidence to recommend this practice.

The outcome of newly diagnosed pediatric ALL has increased significantly over the past decades. More than 95 % of children achieve remission, and approximately 80 % are expected to be long-term event-free survivors (Hunger et al. 2012). The 5-year EFS varies considerably depending on risk category and ranges from 95 %

for low risk to 30 % for very high risk, with infant leukemia having the worst outcomes. Infants younger than 1 year (about 3 % of patients) have EFS of less than 50 %, and around 20 % for those patients aged less than 90 days (Pui et al. 1995).

In low-income countries, pediatric ALL can also be a highly curable malignancy when intensive chemotherapy protocols are used together with appropriate supportive therapy, and recent studies have reported overall survival rates from 60 % to almost 80 % (Bajel et al. 2008; Arya et al. 2011; Muwakkit et al. 2012).

However, not all countries, regions, or social sectors have benefited to the same extent from the progress in ALL treatment. Limited health access, insufficient infrastructure, understaffed units, low sanitary conditions, malnutrition, cultural barriers, and other socioeconomic factors affect the outcome of many children who live in low- and middle-income countries, and the current survival rates for ALL continue to be lower than 35 % in some of these countries (Farmer et al. 2010; Metzger et al. 2003; Rajajee et al. 1999; Ribeiro et al. 2007; Gupta et al. 2011; Howard et al. 2008). In view of this, therapy protocols should be adapted to local resources and conditions to limit toxic deaths while maximizing treatment efficacy (Hunger et al. 2009; Bonilla et al. 2000; Magrath et al. 2005).

Relapsed ALL

Similarly to frontline ALL therapy, treatment outcome for relapsed patients depends on clinical and biological characteristics of the disease, the time from diagnosis, and site of relapse. Only about one-third of all children with first relapsed ALL can be cured by risk-oriented therapies using conventional intensive chemotherapy and radiotherapy, with percentages ranging from 0 to 70 % depending on the pattern of prognostic factors present at relapse (Nguyen et al. 2008; Roy et al. 2005; Schroeder et al. 1995). The use of novel therapies such as monoclonal antibodies, targeted molecules, and some new chemotherapeutic agents is opening new opportunities for some patients. Clofarabine, a second-generation purine analog approved in pediatric leukemia, alone or in combination with other chemotherapy agents, is replacing HSCT for intermediate- and high-risk patients (Tallen et al. 2010; Eckert et al. 2013).

Acute Myeloid Leukemia

AML is less common than ALL, at a ratio of one case of AML for every four of ALL. This relationship is reversed in the neonatal period, where 95 % of all leukemias are AML, whereas in infants younger than 1 year, the ratio is 1:2. The annual standardized rate is usually between 4 and 7 per million. Its peak incidence occurs in the first year of life and then decreases until age 4 and remains relatively uniform thereafter (Gurney et al. 1995).

Although there is no clear variation between geographical regions, the promyelocytic subtype seems to have a higher incidence rate among Hispanic/Latino population (Malta Corea et al. 1993; Hernandez et al. 2000; Douer et al. 1996), and the higher relative frequency of AML in some series appears to be due to a deficit of ALL.

Genetics and Biology

Characterization of leukemia-associated chromosome translocations has contributed to the understanding of AML pathogenesis. The genes involved in such alterations encode proteins normally implicated in the control of hematopoietic cell growth and differentiation.

The t(8;21)(q22;q22)/*RUNX1*(*AML1*,*CBFA2*)-*ETO*(*MTG8*, *RUNX1T*) translocation, which is associated with the AML subtype M2, is the most commonly detected recurrent cytogenetic and molecular abnormality in AML. It results from the fusion of the *RUNX1* (*AML1*, *CBFA2*) gene, located on chromosome 21, with the *ETO* (*MTG8*, *RUNX1T1*) gene, located on chromosome 8. *RUNX1* is a member of the core-binding factor (CBF) family of transcription factors required for the homeostasis of hematopoietic stem and progenitor cells and expansion of hematopoietic stem and progenitor cells, and ETO is a member of the E-box family of transcription factors (Erickson et al. 1992). The chimeric protein resulting from the fusion gene *AML1-ETO* (*RUNX1-RUNX1T1*) functions principally by transactivating or repressing RUNX1 target genes including critical regulators of myeloid progenitor expansion (Lam et al. 2014) or genes that may prolong cell life span such as *BCL-2* (Klampfer et al. 1996). It has also been shown that *AML1-ETO* is able to promote leukemogenesis in p21WAF1-deficient cells (Peterson et al. 2007).

The recurrent chromosomal abnormality inv(16)(p13q22) and the less common t(16;16)(p13q22) translocation create a fusion between the CBFβ gene on 16q22 and MYH11 on 16p13, the gene encoding smooth muscle myosin heavy chain (SMMHC) (Liu et al. 1993). The resulting CBFβ-MYH11 fusion gene, which encodes the oncoprotein CBFβ-SMMHC, is found in practically all patients with the FAB M4 with eosinophilia subtype AML (Liu et al. 1995).

AML with t(15;17)(q22;q21)/*PML-RARA* (also known as APL) is a distinct clinicopathological entity defined by the presence of the *PML-RARA* fusion, regardless of blast count (Arber et al. 2008). This translocation leads to the fusion of the retinoic acid receptor α (RARA) to various partner genes. The genes involved are *PML*, located on chromosome 15, and the *RARA* gene on chromosome 17, which form the fusion *PML-RARA* gene expressed exclusively in the APL subtype. The normal RARA is a transcription factor involved in the differentiation of myeloid cells; it needs all-*trans* retinoic acid (ATRA) (its ligand) to transactivate genes. The resultant RARA chimeric oncoproteins are involved in the pathogenesis of the APL and contribute to leukemic transformation by dominant inhibition of the expression

of target genes that are important for cellular differentiation. Fusion PML-RARA protein remains sensitive to ATRA as do most RARA fusion proteins; however, the non-*PML-RARA* promyelocytic leukemic cells need very high doses of ATRA to differentiate.

Approximately 15–20 % of all pediatric AML patients harbor translocations involving the *MLL* gene (Balgobind et al. 2011), and most infants with acute monoblastic leukemias have *MLL* rearrangements (Sorensen et al. 1994). The more common translocations in childhood AML include t(9;11)(p22;q23), t(11;19)(q23;p13.1), t(11;19)(q23;p13.3), and t(10;11)(p12;q23), (Raimondi et al. 1999).

AML with t(9;11)(p22;q23);*MLLT3-MLL* is the only translocation involving the *MLL* gene included in the WHO 2008 classification of AML as a distinct biological category. This subtype characteristically manifests with proliferation of monoblasts and/or promonocytes (blasts or blast equivalents ≥20 %) and has the morphological, cytochemical, and immunophenotypic features of these cells, although full monocytic differentiation is not often present.

AML with the t(1;22)(p13;q13)/*RBM15/MKL1* translocation is quite uncommon (1–3 % of the cases). So far, it has only been found in acute megakaryocytic leukemia, specifically in children younger than 3 years, and is not associated with DS (Martinez-Climent et al. 1995). The t(1;22) involves the *RBM15* and *MKL1* genes. The fusion protein may modulate chromatin organization, HOX differentiation pathways, or extracellular signaling pathways (Ma et al. 2001).

In 20–25 % of childhood AML cases, no chromosomal abnormalities are visible by conventional karyotyping and are referred to as cytogenetically normal AML. Several mutations have been identified in the normal karyotype subgroup, including mutations in the *FLT3* (FMS-like tyrosine kinase 3), *NPM1* (nucleophosmin), *CEPBA* (CCAAT/enhancer-binding protein alpha), and *WT1* (Wilms' tumor 1) genes.

FLT3 is a protein originally identified in bone marrow CD34-positive cells that has been found in blast cells from most AML and BCP-ALL (Hunte et al. 1995; Carow et al. 1996). Its ligand (FL) is an early-acting factor that promotes survival, proliferation, and differentiation of primitive hematopoietic progenitor cells (Lyman et al. 1993). It participates in the activation of several downstream signaling pathways, such as the Ras/Raf/MAPK and PI3 kinase cascades.

Internal tandem duplications and/or insertions and, rarely, deletions in the *FLT3* gene are the most frequent genetic aberration described in AML, implicated in 20–25 % of all AMLs. Infants with *MLL* rearrangements often have *FLT3* mutations; they are also seen in 4–12 % of AML cases with t(8;21), and some reports suggest that this mutation is an adverse prognostic finding in AML with t(8;21) translocation (Boissel et al. 2006).

Mutations in the *NPM1* gene occur in approximately 7 % of children with AML. The nucleophosmin protein regulates the alternate reading frame (ARF)-p53 tumor-suppressor pathway (Colombo et al. 2002). These mutations have been associated with all morphological subtypes with the exception of M5 AML and are characteristically CD34-negative at diagnosis (Cazzaniga et al. 2005; Cordell et al. 1999).

Another group of mutations that occur in AML patients with normal karyotype are *CEPBA* (CCAAT/enhancer-binding protein alpha) gene mutations, present in nearly 5 % of pediatric AML patients and 17 % of those with normal karyotypes (Ho et al. 2009). The *CEPBA* gene encodes the protein C/EBPα, a transcription factor that regulates proliferation and controls terminal granulocytic differentiation (Lekstrom-Himes and Xanthopoulos 1998).

WT1 mutations have been reported in 6.5–12 % of childhood AML patients (Sano et al. 2013; Hollink et al. 2009a). Wt1 is a transcriptional activator of the erythropoietin gene. Loss of *WT1* expression results in diminished erythropoietin receptor (EpoR) expression in hematopoietic progenitors, suggesting that activation of the EpoR gene by Wt1 is an important mechanism in normal hematopoiesis (Dame et al. 2006). *WT1* mutations cause translation of an aberrant protein with loss of normal function and might therefore result in stem cell proliferation and blocking of differentiation, thereby contributing to leukemogenesis (Hollink et al. 2009a).

Clinical Presentation

Most of the signs and symptoms are common to both acute leukemias in children; however, some AML subtypes are associated with specific features.

In acute monocytic leukemia, extramedullary infiltration occurs more commonly than in other subtypes of AML and includes the lungs, colon, meninges, lymph nodes, orbit, and gums.

Disseminated intravascular coagulation can occur in any subtype of acute leukemia at initial presentation because of rapid cell turnover or sepsis, although it is characteristic of promyelocytic AML. This condition can result in severe, life-threatening hemorrhagic and thrombotic events; therefore, it is essential that cases of APL be rapidly identified.

Rarely, the first sign of AML is the development of a solid leukemic mass outside of the bone marrow. This tumor, known as myeloid sarcoma, chloroma, or granulocytic sarcoma, may occur in almost any part of the body.

Diagnosis

Some specific morphological findings, such as Auer rods, make obvious that cells belong to one or more of the myeloid lineage, and negative MPO or SBB stains help to diagnose ALL, although acute monocytic leukemia usually gives a negative stain with MPO; therefore, nonspecific esterase activity might be useful for discernment when immunophenotype and other more reliable diagnostic resources are not available.

Immunophenotype

The myeloid-related antigens CD13, CD33, CD65, CD117, and myeloperoxidase identify one or more of the myeloid lineages in the leukemic cells. For identification of AML with monocytic differentiation, the specific markers include CD4, CD11b, CD14, CD16, CD36, CD56, CD64, and HLA-DR, and less often expressed CD34, compared with non-monocytic AML. As single markers, none of these is sufficiently sensitive and specific for identifying monocytic AML (Xu et al. 2006).

The minimal panel required to diagnose AML according to the WHO and EGIL includes CD34, CD117, CD11b, CD11c, CD13, CD14, CD15, CD33, CD64, CD65, MPO, lysozyme, CD41, and CD61.

Prognostic Factors

The aim of defining risk groups in AML is to identify both those patients with a high probability of treatment response and a low relapse rate and those with a lower response rate and higher probability of relapse.

Age is considered one of the most significant prognostic factors. Several groups have found that younger children have a lower risk of relapse and EFS no worse than older children (Abrahamsson et al. 2005; Estey et al. 1987; Rubnitz et al. 2012; Smith et al. 2005; Webb et al. 2001; Medina-Sanson et al. 2015).

The AML-BFM 83 and 87 studies identified that a WBC count >100,000/μL was an independent prognostic factor, indicating a high risk, especially for early failure (Creutzig et al. 1999).

A correlation between response to the first induction course and disease outcome has been described by the BFM and other groups (Creutzig et al. 1999; Lie et al. 2005).

t(8;21), inv(16)(p13.1;q22)/*CBF*β-*MYH11*, and t(16;16)(p13.1;q22)/*CBF*β-*MYH11* are the only favorable genetic abnormalities for which there are strong data based on large numbers of pediatric patients (Harrison et al. 2010; von Neuhoff et al. 2010).

AMLs with *MLL* rearrangements conform a heterogeneous group with several outcomes, mostly dependent on the type of translocation. The 5-year OS that ranges from 100 % for the t(1;11) to 22 % for t(6;11) translocation may depend on the translocation partner (Balgobind et al. 2009). Another analysis found that cases with the t(9;11) have a better prognosis than those who carry other *MLL* rearrangements, and in fact this translocation represents one of the most favorable genetic factors for patients with AML (Rubnitz et al. 2002).

In the subset of AML patients with normal karyotype, the mutations with proven prognostic significance include *FLT3-ITD*, biallelic *CEBPA* mutations, and *NPM1*.

A study of 91 pediatric patients with AML trialed in the Children's Cancer Group (CCG)-2891 found that *FLT3-ITD* mutations represent the single most significant, independent prognostic factor for poor outcome in pediatric AML, regardless of

diagnostic WBC counts, induction regimen, or cytogenetic markers. EFS at 8 years for patients with and without *FLT3/ITD* mutations were 7 % and 44 %, respectively (Meshinchi et al. 2001). However, a recent study suggests that the poor prognosis of *FLT3*/ITD in pediatric AML depends on the allelic ratio between mutant and wild-type FLT3, and only those cases with an allelic ratio >0.4 have a very poor outcome (Meshinchi et al. 2006).

The presence of biallelic *CEBPA* mutations is an independent prognostic factor for improved outcome (Ho et al. 2009).

NPM1 mutations have been associated with a favorable prognosis (OS rates >80 %), but only in the absence of karyotype abnormalities and *FLT3* mutations (Hollink et al. 2009b; Staffas et al. 2011; Brown et al. 2007).

It has also been found that patients with *WT1* mutation have a dismal prognosis (5-year OS 21 %). However, the impact of this mutation has not been completely elucidated and is probably dependent on the *FLT3* status (Ho et al. 2010).

For PML patients, the most important adverse prognostic factor is the presenting WBC count. A leukocyte count greater than 10,000 cells/μL is associated with an EFS of approximately 60 % (Ortega et al. 2005). In the current pediatric protocols, patients are considered low or high risk on the basis of WBC counts lower or higher than 10,000 cells/μL, respectively.

The microgranular variant (M3v), the presence of a bcr3 PML breakpoint, and *FLT3-ITD* mutations have also been associated with a poor prognosis (Tallman 2008; Creutzig et al. 2001; Kaspers and Creutzig 2005; Lange et al. 2008; Abrahamsson et al. 2011; Stevens et al. 1998; Rubnitz et al. 2012).

Children with DS, who characteristically present the FAB subtypes M7, M6, and M0, start before the age of 5 years, have a low WBC at diagnosis and mutations in the *GATA1* gene (Lange et al. 1998), and exhibit high sensitivity to chemotherapy (Ravindranath et al. 1992; Lie et al. 1996) and a good outcome when moderate intensity chemotherapy is used (Creutzig et al. 2005b).

It has been reported that ethnicity may also influence survival. A COG study found that Hispanic and black children with AML have worse survival than white children (Aplenc et al. 2006) and that this might be related to genetic differences (Medina-Sanson et al. 2015; Davies et al. 2001).

Risk-Group Assignment

Risk-adjusted therapy approaches in childhood AML are becoming as important as in ALL. In the past, clinical, laboratory, and blast features in addition to morphological response were the basis of risk assignment. However, novel significant predictors of disease outcome are emerging, and the current criteria for risk stratification are based on cytogenetic on molecular characteristics, together with assessment of response measured either by MRD or bone marrow response (Abrahamsson et al. 2011; Wheatley et al. 1999; Langebrake et al. 2006, Rubnitz et al. 2010).

In the COG trials, the combination of cytogenetic, molecular, and MRD information is being used to stratify patients into two groups. *Low-risk* AML includes cases with mutations involving *CBF*, *CEBPA*, and *NPM* and those with no MRD at the end of induction therapy. This group represents approximately 73 % of patients, with a predicted survival close to 75 %. The *high-risk* group includes patients with adverse cytogenetic abnormalities such as monosomy 7, del(5q), high *FLT3-ITD* to wild-type allelic ratio, or MRD positive at the end of induction and has a survival rate of <35 % (Pui et al. 2011; Meshinchi et al. 2006).

According to the AML-BFM 83 and 87 studies, a combination of morphological and response criteria was sufficient to stratify AML patients. The s*tandard-risk* group defined by favorable morphology and a blast cell reduction on day 15 (not required for M3) comprises 31 % of all patients with OS, EFS, and disease-free survival (DFS) at 5 years of 73 % (standard error [SE] 4 %), 68 % (SE 5 %), and 76 % (SE 4 %), respectively (Creutzig et al. 1999). In the last decade, the BFM-AML protocols have incorporated standardized quantitative assessment of MRD for stratification (Langebrake et al. 2006), and it seems that MRD monitoring using methods that target leukemia-associated genes such as *WT1*, *PRAME*, *CCL23*, *GAGED2*, *MSLN*, *SPAG6*, and *ST18* has a strong independent prognostic significance (Steinbach et al. 2015).

The MRC developed an index to stratify AML patients into three risk groups based on morphological response after course one and cytogenetics: *good*, favorable karyotype or M3, irrespective of response status or presence of additional abnormalities; *standard*, neither good nor poor; and *poor*, adverse karyotype or resistant disease and no good-risk features. Survival for these three groups was 70 %, 48 %, and 15 %, respectively, and relapse rates were 33 %, 50 %, and 78 % (Wheatley et al. 1999).

Risk stratification of those patients whose leukemia lack favorable or unfavorable genetic features should be assessed based on response to therapy.

In many developing countries, sophisticated diagnostic resources are unavailable, and it has been necessary to develop more realistic approaches.

In Mexico, for instance, a modification of the NOPHO-AML 93 schedule was adopted as a feasible national protocol, where the risk stratification is based on the morphological response to the first induction course assessed in bone marrow by morphology on day 16. Good-risk patients are those with less than 5 % blasts after the first cycle. Therapy is intensified until response, and if remission is not achieved, the child is classified as a nonresponder (Lie et al. 2005).

Treatment and Outcome

Current pediatric AML protocols result in 85–90 % complete remission rates (Kaspers and Creutzig 2005). The long-term survival rates for patients who achieve remission are in the range of 60–70 % with EFS rates between 45 % and 55 % (Lange et al. 2008; Lie et al. 2005; Abrahamsson et al. 2011; Creutzig et al. 2001; Stevens et al. 1998).

Improvement in pediatric AML survival is to a large extent the result of intensive supportive care, better risk-group stratification, and better use of the old drugs rather than the introduction of new agents. Short drug-intensive regimens are the basis of AML therapy. Treatment generally consists of remission induction, followed by consolidation with either chemotherapy or stem cell transplantation, and includes CNS-directed therapy.

Induction This phase is intended to obtain remission and restore normal hematopoiesis. Most pediatric induction regimens are based on the intensive use of a nucleoside analog (usually cytarabine) and an anthracycline (daunorubicin or idarubicin) or mitoxantrone, with or without etoposide or thioguanine (Gibson et al. 2005; Lie et al. 2005). The intensification of this phase by increasing the dose of cytarabine or the number of doses has not proved to be superior to standard doses (Becton et al. 2006; Rubnitz et al. 2010), and when etoposide was compared with thioguanine, similar results were obtained in complete remission rates and DFS (Hann et al. 1997).

The use of G-CSF after induction therapy did not decrease the incidence of infectious complications or treatment-related mortality in the AML-BFM 98 trial (Creutzig et al. 2006).

Consolidation Post-remission chemotherapy is limited by acute toxicity and late effects, including secondary malignancies. Once remission is achieved, most patients are treated with intensive chemotherapy, generally high-dose cytarabine, anthracyclines with or without mitoxantrone, and other non-cross-resistant drugs.

There are important differences among the pediatric clinical trial groups in the approach to treating AML after remission. The Medical Research Council (MRC) Study Group uses high doses of anthracyclines (Gibson et al. 2005), whereas consolidation regimens of the NOPHO (Lie et al. 2005) and the St Jude AML Study Group are based on high-dose cytarabine. The AML-BFM study group has used both drugs in relatively high doses (Creutzig et al. 2005a). However, survival rates are similar despite these different approaches.

CNS-directed therapy Although overt CNS leukemia is relatively rare in AML, the use of high-dose systemic chemotherapy and intrathecal chemotherapy, with or without cranial irradiation, is considered part of the standard treatment for AML (Creutzig et al. 2005a; Pui et al. 1985).

Allogenic Hematopoietic Stem Cell Transplant (allo-HSCT) There is general agreement among pediatric AML study groups to postpone allo-HSCT to second remission in low-risk patients, but the role of HSCT for intermediate-risk and high-risk AML patients in first remission is controversial. Some researchers have shown an advantage for allo-HSCT on survival probability for patients with intermediate- and high-risk AML (Horan et al. 2008; Woods et al. 2001). The procedure is being abandoned by several groups, even for high-risk cases, since it does not seem to be superior to current conventional chemotherapy (Gibson et al. 2005; Creutzig and Reinhardt 2002; Kelly et al. 2014). A recent review of clinical trials showed that the

OS rates of patients who underwent HSCT have been similar to those who received standard chemotherapy (Niewerth et al. 2010).

Acute Promyelocytic Leukemia

The treatment of the APL subtype is very different and generally includes three phases: induction, consolidation/intensification, and maintenance for a total duration of 1–2 years (Sanz et al. 2010; Creutzig et al. 2010). Standard therapy consists of the combination of ATRA with chemotherapy (Testi et al. 2005; Fenaux et al. 2000; Gregory et al. 2009). ATRA is a differentiation therapy used for the cases carrying the t(15;17). It acts by binding to the PML/RARA fusion protein overcoming the differentiation block and allowing the blasts to terminally differentiate and to undergo apoptosis. Arsenic trioxide (ATO) is an alternative differentiating agent that has been particularly used in cases that do not respond to ATRA and in those that harbor the t(11;17). Although experience with ATO in children is limited, the results of its use as single agent are similar to those obtained with ATRA plus chemotherapy, with minimal toxicity (Zhou et al. 2010; George et al. 2004).

The intensive chemotherapy regimens often result in significant toxicity and relatively high rates of treatment-related deaths. Therefore, less toxic and more effective therapies for pediatric AML are being investigated (Moore et al. 2013).

Refractory and Relapsed AML

Although chemotherapy will induce complete remission in approximately 90 % of children, approximately one-third will relapse. The probability of OS ranges from 16 to 34 % after relapse (Gorman et al. 2010; Rubnitz et al. 2007; Abrahamsson et al. 2007; Aladjidi et al. 2003; Wells et al. 2003; Webb et al. 1999).

Optimal reinduction therapy for pediatric relapsed AML is unknown. The first randomized study in pediatric relapsed AML showed the benefit of liposomal daunorubicin to the FLAG (fludarabine, cytarabine, and granulocyte colony-stimulating factor) reinduction regimen in AML (Kaspers et al. 2013).

Allo-HSCT is generally indicated for patients who achieve complete remission.

Novel strategies are being tested for refractory and relapsed AML, including the use of clofarabine and tyrosine kinase inhibitors.

Concluding Remarks

Over the past 50 years, improvements in therapy and supportive care by interdisciplinary teamwork and cooperation on the national or international level have increased the cure rates for childhood acute leukemia in industrialized countries

from near zero to about 80–85 % in the case of childhood ALL and to around 60 % for pediatric patients with AML.

However, global disparities in incidence and mortality between population groups have been documented. Differences in survival are largely explained by factors such as inadequate or delayed access to health care, availability of resources, and sanitation.

From the public health point of view, mortality from leukemia is an important co-indicator in assessing the quality of health care, and the contrast in childhood leukemia survival strongly correlates with the development of the countries. It is a reality that improvements in survival have not benefited all leukemia children from different geographical locations to the same extent, as a result of deprived socioeconomic conditions. Almost four-fifths of the 185,000–250,000 children diagnosed with cancer worldwide each year live in low- and middle-income countries, where there is still a strong need for resources to provide the minimum standards for cancer therapy.

In the United States, Canada, Western Europe, and Australia, more than 90 % of children and adolescents who are diagnosed with cancer each year are treated at specialized hospitals or Pediatric Oncology units that have enough resources to diagnose, treat, and provide comprehensive care for children and adolescents with cancer, and most of them are enrolled in clinical trials. However, minority populations are often underrepresented in these trials and therefore may be difficult to appropriately assess whether the existing cancer therapies provide equal benefit to all population groups.

On the other hand, it is the Pediatric Oncology of the low-income and many middle-income countries which often lacks the minimum diagnostic and treatment standards resources to treat pediatric cancer patients, such as immunophenotyping, cytogenetics, linear accelerators, MRI and positron-emission tomography scanners, and enough number of trained staff. Some of these resources are available in pediatric oncology services of a few tertiary centers, mainly in the largest cities, but even when present remain inaccessible to most of the population.

The main goal continues to be improvement in treatment results. Even if pediatric oncology has a low priority, the institution, in each country or large province, of at least one pediatric cancer unit may improve not only cancer treatment but also medical care in general. Care standards must be improved by promoting education among the first-contact health personnel and staff involved in childhood cancer treatment and by setting algorithms for the diagnosis and initial approach to children with suspected cancer.

International organizations are already contributing to the development of pediatric oncology worldwide, but much still remains to be done, and a great challenge for the future will be the planning of pediatric oncology in developing countries to exploit the existing health-care infrastructure and complement resources between the existing pediatric oncology services (human resources, infrastructure for diagnosis and treatment, support therapy), adapt the standard therapies, and implement logistic strategies to build regional network systems that may allow an equal and just delivery of health care for pediatric cancer patients in countries with lower resources.

References

General Issues and Classification of Leukemia

Bene MC, Castoldi G, Knapp W, et al. Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). Leukemia. 1995;9:1783–6.

Bennet JM, Catovsky D, Daniel MT, et al. Proposed revised criteria for the classification of acute myeloid leukemia. A report of the French-American-British Cooperative Group. Ann Intern Med. 1985;103:620–5.

Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of the acute leukaemias (FAB cooperative group). Br J Haematol. 1976;33:451–8.

Buffler PA, Kwan ML, Reynolds P, Urayama KY. Environmental and genetic risk factors for childhood leukemia: appraising the evidence. Cancer Invest. 2005;23:60–75.

Gloeckler L, Percy C, Bunin GR. Introduction. In: Cancer incidence and survival among children and adolescents: United States SEER Program 1975–1995. Bethesda: Surveillance Epidemiology and End Results. US Department of Health & Human Services. NIH Publication (99–4649). 1999. p. 1–16.

Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood. 1994;84:1361–92.

Swerdlow SH, Campo E, Harris NL, et al., editors. World Health Organization classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon: IARC Press; 2008.

Vardiman JW, Thiele J, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rational and important changes. Blood. 2009;114:937–51.

Acute Lymphoblastic Leukemia

Advani S, Pai S, Venzon D, et al. Acute lymphoblastic leukemia in India: An analysis of prognostic factors using a single treatment regimen. Ann Oncol. 1999;10:167–76.

Aifantis I, Raetz E, Buonamici S. Molecular pathogenesis of T-cell leukaemia and lymphoma. Nat Rev Immunol. 2008;8:380–90.

Armstrong SA, Staunton JE, Silverman LB, et al. MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. Nat Genet. 2002;30:41–7.

Arya LS, Padmanjali KS, Sazawal S, et al. Childhood T-lineage acute lymphoblastic leukemia: management and outcome at a tertiary care center in North India. Indian Pediatr. 2011;48:785–90.

Asim M, Zaidi A, Ghafoor T, Qureshi Y. Death analysis of childhood acute lymphoblastic leukaemia; experience at Shaukat Khanum Memorial Cancer Hospital and Research Centre, Pakistan. J Pak Med Assoc. 2011;61:666–70.

Avramis VI, Sencer S, Periclou AP, et al. A randomized comparison of native Escherichia coli asparaginase and polyethylene glycol conjugated asparaginase for treatment of children with newly diagnosed standard-risk acute lymphoblastic leukemia: a Children's Cancer Group study. Blood. 2002;99:1986–94.

Babatunde A, Amiwero C, Olatunji P and Durotoye I. Pattern of haematological malignancies in Ilorin, Nigeria: a ten year review. Int J Hematol 2008;5(2), p 1–7. https://ispub.com/IJHE/5/2/13470.

Bachir F, Bennani S, Lahjouji A, et al. Characterization of acute lymphoblastic leukemia subtypes in Moroccan children. Int J Pediatr. 2009;2009, 674801.

Bain BJ, Barnett D, Linch D, et al. Revised guideline on immunophenotyping in acute leukaemias and chronic lymphoproliferative disorders. Clin Lab Haematol. 2002;24:1–13.

Bajel A, George B, Mathews V, et al. Treatment of children with acute lymphoblastic leukemia in India using a BFM protocol. Pediatr Blood Cancer. 2008;51:621–5.

Bene MC, Nebe T, Bettelheim P, Buldini B, Bumbea H, Kern W, et al. Immunophenotyping of acute leukemia and lymphoproliferative disorders: a consensus proposal of the European LeukemiaNet Work Package 10. Leukemia. 2011;25:567–74.

Bercovich D, Ganmore I, Scott LM, et al. Mutations of JAK2 in acute lymphoblastic leukaemias associated with Down's syndrome. Lancet. 2008;372:1484–92.

Bernard OA, Busson-LeConiat M, Ballerini P, et al. A new recurrent and specific cryptic translocation, t(5;14)(q35;q32), is associated with expression of the Hox11L2 gene in T acute lymphoblastic leukemia. Leukemia. 2001;15:1495–504.

Bhargava M, Kumar R, Karak A, et al. Immunological subtypes of acute lymphoblastic leukemia in North India. Leuk Res. 1988;12:673–8.

Bhojwani D, Howard SC, Pui CH. High-risk childhood acute lymphoblastic leukemia. Clin Lymphoma Myeloma. 2009;9 Suppl 3:S222–30.

Bonilla M, Moreno N, Marina N, et al. Acute lymphoblastic leukemia in a developing country: preliminary results of a nonrandomized clinical trial in El Salvador. J Pediatr Hematol Oncol. 2000;22:495–501.

Borowitz MJ, Hunger SP, Carroll AJ, et al. Predictability of the t(1;19)(q23;p13) from surface antigen phenotype: implications for screening cases of childhood acute lymphoblastic leukemia for molecular analysis: a Pediatric Oncology Group study. Blood. 1993;82:1086–91.

Borowitz MJ, Devidas M, Hunger SP, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's Oncology Group study. Blood. 2008;111:5477–85.

Bostrom BC, Sensel MR, Sather HN, et al. Dexamethasone versus prednisone and daily oral versus weekly intravenous mercaptopurine for patients with standard-risk acute lymphoblastic leukemia: a report from the Children's Cancer Group. Blood. 2003;101:3809–17.

Bruggemann M, Gokbuget N, Kneba M. Acute lymphoblastic leukemia: monitoring minimal residual disease as a therapeutic principle. Semin Oncol. 2012;39:47–57.

Bürger B, Zimmermann M, Mann G, et al. Diagnostic cerebrospinal fluid examination in children with acute lymphoblastic leukemia: significance of low leukocyte counts with blast or traumatic lumbar puncture. J Clin Oncol. 2003;21:184–8.

Burke MJ, Bhatla T. Epigenetic modifications in pediatric acute lymphoblastic leukemia. Front Pediatr. 2014;2:42.

Cario G, Zimmermann M, Romey R, et al. Presence of the P2RY8-CRLF2 rearrangement is associated with a poor prognosis in non-high-risk precursor B-cell acute lymphoblastic leukemia in children treated according to the ALL-BFM 2000 protocol. Blood. 2010;115:5393–7.

Carroll AJ, Crist WM, Link MP, et al. The t(1;14)(p34;q11) is nonrandom and restricted to T-cell acute lymphoblastic leukemia: a Pediatric Oncology Group study. Blood. 1990;76:1220–4.

Chauvenet AR, Martin PL, Devidas M, et al. Anti–metabolite therapy for lesser risk B-lineage acute lymphoblastic leukemia of childhood: a report from Children's Oncology Group Study P9201. Blood. 2007;110:1105–11.

Chen Q, Cheng JT, Tasi LH, et al. The tal gene undergoes chromosome translocation in T cell leukemia and potentially encodes a helix-loop-helix protein. EMBO J. 1990;9:415–24.

Chen CL, Liu Q, Pui CH, et al. Higher frequency of glutathione S-transferase deletions in black children with acute lymphoblastic leukemia. Blood. 1997;89:1701–7.

Chen IM, Harvey RC, Mullighan CG, et al. Outcome modeling with CRLF2, IKZF1, JAK, and minimal residual disease in pediatric acute lymphoblastic leukemia: A Children's Oncology Group Study. Blood. 2012;119:3512–22.

Childhood Acute Lymphoblastic Leukaemia Collaborative Group (CALLCG). Beneficial and harmful effects of anthracyclines in the treatment of childhood acute lymphoblastic leukaemia: a systematic review and meta-analysis. Br J Haematol. 2009;145:376–88.

Childhood ALL Collaborative Group. Duration and intensity of maintenance chemotherapy in acute lymphoblastic leukaemia: overview of 42 trials involving 12,000 randomized children. Lancet. 1996;347:1783–8.

Conter V, Bartram CR, Valsecchi MG, et al. Molecular response to treatment redefines all prognostic factors in children and adolescents with B-cell precursor acute lymphoblastic leukemia: results in 3184 patients of the AIEOP-BFM ALL 2000 study. Blood. 2010;115:3206–14.

Coustan-Smith E, Ribeiro RC, Rubnitz JE, et al. Clinical significance of residual disease during treatment in childhood acute myeloid leukaemia. Br J Haematol. 2003;123:243–52.

Coustan-Smith E, Mullighan CG, Onciu M, Behm FG, Raimondi SC, Pei D, et al. Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. Lancet Oncol. 2009;10:147–56.

De Braekeleer E, Meyer C, Douet-Guilbert N, et al. Complex and cryptic chromosomal rearrangements involving the MLL gene in acute leukemia: A study of 7 patients and review of the literature. Blood Cells Mol Dis. 2010;44:268–74.

Den Boer ML, van Slegtenhorst M, De Menezes RX, et al. A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. Lancet Oncol. 2009;10:125–34.

Diakos C, Xiao Y, Zheng S, et al. Direct and indirect targets of the E2A-PBX1 leukemia- specific fusion protein. PLoS. 2014;9, e87602.

Dijon M, Bardin F, Murati A, et al. The role of Ikaros in human erythroid differentiation. Blood. 2008;111:1138–46.

Dördelmann M, Reiter A, Borkhardt A, et al. Prednisone response is the strongest predictor of treatment outcome in infant acute lymphoblastic leukemia. Blood. 1999;94:1209–17.

Dörge P, Meissner B, Zimmermann M, et al. IKZF1 deletion is an independent predictor of outcome in pediatric acute lymphoblastic leukemia treated according to the ALL-BFM 2000 protocol. Haematologica. 2013;98:428–32.

Dumortier A, Kirstetter P, Kastner P, Chan S. Ikaros regulates neutrophil differentiation. Blood. 2003;101:2219–26.

Eckert C, Henze G, Seeger K, et al. Use of allogeneic hematopoietic stem-cell transplantation based on minimal residual disease response improves outcomes for children with relapsed acute lymphoblastic leukemia in the intermediate-risk group. J Clin Oncol. 2013;31:2736–42.

Enciso-Mora V, Hosking FJ, Sheridan E, et al. Common genetic variation contributes significantly to the risk of childhood B-cell precursor acute lymphoblastic leukemia. Leukemia. 2012;26:2212–5.

Ensor HM, Schwab C, Russell LJ, et al. Demographic, clinical and outcome features of children with acute lymphoblastic leukemia and CRLF2 deregulation: Results from the MRC ALL97 clinical trial. Blood. 2010;117:2129–36.

Evans WE, Relling MV. Moving towards individualized medicine with pharmacogenomics. Nature. 2004;429:464–8.

Farmer P, Frenk J, Knaul FM, et al. Expansion of cancer care and control in countries of low and middle income: A call to action. Lancet. 2010;376:1186–93.

Forestier E, Johansson B, Borgström G, et al. Cytogenetic findings in a population-based series of 787 childhood acute lymphoblastic leukemias from the Nordic countries. The NOPHO Leukemia Cytogenetic Study Group. Eur J Haematol. 2000;64:194–200.

Forestier E, Heyman M, Andersen MK, et al. Outcome of *ETV6L-RUNX1* –positive childhood acute lymphoblastic leukaemia in the NOPHO-ALL-1992 protocol: frequent late relapses but good overall survival. Br J Haematol. 2008;140:665–72.

Fronkova E, Mejstrikova E, Avigad S, et al. Minimal residual disease (MRD) analysis in the non-MRD-based ALL IC-BFM 2002 protocol for childhood ALL: is it possible to avoid MRD testing? Leukemia. 2008;22:989–97.

Gadner H, Masera G, Schrappe M, et al. The eighth international childhood acute lymphoblastic leukemia workshop ('Ponte di legno meeting') report: Vienna, Austria, April 27–28, 2005. Leukemia. 2006;20:9–17.

Garrido C, Santizo VG, Müllers P, et al. Frequency of thiopurine S-methyltransferase mutant alleles in indigenous and admixed Guatemalan patients with acute lymphoblastic leukemia. Med Oncol. 2013;30:474.

Gaynon PS, Angiolillo AL, Carroll WL, et al. Long-term results of the children's cancer group studies for childhood acute lymphoblastic leukemia 1983–2002: a Children's Oncology Group Report. Leukemia. 2010;24:285–97.

Georgopoulos K. Acute lymphoblastic leukemia-on the wings of IKAROS. N Engl J Med. 2009;360:524–6.

Georgopoulos K, Bigby M, Wang JH, et al. The Ikaros gene is required for the development of all lymphoid lineages. Cell. 1994;79:143–56.

Goggins WB, Fiona FKL. Racial and ethnic disparities in survival of US children with acute lymphoblastic leukemia: evidence from the SEER database 1988–2008. Cancer Causes Control. 2012;23:737–43.

Golub TR, Barker GF, Bohlander SK, et al. Fusion of the TEL gene on 12p13 to the AML1 gene on 21q22 in acute lymphoblastic leukemia. Proc Natl Acad Sci U S A. 1995;92:4917–21.

Greaves MF, Wiemels J. Origins of chromosome translocations in childhood leukaemia. Nat Rev Cancer. 2003;3:639–49.

Greaves MF, Colman SM, Beard ME, et al. Geographical distribution of acute lymphoblastic leukaemia subtypes: second report of the collaborative group study. Leukemia. 1993;7:27–34.

Gupta S, Antillon FA, Bonilla M, et al. Treatment-related mortality in children with acute lymphoblastic leukemia in Central America. Cancer. 2011;117:4788–95.

Gupta S, Wilejto M, Pole JD, et al. Low socioeconomic status is associated with worse survival in children with cancer: a systematic review. PLoS One. 2014;9, e89482.

Gurney JG, Severson RK, Davis S, Robison LL. Incidence of cancer in children in the United States. Sex-, race-, and 1-year age-specific rates by histologic type. Cancer. 1995;75:2186–219.

Gustafsson G, Schmiegelow K, Forestier E, et al. Improving outcome through two decades in childhood ALL in the Nordic countries: the impact of high-dose methotrexate in the reduction of central nervous system irradiation. Nordic Society of Pediatric Haematology and Oncology (NOPHO). Leukemia. 2000;14:2267–75.

Harrison CJ, Moorman AV, Barber KE, et al. Interphase molecular cytogenetic screening for chromosomal abnormalities of prognostic significance in childhood acute lymphoblastic leukaemia: a UK Cancer Cytogenetics Group Study. Br J Haematol. 2005;129:520–30.

Harvey RC, Mullighan CG, Chen IM, et al. Rearrangement of CRLF2 is associated with mutation of JAK kinases, alteration of IKZF1, Hispanic/Latino ethnicity, and a poor outcome in pediatric B-progenitor acute lymphoblastic leukemia. Blood. 2010;115:5312–21.

Heerema NA, Nachman JB, Sather HN, et al. Hypodyploid with less than 45 chromosomes confers adverse risk in childhood acute lymphoblastic leukemia: a report of the children's cancer group. Blood. 1999;94:4036–45.

Heerema NA, Sather HN, Sensel MG, et al. Prognostic impact of trisomies of chromosomes 10, 17, and 5 among children with acute lymphoblastic leukemia and high hyperdiploidy (>50 chromosomes). J Clin Oncol. 2000;18:1876–87.

Heerema NA, Raimondi SC, Anderson JR, et al. Specific extra chromosomes occur in a modal number dependent pattern in pediatric acute lymphoblastic leukemia. Genes Chromosomes Cancer. 2007;46:684–93.

Hijiya N, Liu W, Sandlund JT, et al. Overt testicular disease at diagnosis of childhood acute lymphoblastic leukemia: lack of therapeutic role of local irradiation. Leukemia. 2005;19: 1399–403.

Hossain MJ, Xie L, McCahan SM. Characterization of pediatric acute lymphoblastic leukemia survival patterns by age at diagnosis. J Cancer Epidemiol. 2014;2014, 865979.

Howard SC, Metzger ML, Wilimas JA, et al. Childhood cancer epidemiology in low-income countries. Cancer. 2008;112:461–72.

Hunger SP, Sung L, Howard SC. Treatment strategies and regimens of graduated intensity for childhood acute lymphoblastic leukemia in low-income countries: a proposal. Pediatr Blood Cancer. 2009;52:559–65.

Hunger SP, Lu X, Devidas M, et al. Improved survival for children and adolescents with acute lymphoblastic leukemia between 1990 and 2005: a report from the children's oncology group. J Clin Oncol. 2012;30:1663–9.

Igarashi S, Manabe A, Ohara A, et al. No advantage of dexamethasone over prednisolone for the outcome of standard- and intermediate-risk childhood acute lymphoblastic leukemia in the Tokyo Children's Cancer Study Group L95-14 protocol. J Clin Oncol. 2005;23:6489–98.

Kadan-Lottick NS, Ness KK, Bhatia S, Gurney JG. Survival variability by race and ethnicity in childhood acute lymphoblastic leukemia. JAMA. 2003;290:2008–14.

Kamat DM, Gopal R, Advani SH, et al. Pattern of subtypes of acute lymphoblastic leukemia in India. Leuk Res. 1985;9:927–34.

Kamel AM, Ghaleb FM, Assem MM, et al. Phenotypic analysis of T-cell acute lymphoblastic leukemia in Egypt. Leuk Res. 1990;14:602–9.

Kamps WA, Bökkerink JPM, Hakvoort-Cammel FGAJ, Veerman AJP, et al. BFM-oriented treatment for children with acute lymphoblastic leukemia without cranial irradiation and treatment reduction for standard risk patients: results of DCLSG protocol ALL-8 (1991–1996). Leukemia. 2002;16:1099–111.

Kanerva J, Saarinen-Pihkala UM, Nini T, et al. Favorable outcome in 20-year follow-up of children with very-low-risk ALL and minimal standard therapy, with special reference to *TEL-AML1* fusion. Pediatr Blood Cancer. 2004;42:30–5.

Kang H, Wilson CS, Harvey RC, et al. Gene expression profiles predictive of outcome and age in infant acute lymphoblastic leukemia: a Children's Oncology Group study. Blood. 2012;119:1872–81.

Kelly MJ, Trikalinos TA, Dahabreh IJ, et al. Cranial radiation for pediatric T-lineage acute lymphoblastic leukemia: a systematic review and meta-analysis. Am J Hematol. 2014a;89:992–7.

Kishi S, Cheng C, French D, et al. Ancestry and pharmacogenetics of antileukemic drug toxicity. Blood. 2007;109:4151–7.

Kroll ME, Stiller CA, Richards S, et al. Evidence for under-diagnosis of childhood acute lymphoblastic leukaemia in poorer communities within Great Britain. Br J Cancer. 2012;106:1556–9.

Kuiper RP, Waanders E, van der Velden VH, et al. IKZF1 deletions predict relapse in uniformly treated pediatric precursor B-ALL. Leukemia. 2010;24:1258–64.

Larson Gedman A, Chen Q, Kugel Desmoulin S, et al. The impact of NOTCH1, FBW7 and PTEN mutations on prognosis and downstream signaling in pediatric T-cell acute lymphoblastic leukemia: a report from the Children's Oncology Group. Leukemia. 2009;23:1417–25.

Levin SD, Koelling RM, Friend SL, et al. Thymic stromal lymphopoietin: a cytokine that promotes the development of IgM+ B cells *in vitro* and signals via a novel mechanism. J Immunol. 1999;162:677–83.

Linabery AM, Ross JA. Trends in childhood cancer incidence in the U.S. (1992–2004). Cancer. 2008;112:416–32.

Lobato-Mendizábal E, López-Martínez B, Ruiz-Argüelles GJ. A critical review of the prognostic value of the nutritional status at diagnosis in the outcome of therapy of children with acute lymphoblastic leukemia. Rev Invest Clin. 2003;55:31–5.

Macharia WM. Comparison of prognostic determinants in childhood acute lymphoblastic leukemia in negroid and Caucasian populations. East Afr Med J. 1996;73:638–42.

Magrath I, Shanta V, Advani S, et al. Treatment of acute lymphoblastic leukaemia in countries with limited resources; lessons from use of a single protocol in India over a twenty year period [corrected]. Eur J Cancer. 2005;41:1570–83.

Malinge S, Ben-Abdelali R, Settegrana C, et al. Novel activating JAK2 mutation in a patient with Down syndrome and B-cell precursor acute lymphoblastic leukemia. Blood. 2007;109: 2202–4.

Maloney KW, Carroll WL, Carroll AJ, et al. Down syndrome childhood acute lymphoblastic leukemia has a unique spectrum of sentinel cytogenetic lesions that influences treatment outcome: a report from the Children's Oncology Group. Blood. 2010;116:1045–50.

Matasar MJ, Ritchie EK, Consedine N. Incidence rates of the major leukemia subtypes among US Hispanics, Blacks, and non-Hispanic Whites. Leuk Lymphoma. 2006;47:2365–70.

Mc Nally RJ, Birch JM, Taylor GM, Eden OB. Incidence of childhood precursor B-cell acute lymphoblastic leukaemia in north-west England. Lancet. 2000;356:485–6.
Mejia-Arangure JM, Fajardo-Gutierrez A, Reyes-Ruiz NI, et al. Malnutrition in childhood lymphoblastic leukemia: a predictor of early mortality during the induction-to-remission phase of the treatment. Arch Med Res. 1999;30:150–3.
Metzger ML, Howard SC, Fu LC, et al. Outcome of childhood acute lymphoblastic leukaemia in resource-poor countries. Lancet. 2003;362:706–8.
Mi JQ, Wang X, Yao Y, et al. Newly diagnosed acute lymphoblastic leukemia in China (II): prognosis related to genetic abnormalities in a series of 1091 patients. Leukemia. 2012;26:1507–16.
Mitchell CD, Richards SM, Kinsey SE, Medical Research Council Childhood Leukaemia Working Party, et al. Benefit of dexamethasone compared with prednisolone for childhood acute lymphoblastic leukaemia: results of the UK medical research council ALL97 randomised trial. Br J Haematol. 2005;129:734–45.
Mitchell C, Richards S, Harrison CJ, Eden T. Long-term follow-up of the United Kingdom Medical Research Council protocols for childhood acute lymphoblastic leukaemia, 1980–2001. Leukemia. 2010;24:406–18.
Moorman AV, Richards SM, Martineau M, et al. United Kingdom Medical Research Council's Childhood Leukemia Working Party. Blood. 2003;102:2756–62.
Moreno-Guerrero SS, Ramírez-Pacheco A, Dorantes-Acosta EM, Medina-Sanson A. Analysis of genetic polymorphisms of thiopurine S-methyltransferase (TPMT) in Mexican pediatric patients with cancer. Rev Invest Clin. 2013;65:156–64.
Möricke A, Zimmermann M, Reiter A, et al. Prognostic impact of age in children and adolescents with acute lymphoblastic leukemia: data from the trials ALL-BFM 86, 90 and 95. Klin Padiatr. 2005;217:310–20. PubMed Abstract.
Möricke A, Reiter A, Zimmermann M, et al. Risk-adjusted therapy of acute lymphoblastic leukemia can decrease treatment burden and improve survival: treatment results of 2169 unselected pediatric and adolescent patients enrolled in the trial ALL-BFM 95. Blood. 2008; 111:4477–89.
Mrózek K, Harper DP, Aplan PD. Cytogenetics and molecular genetics of acute lymphoblastic leukemia. Hematol Oncol Clin North Am. 2009;23:991–1010.
Mullighan CG, Goorha S, Radtke I, et al. Genome-wide analysis of genetic alterations in acute lymphoblastic leukemia. Nature. 2007;446:758–64.
Mullighan CG, Miller CB, Radtke I, et al. BCR-ABL1 lymphoblastic leukaemia is characterized by the deletion of Ikaros. Nature. 2008;453:110–4.
Mullighan CG, Su X, Zhang J, et al. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. N Engl J Med. 2009a;360:470–80.
Mullighan CG, Zhang J, Harvey RC, et al. JAK mutations in high-risk childhood acute lymphoblastic leukemia. Proc Natl Acad Sci U S A. 2009b;106:9414–8.
Muwakkit S, Al-Aridi C, Samra A, Saab R, Mahfouz RA, Farra C, Jeha S, Abboud MR. Implementation of an intensive risk-stratified treatment protocol for children and adolescents with acute lymphoblastic leukemia in Lebanon. Am J Hematol. 2012;87:678–83.
Nguyen K, Devidas M, Cheng SC, et al. Factors influencing survival after relapse from acute lymphoblastic leukemia: a Children's Oncology Group Study. Leukemia. 2008;22:2142–50.
Oudot C, Auclerc MF, Levy V, et al. Prognostic factors for leukemic induction failure in children with acute lymphoblastic leukemia and outcome after salvage therapy: the FRALLE 93 study. J Clin Oncol. 2008;26:1496–503.
Palmi C, Vendramini E, Silvestri D, et al. Poor prognosis for P2RY8-CRLF2 fusion but not for CRLF2 over-expression in children with intermediate risk B-cell precursor acute lymphoblastic leukemia. Leukemia. 2012;26:2245–53.
Papaemmanuil E, Rapado I, Li Y, et al. RAG-mediated recombination is the predominant driver of oncogenic rearrangement in ETV6-RUNX1 acute lymphoblastic leukemia. Nat Genet. 2014;46:116–25.
Parkin DM, Stiller CA, Draper GJ, Bieber CA, et al., editors. International incidence of childhood cancer. IARC scientific publication no. 87. International Agency for Research on Cancer, Lyon; 1988a. p. 44–8.

Parkin DM, Stiller CA, Draper GJ, Bieber CA. The international incidence of childhood cancer. Int J Cancer. 1988b;42:511–20.
Parkin DM, Ferlay J, Hamdi-Cherif M, et al., editors. Cancer in Africa: epidemiology and prevention. IARC scientific publications no. 153. Lyon: IARC Press; 2003. p. 381–2.
Paulsson K, Johanson B. High hyperdiploid childhood acute lymphoblastic leukemia. Genes Chromosomes Cancer. 2009;48:637–60.
Paulsson K, Horvat A, Strombeck B, et al. Mutations of FLT3, NRAS, KRAS, and PTPN11 are frequent and possibly mutually exclusive in high hyperdiploid childhood acute lymphoblastic leukemia. Genes Chromosomes Cancer. 2008;47:26–33.
Paulsson K, Forestier E, Lilljebjörn H, et al. Genetic landscape of high hyperdiploid childhood acute lymphoblastic leukemia. Proc Natl Acad Sci U S A. 2010;107:21719–24.
Perez-Saldivar AAA, Fajardo-Gutiérrez A, Bernáldez-Ríos R, et al. Childhood acute leukemias are frequent in Mexico City: descriptive epidemiology. BMC Cancer. 2011;11:355.
Pfeiffer P, Goedecke W, Obe G. Mechanisms of DNA double-strand break repair and their potential to induce chromosomal aberrations. Mutagenesis. 2000;15:289–302.
Pieters R, Schrappe M, de Lorenzo P, et al. A treatment protocol for infants younger than 1 year with acute lymphoblastic leukaemia (Interfant-99): an observational study and a multicenter randomized trial. Lancet. 2007;370:240–50.
Pollock BH, DeBaun MR, Camitta BM, et al. Racial differences in the survival of childhood B-precursor acute lymphoblastic leukemia: a pediatric oncology group study. J Clin Oncol. 2000;18:813–23.
Poole C, Greenland S, Luetters C, et al. Socioeconomic status and childhood leukaemia: a review. Int J Epidemiol. 2006;35:370–8.
Pui CH. Acute lymphoblastic leukemia. In: Pui CH, editor. Childhood leukemias. New York: Cambridge University Press; 2006. p. 439–72.
Pui CH, Pei D, Sandlund JT, et al. Long-term results of St Jude Total Therapy Studies 11, 12, 13A, 13B, and 14 for childhood acute lymphoblastic leukemia. Leukemia. 2010;24:371–82.
Pui CH, Evans WE. Acute lymphoblastic leukemia. N Engl J Med. 1998;339:605–15.
Pui CH, Howard SC. Current management and challenges of malignant disease in the CNS in paediatric leukaemia. Lancet Oncol. 2008;9:257–68.
Pui CH, Hancock ML, Head DR, et al. Clinical significance of CD34 expression in childhood acute lymphoblastic leukemia. Blood. 1993;82:889–94.
Pui CH, Kane JR, Crist WM. Biology and treatment of infant leukemias. Leukemia. 1995;9:762–9.
Pui CH, Boyett JM, Relling MV, et al. Sex differences in prognosis for children with acute lymphoblastic leukemia. J Clin Oncol. 1999;17:818–24.
Pui CH, Boyett JM, Rivera GK, et al. Long-term results of Total Therapy studies 11, 12, 13A for childhood acute lymphoblastic leukemia at St Jude Children's Research Hospital. Leukemia. 2000;14:2286–94.
Pui CH, Chessells JM, Camita B, et al. Clinical heterogeneity in childhood acute lymphoblastic leukemia with 11q23 rearrangements. Leukemia. 2003a;17:700–6.
Pui CH, Sandlund JT, Pei D, et al. Results of therapy for acute lymphoblastic leukemia in black and white children. JAMA. 2003b;290:2001–7.
Pui CH, Relling MV, Downing JR. Acute lymphoblastic leukemia. N Engl J Med. 2004;350:1535–48.
Pui CH, Campana D, Pei D, et al. Treating childhood acute lymphoblastic leukemia without cranial irradiation. N Engl J Med. 2009;360:2730–41.
Pui CH, Pei D, Sandlund JT, et al. Long-term results of St Jude Total Therapy Studies 11, 12, 13A, 13B, and 14 for childhood acute lymphoblastic leukemia. Leukemia. 2010;24:371–82.
Pullen J, Shuster JJ, Link M, et al. Significance of commonly used prognostic factors differs for children with T-cell acute lymphocytic leukemia (ALL), as compared to those with B-precursor ALL. A Pediatric Oncology Group (POG) study. Leukemia. 1999;13:1696–707.
Raaschou-Nielsen O, Obel J, Dalton S, et al. Socioeconomic status and risk of childhood leukaemia in Denmark. Scand J Public Health. 2004;32:279–86.

Raimondi SC. Current status of cytogenetic research in childhood acute lymphoblastic leukemia. Blood. 1993;81:2237–51.
Raimondi SC, Peiper SC, Kitchingman GR, et al. Childhood acute lymphoblastic leukemia with chromosomal breakpoints at 11q23. Blood. 1989;73:1627–34.
Raimondi SC, Behm FG, Roberson PK, et al. Cytogenetics of pre-B-cell acute lymphoblastic leukemia with emphasis on prognostic implications of the t(1;19). J Clin Oncol. 1990;8:1 380–8.
Raimondi SC, Zhou Y, Mathew S, et al. Reassessment of the prognostic significance of hypodiploidy in pediatric patients with acute lymphoblastic leukemia. Cancer. 2003;98:2715–22.
Rajajee S, Desikulu MV, Pushpa V. Survival of childhood acute lymphoblastic leukemia: experience in Chennai. J Trop Pediatr. 1999;45:367–70.
Rajalakshmy KR, Abitha AR, Pramila R, et al. Immunophenotypic analysis of T-cell acute lymphoblastic leukemia in Madras, India. Leuk Res. 1997;21:119–24.
Reiter A, Schrappe M, Ludwing WD, et al. Chemotherapy in 998 unselected childhood acute lymphoblastic leukemia patients. Results and conclusions of the multicenter trial ALL BFM 86. Blood. 1994;84:3122–33.
Ribeiro KB, Lopes LF, de Camargo B. Trends in childhood leukemia mortality in Brazil and correlation with social inequalities. Cancer. 2007;110:1823–31.
Romana SP, Mauchauffe M, Le Coniat M, et al. The t(12;21) of acute lymphoblastic leukemia results in a tel-AML1 gene fusion. Blood. 1995;85:3662–70.
Roy A, Cargill A, Love S, et al. Outcome after first relapse in childhood acute lymphoblastic leukemia – lessons from the United Kingdom R2 trial. Br J Haematol. 2005;130:67–75.
Rubnitz JE, Downing JR, Pui CH, et al. *TEL* gene rearrangement in acute lymphoblastic leukemia: a new genetic marker with prognostic significance. J Clin Oncol. 1997;15:1150–7.
Rubnitz JE, Wichlan D, Devidas M, et al. Prospective analysis of TEL gene rearrangements in childhood acute lymphoblastic leukemia: a Children's Oncology Group study. J Clin Oncol. 2008;26:2186–91.
Russell LJ, Capasso M, Vater I, et al. Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukemia. Blood. 2009;114:2688–98.
Sala A, Pencharz P, Barr RD. Children, cancer, and nutrition-A dynamic triangle in review. Cancer. 2004;100:677–87.
Salzer WL, Devidas M, Carroll WL, et al. Long-term results of the Pediatric Oncology Group studies for childhood acute lymphoblastic leukemia 1984–2001: a report from the Children's Oncology Group. Leukemia. 2010;24:355–70.
Schmiegelow K, Forestier E, Hellebostad M, et al. Long-term results of NOPHO ALL-92 and ALL-2000 studies of childhood acute lymphoblastic leukemia. Leukemia. 2010;24: 345–54.
Schrappe M, Hunger SP, Pui CH, et al. Outcomes after induction failure in childhood acute lymphoblastic leukemia. N Engl J Med. 2012;366:1371–81.
Schroeder H, Garwicz S, Kristinsson J, et al. Outcome after first relapse in children with acute lymphoblastic leukemia: a population-based study of 315 patients from the Nordic Society of Pediatric Hematology and Oncology (NOPHO). Med Pediatr Oncol. 1995;25:372–8.
Schultz KR, Pullen J, Sather HN, et al. Risk and response-based classification of childhood B-precursor acute lymphoblastic leukemia: a combined analysis of prognostic markers from the Pediatric Oncology Group (POG) and Children's Cancer Group (CCG). Blood. 2007;109:926–35.
Schultz KR, Bowman WP, Aledo A, et al. Improved early event-free survival with imatinib in Philadelphia chromosome –positive acute lymphoblastic leukemia: a children's oncology group study. J Clin Oncol. 2009;27:5175–81.
Shah A, Coleman MP. Increasing incidence of childhood leukaemia: a controversy re-examined. Br J Cancer. 2007;97:1009–12.

Shurtleff SA, Buijs A, Behm FG, et al. TEL/AML1 fusion resulting from a cryptic t(12;21) is the most common genetic lesion in pediatric ALL and defines a subgroup of patients with an excellent prognosis. Leukemia 1995;9:1985–9.

Sievers EL, Lange BJ, Alonzo TA, et al. Immunophenotypic evidence of leukemia after induction therapy predicts relapse: results from a prospective Children's Cancer Group study of 252 patients with acute myeloid leukemia. Blood. 2003;101:3398–406.

Silverman LB, Gelber RD, Young ML, et al. Induction failure in acute lymphoblastic leukemia of childhood. Cancer. 1999;85:1395–404.

Sirvent N, Suciu S, Bertrand Y, et al. Overt testicular disease (OTD) at diagnosis is not associated with a poor prognosis in childhood acute lymphoblastic leukemia: result of the EORT CLG Study 58881. Pediatr Blood Cancer. 2007;49:344–8.

Smith M, Arthur D, Camitta B, et al. Uniform approach to risk classification and treatment assignment for children with acute lymphoblastic leukemia. J Clin Oncol. 1996;14:18–24.

Smith A, Roman E, Simpson J, Ansell P, Fear NT, Eden T. Childhood leukaemia and socioeconomic status: fact or artefact? A report from the United Kingdom childhood cancer study (UKCCS). Int J Epidemiol. 2006;35:1504–13.

Stewart CC, Behm FG, Carey JL, et al. US–Canadian consensus recommendations on the immunophenotypic analysis of hematologic neoplasia by flow cytometry: selection of antibody combinations. Cytometry. 1997;30:231–5.

Stiller CA, Parkin DM. Geographic and ethnic variations in the incidence of childhood cancer. Br Med Bull. 1996;52:682–703.

Sun L, Heerema N, Crotty L, et al. Expression of dominant-negative and mutant isoforms of the antileukemic transcription factor Ikaros in infant acute lymphoblastic leukemia. Proc Natl Acad Sci U S A. 1999;96:680–5.

Surveillance Epidemiology and End Results (SEER) cancer statistics review, 1975–2010. Section 29 childhood cancer by the ICCC. Bethesda: National Cancer Institute. http://seer.cancer.gov/archive/csr/1975_2010/results_merged/sect_29_childhood_cancer_iccc.pdf. Accessed 31 Oct 2014.

Sutcliffe MJ, Shuster JJ, Sather HN, et al. High concordance from independent studies by the Children's Cancer Group (CCG) and Pediatric Oncology Group (POG) associating favorable prognosis with combined trisomies 4, 10, and 17 in children with NCI Standard-Risk B-precursor Acute Lymphoblastic Leukemia: a Children's Oncology Group (COG) initiative. Leukemia. 2005;19:734–40.

Taja-Chayeb L, Vidal-Millán S, Gutiérrez O, et al. Thiopurine S-methyltransferase gene (TMPT) polymorphisms in a Mexican population of healthy individuals and leukemic patients. Med Oncol. 2008;25:56–62.

Tallen G, Ratei R, Mann G, et al. Long-term outcome in children with relapsed acute lymphoblastic leukemia after time-point and site-of-relapse stratification and intensified short-course multidrug chemotherapy: results of trial ALL-REZ BFM 90. J Clin Oncol. 2010;28:2339–47.

Taskov H, Dimitrova E, Servinova M, et al. Immunological subtypes of childhood acute lymphoblastic leukemia in Bulgaria. Leuk Res. 1995;19:877–81.

Trueworthy R, Shuster J, Look T, et al. Ploidy of lymphoblasts is the strongest predictor of treatment outcome in B-progenitor cell acute lymphoblastic leukemia of childhood: a pediatric oncology group study. J Clin Oncol. 1992;10:606–13.

van Dongen JJM, Lhermitte L, Böttcher S, et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. Leukemia. 2012;26:1908–75.

van Grotel M, Meijerink JPP, van Wering ER, et al. Prognostic significance of molecular-cytogenetic abnormalities in pediatric T-ALL is not explained by immunophenotypic differences. Leukemia. 2008a;22:124–31.

van Grotel M, van den Heuvel-Eibrink MM, van Wering ER, et al. CD34 expression is associated with poor survival in pediatric T-cell acute lymphoblastic leukemia. Pediatr Blood Cancer. 2008b;51:737–40.

Viana MB, Murao M, Ramos G, et al. Malnutrition as a prognostic factor in lymphoblastic leukaemia: a multivariate analysis. Arch Dis Child. 1994;71:304–10.

Webb KH, Harrison G, Stevens RF, et al. Relationships between age at diagnosis, clinical features, and outcome of therapy in children treated in the Medical Research Council AML 10 and 12 trials for acute myeloid leukemia. Blood. 2001;98:1714–20.
Williams CK. Some biological and epidemiological characteristics of human leukaemia in Africans. IARC Sci Publ. 1984;63:687–712.
Xu H, Cheng C, Devidas M, et al. *ARID5B* genetic polymorphisms contribute to racial disparities in the incidence and treatment outcome of childhood acute lymphoblastic leukemia. J Clin Oncol. 2012;30:751–7.
Yeoh EJ, Ross ME, Shurtleff SA, et al. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. Cancer Cell. 2002;1:133–43.
Yoda A, Yoda Y, Chiaretti S, et al. Functional screening identifies CRLF2 in precursor B-cell acute lymphoblastic leukemia. Proc Natl Acad Sci U S A. 2010;107:252–7.
Yoshida T, Ng SY, Zuniga-Pflucker JC, Georgopoulos K. Early hematopoietic lineage restrictions directed by Ikaros. Nat Immunol. 2006;7:382–91.
Yung YL, Hung CC, Chen JS, et al. IKZF1 deletions predict a poor prognosis in children with B-cell progenitor acute lymphoblastic leukemia: a multicenter analysis in Taiwan. Cancer Sci. 2011;102:1874–81.
Zelent A, Greaves M, Denver T. Role of the TEL-AML1 fusion gene in the molecular pathogenesis of childhood acute lymphoblastic leukaemia. Oncogene. 2004;23:4275–83.

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Abrahamsson J, Clausen N, Forestier E, et al. Long-term results in children with AML: NOPHO-AML Study Group – report of three consecutive trials. Leukemia. 2005;19:2090–100.
Abrahamsson J, Clausen N, Gustafsson G, et al. Improved outcome after relapse in children with acute myeloid leukaemia. Br J Haematol. 2007;136:229–36.
Abrahamsson J, Forestier E, Heldrup J, et al. Response-guided induction therapy in pediatric acute myeloid leukemia with excellent remission rate. J Clin Oncol. 2011;29:310–5.
Aladjidi N, Auvrignon A, Leblanc T, et al. Outcome in children with relapsed acute myeloid leukemia after initial treatment with the French Leucemie Aique Myeloide Enfant (LAME) 89/91 protocol of the French Society of Pediatric Hematology and Immunology. J Clin Oncol. 2003;21:4377–85.
Aplenc R, Alonzo TA, Gerbing RB, et al. Ethnicity and survival in childhood acute myeloid leukemia: a report from the Children's Oncology Group. Blood. 2006;108:74–80.
Arber DA, Brunning RD, Le Beau MM, et al. Acute myeloid leukaemia with recurrent genetic abnormalities. In: Swerdlow SH, Campo E, Harris NL, et al., editors. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon: International Agency for Research on Cancer (IARC); 2008. p. 110–23.
Balgobind BV, Raimondi SC, Harbott J, et al. Novel prognostic subgroups in childhood 11q23/MLL-rearranged acute myeloid leukemia: results of an international retrospective study. Blood. 2009;114:2489–96.
Balgobind BV, Zwaan CM, Pieters R, Van den Heuvel-Eibrink MM. The heterogeneity of pediatric MLL-rearranged acute myeloid leukemia. Leukemia. 2011;25:1239–48.
Becton D, Dahl GV, Ravindranath Y, et al. Randomized use of cyclosporin A (CsA) to modulate P-glycoprotein in children with AML in remission: Pediatric Oncology Group Study 9421. Blood. 2006;107:1315–24.
Boissel N, Leroy H, Brethon B, et al. Incidence and prognostic impact of c-Kit, FLT3, and Ras gene mutations in core binding factor acute myeloid leukemia (CBF-AML). Leukemia. 2006;20:965–70.
Brown P, McIntyre E, Rau R, et al. The incidence and clinical significance of nucleophosmin mutations in childhood AML. Blood. 2007;110:979–85.
Carow CE, Levenstein M, Kaufmann SH, et al. Expression of the hematopoietic growth factor receptor FLT3 (STK-lmk2) in human leukemias. Blood. 1996;87:1089–96.

Cazzaniga G, Dell'Oro MG, Mecucci C, et al. Nucleophosmin mutations in childhood acute myelogenous leukemia with normal karyotype. Blood. 2005;106:1419–22.
Colombo E, Marine JC, Danovi D, et al. Nucleophosmin regulates the stability and transcriptional activity of p53. Nat Cell Biol. 2002;4:529–33.
Cordell JL, Pulford KA, Bigerna B, et al. Detection of normal and chimeric nucleophosmin in human cells. Blood. 1999;93:632–42.
Creutzig U, Reinhardt D. Current controversies: which patients with acute myeloid leukaemia should receive a bone marrow transplantation?–A European view. Br J Haematol. 2002; 118:365–77.
Creutzig U, Zimmermann M, Ritter J, et al. Definition of a standard-risk group in children with AML. Br J Haematol. 1999;104:630–9.
Creutzig U, Ritter J, Zimmermann M, et al. Improved treatment results in high-risk pediatric acute myeloid leukemia patients after intensification with high-dose cytarabine and mitoxantrone: results of Study Acute Myeloid Leukemia-Berlin-Frankfurt-Münster 93. J Clin Oncol. 2001;19:2705–13.
Creutzig U, Zimmermann M, Ritter J, et al. Treatment strategies and long-term results in paediatric patients treated in four consecutive AML-BFM trials. Leukemia. 2005a;19:2030–42.
Creutzig U, Reinhardt D, Diekamp S, Dworzak M, Stary J, Zimmermann M. AML patients with Down syndrome have a high cure rate with AML-BFM therapy with reduced dose intensity. Leukemia. 2005b;19:1355–60.
Creutzig U, Zimmermann M, Lehrnbecher T, et al. Less toxicity by optimizing chemotherapy, but not by addition of granulocyte colony-stimulating factor in children and adolescents with acute myeloid leukemia: results of AML-BFM 98. J Clin Oncol. 2006;24:4499–506.
Creutzig U, Zimmermann M, Dworzak M, et al. Favourable outcome of patients with childhood acute promyelocytic leukaemia after treatment with reduced cumulative anthracycline doses. Report from AML-BFM Study Group. Br J Haematol. 2010;149:399–409.
Dame C, Kirschner KM, Bartz KV, et al. Wilms tumor suppressor, Wt1, is a transcriptional activator of the erythropoietin gene. Blood. 2006;107:4282–90.
Davies SM, Robison LL, Buckley JD, et al. Glutathione S-transferase polymorphisms and outcome of chemotherapy in childhood acute myeloid leukemia. J Clin Oncol. 2001;19:1279–87.
Douer D, Preston-Martin S, Chang E, Nichols PW, Watkins KJ, Levine AM. High frequency of acute promyelocytic leukemia among Latinos with acute myeloid leukemia. Blood. 1996;87:308–13.
Erickson P, Gao J, Chang KS, et al. Identification of breakpoints in t(8;21) acute myelogenous leukemia and isolation of a fusion transcript, AML1/ETO, with similarity to Drosophila segmentation gene, runt. Blood. 1992;80:1825–31.
Estey E, Plunkett W, Dixon D, Keating M, et al. Variables predicting response to high dose cytosine arabinoside therapy in patients with refractory acute leukemia. Leukemia. 1987;1:580–3.
Fenaux P, Chevret S, Guerci A, et al. Long-term follow-up confirms the benefit of all-trans retinoic acid in acute promyelocytic leukemia. European APL group. Leukemia. 2000;14:1371–7.
George B, Mathews V, Poonkuzhali B, et al. Treatment of children with newly diagnosed acute promyelocytic leukemia with arsenic trioxide: a single center experience. Leukemia. 2004;18: 1587–90.
Gibson BES, Wheatley K, Hann IM, et al. Treatment strategy and long-term results in paediatric patients treated in consecutive UK AML trials. Leukemia. 2005;19:2130–8.
Gorman MF, Ji L, Ko RH, et al. Outcome for children treated for relapsed or refractory acute myelogenous leukemia (rAML): A Therapeutic Advances in Childhood Leukemia (TACL) Consortium study. Pediatr Blood Cancer. 2010;55:421–9.
Gregory J, Kim H, Alonzo T, et al. Treatment of children with acute promyelocytic leukemia: results of the first North American Intergroup trial INT0129. Pediatr Blood Cancer. 2009;53:1005–10.
Gurney JG, Severson RK, Davis S, Robison LL. Incidence of cancer in children in the United States. Sex-, race-, and 1-year age-specific rates by histologic type. Cancer. 1995;75: 2186–95.

Hann IM, Stevens RF, Goldstone AH, et al. Randomized comparison of DAT versus ADE as induction chemotherapy in children and younger adults with acute myeloid leukemia. Results of the Medical Research Council's 10th AML trial (MRC AML10). Adult and Childhood Leukaemia Working Parties of the Medical Research Council. Blood. 1997;89:2311–8.

Harrison C, Hills R, Moorman AV, et al. Cytogenetics of childhood acute myeloid leukemia: United Kingdom Medical Research Council treatment trials AML 10 and 12. J Clin Oncol. 2010;28:2674–81.

Hernandez P, Milanes MT, Svarch E, et al. High relative proportion of acute promyelocytic leukemia in children: experience of a multicenter study in Cuba. Leuk Res. 2000;24:739–40.

Ho PA, Alonzo TA, Gerbing RB, et al. Prevalence and prognostic implications of *CEBPA* mutations in pediatric acute myeloid leukemia (AML): a report from the Children's Oncology Group. Blood. 2009;113:6558–66.

Ho PA, Zeng R, Alonzo TA, et al. Prevalence and prognostic implications of WT1 mutations in pediatric acute myeloid leukemia (AML): a report from the Children's Oncology Group. Blood. 2010;116:702–10.

Hollink IH, van den Heuvel-Eibrink MM, Zimmermann M, et al. Clinical relevance of Wilms tumor 1 gene mutations in childhood acute myeloid leukemia. Blood. 2009a;113:5951–60.

Hollink IH, Zwaan CM, Zimmermann M, et al. Favorable prognostic impact of NPM1 gene mutations in childhood acute myeloid leukemia, with emphasis on cytogenetically normal AML. Leukemia. 2009b;23:262–70.

Horan JT, Alonzo TA, Lyman GH, et al. Impact of disease risk on efficacy of matched related bone marrow transplantation for pediatric acute myeloid leukemia: the Children's Oncology Group. J Clin Oncol. 2008;26:5797–801.

Hunte BE, Hudak S, Campbell D, et al. flk2/ flt3 ligand is a potent cofactor for the growth of primitive B cell progenitors. J Immunol. 1995;156:489–96.

Kaspers GJ, Creutzig U. Pediatric acute myeloid leukemia: international progress and future directions. Leukemia. 2005;19:2025–9.

Kaspers GJ, Zimmermann M, Reinhardt D, et al. Improved outcome in pediatric relapsed acute myeloid leukemia: results of a randomized trial on liposomal daunorubicin by the International BFM Study Group. J Clin Oncol. 2013;31:599–607.

Kelly MJ, Horan JT, Alonzo TA, et al. Comparable survival for pediatric acute myeloid leukemia with poor-risk cytogenetics following chemotherapy, matched related donor, or unrelated donor transplantation. Pediatr Blood Cancer. 2014b;61:269–75.

Klampfer L, Zhang J, Zelenetz AO, Uchida H, Nimer SD. The AML1/ETO fusion protein activates transcription of BCL-2. Proc Natl Acad Sci U S A. 1996;93:14059–64.

Lam K, Muselman A, Du R, et al. *Hmga2* is a direct target gene of RUNX1 and regulates expansion of myeloid progenitors in mice. Blood. 2014;124:2203–12.

Lange BJ, Kobrinsky N, Barnard DR, et al. Distinctive demography, biology, and outcome of acute myeloid leukemia and myelodysplastic syndrome in children with Down syndrome: Children's Cancer Group Studies 2861 and 2891. Blood. 1998;91:608–15.

Lange BJ, Smith FO, Feusner J, et al. Outcomes in CCG-2961, a children's oncology group phase 3 trial for untreated pediatric acute myeloid leukemia: a report from the children's oncology group. Blood. 2008;111:1044–53.

Langebrake C, Creutzig U, Dworzak M, et al. Residual disease monitoring in childhood acute myeloid leukemia by multiparameter flow cytometry: the MRD-AML-BFM Study Group. J Clin Oncol. 2006;24:3686–92.

Lekstrom-Himes J, Xanthopoulos KG. Biological role of the CCAAT/enhancer-binding protein family of transcription factors. J Biol Chem. 1998;273:28545–8.

Lie SO, Jonmundsson G, Mellander L, et al. A population-based study of 272 children with acute myeloid leukaemia treated on two consecutive protocols with different intensity: best outcome in girls, infants, and children with Down's syndrome. Nordic Soc Paediatr Haematol Oncol (NOPHO) Br J Haematol. 1996;94:82–8.

Lie SO, Abrahamsson J, Clausen N, et al. Long-term results in children with AML: NOPHO-AML Study Group – report of three consecutive trials. Leukemia. 2005;19:2090–100.

Liu P, Tarlé SA, Hajra A, Claxton DF, Marlton P, Freedman M, Siciliano MJ, Collins FS. Fusion between transcription factor CBF beta/PEBP2 beta and a myosin heavy chain in acute myeloid leukemia. Science. 1993;261:1041–4.

Liu PP, Hajra A, Wijrnenga C, Collins FS. Molecular pathogenesis of the chromosome 16 inversion in the M4Eo subtype of acute myeloid leukemia. Blood. 1995;85:2289–302.

Lyman SD, James L, Bos TV, et al. Molecular cloning of a ligand for the flt3/flk-2 tyrosine kinase receptor: a proliferative factor for primitive hematopoietic cells. Cell. 1993;75:1157–67.

Ma Z, Morris SW, Valentine V. Fusion of two novel genes, RBM15 and MKL1, in the t(1;22) (p13;q13) of acute megakaryoblastic leukemia. Nat Genet. 2001;28:220–1.

Malta Corea A, Pacheco Espinoza C, Cantu Rajnolid A, et al. Childhood acute promyelocytic leukemia in Nicaragua. Ann Oncol. 1993;4:892–4.

Martinez-Climent JA, Lane NJ, Rubin CM, et al. Clinical and prognostic significance of chromosomal abnormalities in childhood acute myeloid leukemia de novo. Leukemia. 1995;9:95–101.

Medina-Sanson A, Ramírez-Pacheco A, Moreno-Guerrero SS, et al. Role of genetic polymorphisms of deoxycytidine kinase and cytidine deaminase to predict risk of death in children with acute myeloid leukemia. BioMed Res Int. 2015(2015), Article ID 309491, 10 p.

Meshinchi S, Woods WG, Stirewalt DL, et al. Prevalence and prognostic significance of Flt3 internal tandem duplication in pediatric acute myeloid leukemia. Blood. 2001;97:89–94.

Meshinchi S, Alonzo TA, Stirewalt DL, et al. Clinical implications of FLT3 mutations in pediatric AML. Blood. 2006;108:3654–61.

Moore AS, Kearns PR, Knapper S, et al. Novel therapies for children with acute myeloid leukaemia. Leukemia. 2013;27:1451–60.

Niewerth D, Creutzig U, Bierings MB, Kaspers GJ. A review on allogeneic stem cell transplantation for newly diagnosed pediatric acute myeloid leukemia. Blood. 2010;116:2205–14.

Ortega JJ, Madero L, Martin G, et al. Treatment with all-trans retinoic acid and anthracycline monochemotherapy for children with acute promyelocytic leukemia: a multicenter study by the PETHEMA group. J Clin Oncol. 2005;23:7632–40.

Peterson LF, Yan M, Zhang DE. The p21Waf1 pathway is involved in blocking leukemogenesis by the t(8;21) fusion protein AML1-ETO. Blood. 2007;109:4392–8.

Pui CH, Dahl GV, Kalwinsky DK, et al. Central nervous system leukemia in children with acute nonlymphoblastic leukemia. Blood. 1985;66:1062–7.

Pui CH, Carroll WL, Meshinchi S, Arceci RJ. Biology, risk stratification, and therapy of pediatric acute leukemias: an update. J Clin Oncol. 2011;29:551–65.

Raimondi SC, Chang MN, Ravindranath Y, et al. Chromosomal abnormalities in 478 children with acute myeloid leukemia: clinical characteristics and treatment outcome in a cooperative pediatric oncology group study-POG 8821. Blood. 1999;94:3707–16.

Ravindranath Y, Abella E, Krischer JP, et al. Acute myeloid leukemia (AML) in Down's syndrome is highly responsive to chemotherapy: experience on Pediatric Oncology Group AML Study 8498. Blood. 1992;80:2210–4.

Rubnitz JE, Raimondi SC, Tong X, et al. Favorable impact of the t(9;11) in childhood acute myeloid leukemia. J Clin Oncol. 2002;20:2302–9.

Rubnitz JE, Razzouk BI, Lensing S, et al. Prognostic factors and outcome of recurrence in childhood acute myeloid leukemia. Cancer. 2007;109:157–63.

Rubnitz JE, Inaba H, Dahl G, et al. Minimal residual disease-directed therapy for childhood acute myeloid leukemia: results of the AML02 multicenter trial. Lancet Oncol. 2010;11:543–52.

Rubnitz JE, Pounds S, Cao X, et al. Treatment outcome in older patients with childhood acute myeloid leukemia. Cancer. 2012;118:6253–9.

Sano H, Shimada A, Tabuchi K, et al. WT1 mutation in pediatric patients with acute myeloid leukemia: a report from the Japanese Childhood AML Cooperative Study Group. Int J Hematol. 2013;98:437–45.

Sanz MA, Montesinos P, Rayon C, et al. Risk-adapted treatment of acute promyelocytic leukemia based on all-trans retinoic acid and anthracycline with addition of cytarabine in consolidation therapy for high risk patients: further improvements in treatment outcome. Blood. 2010;115: 5137–46.

Smith FO, Alonzo TA, Gerbing RB, et al. Long-term results of children with acute myeloid leukemia: a report of three consecutive Phase III trials by the Children's Cancer Group: CCG 251, CCG 213 and CCG 2891. Leukemia. 2005;19:2054–62.

Sorensen PH, Chen CS, Smith FO, et al. Molecular rearrangements of the MLL gene are present in most cases of infant acute myeloid leukemia and are strongly correlated with monocytic or myelomonocytic phenotypes. J Clin Invest. 1994;93:429–37.

Staffas A, Kanduri M, Hovland R, et al. Presence of FLT3-ITD and high BAALC expression are independent prognostic markers in childhood acute myeloid leukemia. Blood. 2011; 118:5905–13.

Steinbach D, Bader P, Willasch A, et al. Prospective validation of a new method of monitoring minimal residual disease in childhood acute myelogenous leukemia. Clin Cancer Res. 2015;21:1353–9.

Stevens RF, Hann IM, Wheatley K, et al. Marked improvements in outcome with chemotherapy alone in paediatric acute myeloid leukemia: results of the United Kingdom Medical Research Council's 10th AML trial. MRC Childhood Leukaemia Working Party. Br J Haematol. 1998;101:130–40.

Tallman MS. The expanding role of arsenic in acute promyelocytic leukemia. Semin Hematol. 2008;45:S25–9.

Testi AM, Biondi A, Lo Coco F, et al. GIMEMA-AIEOPAIDA protocol for the treatment of newly diagnosed acute promyelocytic leukemia (APL) in children. Blood. 2005;106:447–53.

von Neuhoff C, Reinhardt D, Sander B, et al. Prognostic impact of specific chromosomal aberrations in a large group of pediatric patients with acute myeloid leukemia treated uniformly according to Trial AML-BFM 98. J Clin Oncol. 2010;28:2682–9.

Webb DK, Wheatley K, Harrison G, et al. Outcome for children with relapsed acute myeloid leukaemia following initial therapy in the Medical Research Council (MRC) AML 10 trial. MRC Childhood Leukaemia Working Party. Leukemia. 1999;13:25–31.

Webb DK, Harrison G, Stevens RF, et al. Relationships between age at diagnosis, clinical features, and outcome of therapy in children treated in the Medical Research Council AML 10 and 12 trials for acute myeloid leukemia. Blood. 2001;98:1714–20.

Wells RJ, Adams MT, Alonzo TA, et al. Mitoxantrone and cytarabine induction, high-dose cytarabine, and etoposide intensification for pediatric patients with relapsed or refractory acute myeloid leukemia: Children's Cancer Group Study 2951. J Clin Oncol. 2003;21:2940–7.

Wheatley K, Burnett AK, Goldstone AH, et al. A simple, robust, validated and highly predictive index for the determination of risk-directed therapy in acute myeloid leukaemia derived from the MRC AML 10 trial. United Kingdom Medical Research Council's Adult and Childhood Leukaemia Working Parties. Br J Hematol. 1999;107:69–79.

Woods WG, Neudorf S, Gold S, et al. A comparison of allogeneic bone marrow transplantation, autologous bone marrow transplantation, and aggressive chemotherapy in children with acute myeloid leukemia in remission. Blood. 2001;97:56–62.

Xu Y, McKenna RW, Wilson KS, et al. Immunophenotypic identification of acute myeloid leukemia with monocytic differentiation. Leukemia. 2006;20:1321–4.

Zhou J, Zhang Y, Li J, et al. Single-agent arsenic trioxide in the treatment of children with newly diagnosed acute promyelocytic leukemia. Blood. 2010;115:1697–702.

Chapter 2
Etiology and Prevention of Acute Leukemias in Children

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Abstract Acute leukemia (AL) is the most common type of cancer in children under 15 years of age and represents one of the leading causes of mortality among children worldwide. Despite advancements in the knowledge of the biology and treatment of AL, the etiology remains unresolved. A small number of risk factors have been reported as established for the development of this disease, but they explain less than 10 % of cases, leaving 90 % of cases without an identified causation.

Case-control studies have been the main research designs used to investigate the causes of AL in children. The importance of case-control studies rests on the assumption that data on individuals is essential for gaining an understanding of the environmental causes of childhood leukemia and adds great value to the genetic research.

Genetic or environmental factors alone may not be responsible for causing childhood AL. Rather, it is thought that an interaction between genetic susceptibility and exposures to certain environmental factors in a specific time window can contribute to the development of this disease.

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Identifying the causes of childhood AL would lead to the establishment of effective preventive measures in children who are at high risk of developing this disease, reducing incidence and mortality rates, the costs of medical care, and other consequences associated with childhood leukemia. Therefore, we need to implement a new framework for the etiology of AL. We believe that solving key elements of this puzzle can lead to prevention of the development of AL in children.

Keywords Leukemia • Children • Epidemiology • Etiology • Prevention

Introduction

Acute leukemia (AL) is the most common type of cancer in children under 15 years of age, representing 34 % of all childhood cancers. AL is one of the leading causes of mortality among children worldwide (Siegel et al. 2012). Acute lymphoblastic leukemia (ALL) is the most frequent subtype of AL (80–85 % of cases), followed by acute myeloblastic leukemia (AML; 15–20 % of cases) (Greenlee et al. 2000; Gurney et al. 1995a; Margolin et al. 2006). AL incidence rates vary among countries, the highest being reported for Costa Rica, Hispanics in Los Angeles, and Mexico (Parkin et al. 1998; Pérez-Saldivar et al. 2011).

Despite advancements in the knowledge of the biology and treatment for this disease, the identification of the etiology of AL has not been adequate (Carroll et al. 2003; Ludwig et al. 2003; Rowley 1999). Known risk factors explain less than 10 % of cases (Inaba et al. 2013; Buffler et al. 2005), with the remaining 90 % having an unresolved causation.

Etiology of AL in children is considered to be multicausal, and many studies have been conducted to date to investigate whether environmental, genetic, and other potential risk factors are associated with its development. It has been pointed out that childhood AL seems to result from the interaction between individual genetic susceptibility and exposure to environmental carcinogenic agents within a specific time window (Inaba et al. 2013; Buffler et al. 2005).

The purpose of this chapter is to summarize the current scientific evidence on the etiology and prevention of AL in children.

Research Designs Used to Investigate the Causes of AL in Children

Case-control studies have been the main research design used to investigate the causes of childhood AL. The importance of case-control studies rests on the assumption that data on individuals are essential for gaining an understanding

of the environmental causes of childhood leukemia and add great value to the genetic research.

Observational studies have some limitations with regard to scientific strength when searching for the causes of a disease because they cannot deal definitively with bias, chance, and confounding (Fletcher et al. 2014). One of the most recent approaches to investigate the causes of childhood leukemia takes into account not only the investigation of environmental or genetic risk factors independently but also gene-environmental interactions through the pooling of previously collected data from individual observational studies to improve statistical power (Metayer et al. 2013a).

Risk Factors for the Development of AL in Children

Established Risk Factors

After years of exhaustive investigation on the etiology of childhood AL, a small number of risk factors have been reported as established for the development of this disease (Buffler et al. 2005). Among the established risk factors are ionizing radiation, some genetic syndromes, and some drugs used in chemotherapy.

Ionizing Radiation

Ionizing radiation has properties of an initiator more than a promoter for inducing DNA alterations in blood lymphocytes, subsequently provoking malignant cell transformation (Committee to Assess Health Risks from Exposure to Low Levels of Ionizing Radiation 2006; Bhatti et al. 2008; Harbron 2012). Stewart and Kneale reported for first time a high incidence of leukemia in children whose mothers had been exposed to X-rays during the prenatal period. Moreover, a dose-response relationship between X-ray exposure and childhood AL was observed (Stewart and Kneale 1970; Stewart et al. 1956). Risk of childhood leukemia after exposure to in utero radiation has been estimated as 1.5 times above baseline (Miller 1969). Similar results have been reproduced in multiple studies (Brent 2014), contributing to modifications in medical practice regarding radiographic examination of women during pregnancy. Recommendations include that all women of reproductive age should be evaluated for the possibility of pregnancy in cases requiring radiological examinations and implementation of ultrasonography as a safe and first-line diagnostic resource during pregnancy instead of radiography (American College of Radiology 2008).

On the other hand, further investigation is required to confirm the role played by postnatal exposure to diagnostic X-rays as a risk factor in the development of childhood AL (Infante-Rivard 2003; Chokkalingam et al. 2011).

Childhood Leukemia Predisposition Syndromes

Childhood leukemia predisposition syndromes are Down syndrome (DS), neurofibromatosis type 1 (NF1), Fanconi anemia (FA), ataxia-telangiectasia, Nijmegen breakage syndrome, Bloom syndrome (BS), Noonan syndrome (NS), Diamond-Blackfan anemia, Shwachman-Diamond anemia, and dyskeratosis congenita (Seif 2011). Genetic abnormalities observed in these patients have permitted a better understanding of leukemogenesis (Izraeli 2003). Here we address some of the most studied childhood leukemia predisposition syndromes.

Down Syndrome

DS children are highly susceptible to develop leukemia (10–20 times) in comparison with the general population. Acute myeloid leukemia-M7 (AML-M7) is the most common subtype of leukemia seen in DS children and is associated with high chemosensitivity and toxicity in these patients.

During the neonatal period, about 10 % of DS children may present a transient abnormal myelopoiesis (Hasle 2001), considered as one of the most important candidate preleukemic syndromes (approximately 25 % will develop AML during childhood) to be exhaustively studied, as it could allow the identification of the mechanisms associated with the progression from a preleukemic state to childhood leukemia.

On the other hand, DS children are also affected with ALL, a subtype related to a poor prognosis in these patients (Hitzler and Zipursky 2005; Carroll and Raetz 2012; Patrick et al. 2014).

Leukemogenic mechanisms have been reported in 18–28 % of DS-AL patients (Fonatsch 2010): susceptibility to viral replication, altered DNA repair, chromosome fragility, increased number of copies in *AML1* gene, *GATA1* mutations (related with development of AML), dysregulation in Xp22.33/Yp11.32 region of *CRLF2* gene, and mutations in the Janus kinase (*JAK2*) gene (associated with ALL).

In addition, certain environmental exposures have been associated with a high risk in DS children of developing AL: paternal smoking before pregnancy, paternal alcohol consumption, passive child exposure to tobacco smoke, exposure to magnetic fields, infections requiring hospitalization, and, most recently, asthma (Mezei et al. 2014; Mejía-Aranguré et al. 2003, 2007; Flores-Lujano et al. 2009; Núñez-Enríquez et al. 2013).

Neurofibromatosis Type 1

NF1 is a genetic disorder with an autosomal dominant transmission characterized by the presence of multiple benign neoplasms called neurofibromas, skeletal deformations, and cognitive disorders, among other features. The association between NF1 and childhood acute myeloblastic leukemia was reported for first time by McEvoy and Mann (1971). Other authors have reported a 500-fold increased risk

of developing juvenile myelomonocytic leukemia (JMML) (Stiller et al. 1994) and a higher incidence of ALL in this population (Yohay 2009; Stiller et al. 1994; Zvulunov 1996). The reported alterations in the blasts of NF1 patients are loss of heterozygosity for the *NF1* gene and elevated levels of Ras-GTP (Shannon et al. 1994; Bollag et al. 1996). Moreover, loss of function of neurofibromin promotes uncontrolled cell growth and tumorigenesis in these patients through the activation of the *RAS* proto-oncogene (Yohay 2009). On the other hand, NF1 patients seem to have an increased risk for the development of treatment-related leukemias (Perentesis 2001).

Fanconi Anemia

Fanconi anemia (FA) is an inherited syndrome that frequently begins with manifestations of childhood leukemia. The most frequent leukemia is AML (94 %) in adolescents (Rosenberg et al. 2003; Alter 2003), and patients with FA have a 500-fold increase in risk for the development of AML compared with the general population (Alter et al. 2010; Shimamura and Alter 2010). Genetic abnormalities in these patients include mutations in 17 genes, deletion 7q, gain of 13 q, deletion of 20q, gain of 1q, monosomy 7, and gain of 3q (Schneider et al. 2015; Moldovan and D'Andrea 2009; Vaz et al. 2010; Butturini et al. 1994). The mechanisms reported as associated with predisposition to leukemia in children are disruptions of regular DNA double-strand break (DSB) repair by homologous recombination, which may lead to misrepairs and genetic instability (Popp and Bohlander 2010).

Bloom Syndrome

The most affected populations with BS are the Ashkenazi Jewish from Eastern Europe and Israel, accounting for approximately one-third of BS cases (Seif 2011). These patients frequently present immunodeficiency, infertility, and short stature. The BS-mutated gene is called *BLM*, located at 15q26.1 (German 1993; Straughen et al. 1996), which codes for the DNA repair enzyme RecQL3 helicase, resulting in genomic instability that may progress to leukemia. BS children and adolescents are at high risk of developing both types of leukemia (ALL and AML) that may result in death (Sanz and German 2014). Recently, a higher frequency of monosomy 7 and deletions of the long arm of chromosome 7 have been observed in patients with BS and myeloid neoplasms compared with non-BS patients (Poppe et al. 2001).

Noonan Syndrome

Association between NS and JMML has been most frequently reported during the first years of life. NS patients have somatic mutations in the *PTPN11*, *NRAS*, *KRAS*, *SHOC2*, *NF1*, *SOS1*, *RAF1*, and *CBL* genes encoding components of the

RAS/MAPK pathway (Tartaglia et al. 2010; Hyakuna et al. 2015), the mutations of *PTPN11* gene being the most commonly observed alteration in up to 40 % of cases with NS and JMML (Choong et al. 1999; Kratz et al. 2005; Niihori et al. 2005; Niemeyer et al. 2010). In addition, Thr73Ile and p.Asp61His specific mutations in *PTPN11* gene have been linked with a good and poor prognosis of the JMML in NS patients, respectively (Strullu et al. 2014).

Chemotherapy-Related Acute Myeloid Leukemia in Children (t-AML)

After a child is diagnosed with cancer the initial treatment is based on chemotherapy agents. It is noteworthy that chemotherapy carries some adverse effects in children's health, one of the most serious being the development of secondary leukemia as a result of mutations generated by chemotherapy (Pizzo 2011). Nowadays, this complication is more frequently observed than in the past because the survival rates for children with cancer have substantially improved.

Childhood AML is the most frequent subtype of leukemia following chemotherapy (Hoffmann et al. 1995). The development of t-AML depends on exposure to specific chemotherapeutic agents (alkylating, epipodophyllotoxins, and DNA-topoisomerase II inhibitors) and dose- and time-related characteristics (time of exposure, cumulative dose, and the dose intensity) of the previously used chemotherapeutic agent (Ratain et al. 1987; Levine and Bloomfield 1986; Pedersen-Bjergaard et al. 1998).

In general, the cytogenetic abnormalities and gene mutations that have been reported for children with de novo AML and with t-AML are the same; however, some differences in specific mutations between these two groups (t-AML patients have a higher frequency of *p53* point mutations and lower frequency of *FLT3* and *NPM1* mutations compared with de novo AML patients) have been reported (Grimwade et al. 1998; Slovak et al. 2000; Byrd et al. 2002; Sanderson et al. 2006; Greenberg et al. 1997). Notably, the prognosis of t-AML is directly related to the presence or absence of certain cytogenetic features (Schoch et al. 2004; Joannides and Grimwade 2010).

Possible Risk Factors

The investigation on the causes of childhood leukemia has allowed identification of some possible environmental and/or genetic risk factors for the development of AL in children. Moreover, different periods of life have been explored to investigate the role played by environmental and genetic factors in the development of AL in children (Fig. 2.1).

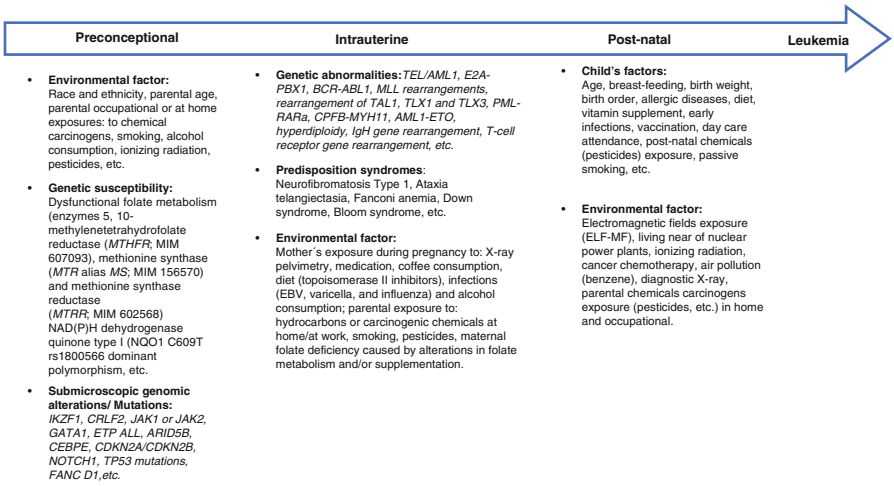

Fig. 2.1 Periods of life explored to investigate the role played by genetic and environmental factors in the development of acute leukemia in children

Environmental Factors

Extremely Low-Frequency Magnetic Fields

The International Agency of Research on Cancer (IARC) classified extremely low-frequency magnetic fields (ELF-MF) as "possible carcinogens to humans" (Gobba et al. 2009). The role played by ELF-MF exposure on the etiology of AL in children has been studied for many years (Mezei et al. 2008; Schüz et al. 2007); however, imprecise estimations have been observed in various meta-analyses (Kheifets et al. 2010) possibly due to measurement errors, selection bias, and confounding factors.

The measurement of the ELF-MF exposure has been different across studies. The proximity to power lines, average of historical current supply data, and wire codes have been used as proxy variables to evaluate the ELF-MF exposure. In this regard, it has been pointed out that the assessment of the individual's ELF-MF exposure through proxy variables may lead to measurement errors (Rothman 1993; Greenland et al. 2000). Alternatively, direct magnetic field measurements (24-h child bedroom and spot measurements at the front door of the residence) by using gaussmeters have been recently introduced (Brain et al. 2003).

Selection bias in studies on ELF-MF exposure and AL are possibly related to a low participation rate of controls with low socioeconomic status (SES) (related to high levels of ELF-MF exposure) in comparison with participation rate of cases (Schüz et al. 2007) and/or because of a lower participation rate of cases compared

with controls (Calvente et al. 2010; Hatch et al. 2000; Kheifets and Shimkhada 2005). Some more recent studies searching for the association between ELF-MF exposure and childhood AL have been designed to avoid selection and differential misclassification biases (Sermage-Faure et al. 2013).

Potential confounders that have been described for the association between ELF-MF are SES, residential mobility, type of residence, degree of social contact, traffic density, and the exposure to electrical contact currents (Jones et al. 1993; Hatch et al. 2000; Gurney et al. 1995b; Feychting et al. 1998; Kheifets et al. 2006, 2010; Does et al. 2011).

Various experimental studies have been conducted until now to evaluate the biological plausibility of the ELF-MF exposure as risk factor for the development of AL in children. However, no effects of ELF-MF have been confirmed either in vitro or in vivo experiments at different levels of exposure (National Research Council (US)/Committee on the Possible Effects of Electromagnetic Fields on Biologic Systems 1997; Lagroye et al. 2011; Swanson and Kheifets 2012).

Allergic Diseases

Associations between allergies and the development of AL in children have been investigated in several epidemiologic studies, but results are not conclusive, as different studies have revealed that allergies are risk factors or protective factors (Schüz et al. 2003; Söderberg et al. 2006; Rosenbaum et al. 2005; Hughes et al. 2007; Chang et al. 2009; Rudant et al. 2010; Spector et al. 2004). Importantly, a case-control study conducted by the Mexican Inter-institutional Group for the Identification of the Causes of Childhood Leukemia in DS children (a population with an intrinsic immune dysregulation) (Kusters et al. 2009) reported that asthma was a risk factor for AL, whereas skin allergy was a protective factor (Núñez-Enríquez et al. 2013). Contrasting results may reflect the heterogeneity of AL and allergies; they seem to share common biological and immune mechanisms.

Three hypotheses have been proposed to explain positive and negative associations between allergic diseases and AL in children. To explain the role of allergies as a risk factor for cancer, the "antigenic stimulation hypothesis" has been proposed, which states that the chronic stimulation of the immune system will provoke randomly occurring pro-oncogenic mutations in actively dividing cells (Söderberg et al. 2004). Two hypotheses put forth to explain inverse allergy-AL association are the "immune-surveillance hypothesis," which suggests that allergic diseases enhance the immune system's ability to detect and eliminate neoplastic cells, and the "adrenal hypothesis" (Schmiegelow et al. 2008), which proposes that infections produce changes in the hypothalamus-pituitary-adrenal axis and subsequently an elevation in plasmatic cortisol, provoking the elimination of leukemic and preleukemic cells. This mechanism would be possible in allergic conditions because the drugs commonly used to treat allergies include corticosteroids (Allergy UK British Allergy Foundation 2012), which could

provide the same protective effect against the development of AL provided by early infections.

Important limitations of studies on the association between allergies and AL have been the study design, exposure data source, and latency period (Linabery et al. 2010). Moreover, the biological plausibility of this association remains unclear.

Infections

The role of infection in the etiology of leukemia was revealed for the first time more than 90 years ago through a series of cases reported by Gordon Ward in 1917. These cases included 1,457 children with AL (Ward 1917).

One of the most relevant researches in this topic was conducted by Kinlen et al., who found a relationship between a high incidence of AL and infectious diseases in children who lived near rural areas. Kinlen's findings resulted in the emergence of a hypothesis proposing that leukemia originates from exposure to an infectious agent in a mixed population (rural-urban), causing an abnormal immune response that increases the risk of developing the disease (Kinlen 1995).

Afterward, Greaves et al. using biological and epidemiological data on AL, suggested the hypothesis of late infection, which comprises two stages. The first stage starts with a mutation in utero at the same time that precursor B cells are developing, and a second stage takes place during the postnatal period, in which the cell that underwent a mutation would be exposed to a common late infection in the first year of the child's life, resulting in the development of AL (Greaves 1988). Moreover, a third hypothesis was then proposed by Smith et al., who considered that AL originates from in utero exposure to infection (Smith 1997).

Case-control studies represent the main type of epidemiological studies conducted so far on the association between infection and AL in children (McNally and Eden 2004). The results of these studies appear to suggest a lower risk of developing AL among children who were exposed to early infections compared with those who were not exposed. No such association, however, has been reported by other authors; therefore, infections that occur during the first year of life are still considered to be a controversial exposure factor (Flores-Lujano et al. 2013).

In addition, different types of infections have been evaluated in epidemiologic studies. The most frequently studied are respiratory tract infections, gastroenteritis, and those caused by specific infectious agents (e.g., retroviruses). However, there is no evidence to date from experimental studies of a specific infectious agent definitively linked with childhood leukemia (Morales-Sánchez et al. 2013).

Over time, some indicators have been used to quantify the exposure to infection. These indicators, designated as "proxies," include socioeconomic status, surgical history, allergic diseases, immunizations, attendance at daycare, breastfeeding, neonatal infections, and prenatal history, among others. A better exposure assessment has been recommended to achieve better epidemiological evidence regarding infection and AL development (Urayama et al. 2010).

Parental Exposures

It is thought that parental exposures may be relevant as risk factors for the development of AL during the preconception period, when germ cells may be damaged, during pregnancy, and postnatally, when carcinogenic agents may provoke mutations (Infante-Rivard et al. 1991; Monge et al. 2007).

Father's Occupational Exposure

The role played by the father's occupational exposure to carcinogenic agents in the risk of developing ALL in his offspring is controversial (Keegan et al. 2012). Pertinent studies had the following weaknesses: (1) information about occupational exposure was obtained from secondary sources or by using the occupation or the industrial branch as an indicator of the exposure; (2) the interviewed workers either had ignored the substances to which they were exposed or could not remember their past exposures; and (3) when exposure was characterized, only the duration of exposure was taken into account, with no consideration given either to the frequency or intensity of exposure or to other variables such as the use of personal protective equipment (Van Maele-Fabry et al. 2010; Keegan et al. 2012; Savitz and Chen 1990; O'Leary et al. 1991; Colt and Blair 1998). This has resulted in a misclassification of the exposure.

Furthermore, in epidemiologic studies, when attempting to prove the occupational effect of a specific position or of exposure to a particular substance, the sample sizes have been unsatisfactory (Savitz and Chen 1990; O'Leary et al. 1991; Colt and Blair 1998). These are difficult problems to solve, because occupations and exposures to substances associated with childhood cancer are infrequently found in the general population; therefore, the risks obtained have been inconsistent and inaccurate (Annegers and Johnson 1992). Ward and colleagues (2003) recommended one way to increase accuracy in this type of studies; they pointed out that it is better to conduct studies with large sample sizes when studying specific substances as risk factors, as these types of exposure are very rare among the general population (Ward et al. 2003). In addition, taking into account the very low frequency of childhood ALL makes it more difficult to investigate associations with rare exposures because it implies a larger sample size (Mejía-Arangure 2013).

Genetic Susceptibility and Gene-Environment Interaction

Recently, it has been reported that genetic factors or environmental factors alone may not be responsible for causing leukemia. Rather, it is thought that an interaction between genetic susceptibility and exposures to certain environmental factors can contribute to the development of this disease in children (Table 2.1).

Genetic susceptibility refers to inherited factors that modulate disease risk, either via the factors' main effects or, more likely, via the interaction with other inherited

factors (gene-gene interactions) or exogenous exposures such as chemicals, dietary factors, and infectious agents (gene-environment interactions) (Chokkalingam and Buffler 2008). Notably, there is evidence of prenatal molecular damage (chromosomal alterations and mutations) in the lymphoid or myeloid progenitors of children with AL (Greaves 2003; Collins-Underwood and Mullighan 2011). Moreover, AL in children has been associated with the presence of various genetic polymorphisms altering the mechanism of the genes that encode enzymes involved in the xenobiotic metabolism (cytochrome P450 [CYP450], NAD(P)H quinone oxidoreductase I [NPQO1], myeloperoxidase [MPO], glutathione S-transferase [GST], and N-acetyltransferase [NAT]) and the membrane transport multidrug resistance [MDR1] gene.

The cancer susceptibility genes belong to one of three classes: (1) gatekeepers, (2) caretakers, and (3) landscapers (Kinzler and Vogelstein 1998). Gatekeeper genes regulate growth and differentiation pathways of the cell through oncogenes and tumor-suppressor genes. These gatekeeper genes either stop the cell from proliferating by repairing damage to the DNA or eliminate the cell via programmed cell death (apoptosis) (Kotnis et al. 2005). In addition, they have the capacity to maintain the integrity of the genome preventing DNA damage from carcinogens through two sets of enzymes: (1) enzymes that detoxify endogenous and exogenous carcinogens, called xenobiotic-metabolizing enzymes, and (2) enzymes that repair damage in the DNA from the carcinogens. Finally, landscaper genes encode gene products that control the microenvironment in which cells grow (Kinzler and Vogelstein 1998).

Xenobiotic Metabolism

Our bodies have evolved host metabolic enzymes and other protective enzymes to protect us against the deleterious effects of carcinogens present in both the diet and the environment. The complete metabolism of xenobiotic comprises two enzymatic phases: phase I (bioactivation) and phase II (detoxification). This metabolism helps to maintain a critical balance of activation and inactivation of a wide range of chemical exposures of relevance to ALL, including chemical carcinogens, insecticides, drugs, petroleum products, nitrosamines, polycyclic aromatic hydrocarbons, and environmental pollutants (Chokkalingam et al. 2012). The cytochrome P450 (*CYP450*) superfamily of genes involve most of the phase I enzyme system where *CYP1* and *CYP2* have been involved in the area of cancer susceptibility. Studies have shown that *CYPA1m1*, *CYP1A1m2*, and polymorphism of *CYP2E1* are more prevalent and increase a child's susceptibility to leukemia. A study published by Infante-Rivard in 2000 evaluated the contribution of gene-environment interaction, *CYP1Am1* and *CYP1A1m2* polymorphisms, and pesticide exposure to the risk of childhood leukemia. The authors reported significant odds ratios of interaction among carriers of the *CYP1Am1* and *CYP1A1m2* genotypes (Labuda et al. 1999; Infante-Rivard et al. 2000).

Table 2.1 Summary of epidemiologic investigations showing a relationship between environmental risk factors and some genetic alterations in acute leukemia in children

Investigated risk factor	Study design	Measurement instruments	Period of life where factor was studied	Confounders	Association with leukemia	Relationship with some genetic alterations	References
Maternal dietary intake of folate during pregnancy, vegetable, protein sources, fruits, legume food groups, vitamin supplements (as well as iron), fish and seafood, DNA-topoisomerase II inhibitors (flavonoids, caffeine, catechins, etc.)	Case-control	Food frequency questionnaires; telephone/ in-person interviews	Before/during pregnancy	Age, sex, state of residence, parental education, maternal age, birth order, maternal alcohol intake during pregnancy, total energy consumption, proportion of foods consumed as large or extra-large portion size, household income, maternal exposure to indoor insecticides during pregnancy, birth weight, occupation, and tobacco smoking during pregnancy	Protective factor	Methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms *and* NAD(P)H:quinone oxidoreductase 1 (NQ01) *and MLL rearrangements*	Bailey et al. (2012), Kwan et al. (2009), Ross et al. (2005), Petridou et al. (2005), Tower and Spector (2007), Jensen et al. (2004), Strick et al. (2000)
Parental exposure to medications (such as the amphetamines, diet pills, and mind-altering drugs)	Case-control	Telephone interview	Before/during pregnancy	Gender, age, household income, parental race, parental education, parental smoking before or during pregnancy, parental drinking before or during pregnancy	Risk factor	Ras proto-oncogene mutations (H-ras, K-ras, N-ras)	Wen et al. (2002), Shu et al. (2004)

Parental exposure to hydrocarbons (solvents, plastic materials) *and* hydrocarbon-related occupations (in particular, motor-vehicle mechanics, motor-vehicle drivers, machinists, miners, and painters)	Case-control	Telephone interview; data obtained from birth certificates	During/after pregnancy	Family income, parental race, education, and age and the child's age and sex, date of birth, place of residence	Risk factor	Methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms *and* NAD(P)H:quinone oxidoreductase 1 (NQ01)	Shu et al. (2004), Perez-Saldivar et al. (2008), Fabia and Thuy (1974), Hakulinen et al. (1976), Zack et al. (1980), Ernster (1987), Begleiter et al. (1992)
Parental smoking (maternal exposure to second-hand smoke, paternal prenatal exposure combined with postnatal passive smoking, paternal smoking of more than 15 cigarettes per day around the time of the child's conception, and maternal smoking during the later trimesters	Case-control	In-person interview using a standardized questionnaire; self-administered questionnaire	Before/during/after pregnancy	Birth order, birth weight, duration of breastfeeding, parental age at delivery, education, and occupational exposure to benzene, child's age at diagnosis, gender, Hispanic ethnicity, and maternal race (i.e., Black, White, and others), household income, state of residence, and family income	Risk factor	CYP1A1 genetic polymorphisms (CYPA1A1*4 and CYP1A1*2B alleles)	Farioli et al. (2014), Metayer et al. (2013b), Milne et al. (2012), Infante-Rivard et al. (2000)

(continued)

Table 2.1 (continued)

Investigated risk factor	Study design	Measurement instruments	Period of life where factor was studied	Confounders	Association with leukemia	Relationship with some genetic alterations	References
Race and ethnicity	Case-control	Meta-analysis	Before/during/after pregnancy	Socioeconomic status, access to health care, quality of health care, sociocultural factors, adherence to therapy, self-advocacy in preventing toxicities, enrollment on cooperative group trials, differences by ethnic group	Risk factor	KIR, HLA-DP, HLA-Bw4, TCF3-PBX1, ARID5B, PIP4K2A	Savage (2014), Bhatia (2011), Lim et al. (2014)

GST and NAT1 and NAT2 polymorphisms are potential risk modifiers of childhood leukemia. The null genotypes of *GST-mu-1* (*GSTM1*), *GST-theta-1* (*GSTT1*), and low-function *GST-pi-1* (*GSTP1*) and slow *NAT2* acetylation genotypes were shown to be associated with an increased risk of childhood ALL (Coles and Kadlubar 2003; Chen et al. 1997; Krajinovic et al. 2000). There are several reports on the interaction of several genes. For example, a case-control study conducted in ALL patients and healthy controls from a French-Canadian population examined the phase I polymorphisms, *CYP1A1* and *CYP2D6*, as well as phase II enzymes *GSTM1*, *GSTT1*, *NAT1*, and *NAT2*. The *NAT2* slow-acetylator, *CYP1A1*2A*, and *GSTM1* null genotypes were shown to be significant risk determinants of ALL (OR=1.6, 1.8, and 1.8, respectively), whereas polymorphisms in *CYP2D6* and *GSTT1* genes did not seem to play an important role in the etiology of ALL (Sinnett et al. 2000).

Membrane transporter genes such a *MDR1* (multidrug resistance 1), also known as *ABCB1* (adenosine triphosphate-binding cassette family B transporter 1), act as efflux pumps to expel compounds from the cell and are strategically expressed in anatomical regions of the body that act as epithelial barriers or perform excretory functions (Yan et al. 2014). Polymorphisms of *ABCD1*/*MDR1* gene have been shown to play a key role in the genetic susceptibility to cancers, including childhood leukemia (Jamroziak et al. 2004, 2005). A study by Jamroziak et al. in 2004 reported a significantly increased risk of ALL in children who carried the homozygous variant genotype of the *C3435T* polymorphism (Jamroziak et al. 2004). Afterward, another study examined the potential interactions between xenobiotic transport and metabolism genes that were significantly associated with childhood ALL, including *ABCB1*, *ARNT*, *CYP2C8*, *CYP1A2*, *CYP1B1*, and *IDH1*, with self-reported household chemical exposures early in childhood in the modulation of childhood ALL risk. This study focused on haplotype findings observed in both Hispanic and non-Hispanic ethnicities. The *MDR1* gene was significantly associated with a higher risk of childhood ALL and showed a significant interaction/association with indoor insecticides (Chokkalingam et al. 2012). The authors concluded that the increased risk of ALL associated with paint and indoor insecticide use was seen only in specific haplotypes of genes that work in concert with chemical use to modulate risk (Chokkalingam et al. 2012).

Folate Metabolism

5,10-Methylenetetrahydrofolate reductase (MTHFR) is of relevance in folate metabolism. Changes in MTHFR activity due to polymorphisms in the *MTHFR* gene could confer susceptibility to cancer. Folate deficiency induces chromosomal damage, formation of fragile sites, and micronuclei, often associated with tumorigenesis (Kim 2000). There are reports of a protective effect against the risk of childhood ALL when folate supplementation is taken during pregnancy (Thompson et al. 2001). Two of the most studied gene variants in folate metabolism

in relation to the risk of ALL are *MTHFR 677C>T* and *MTHFR 1298A>C*. Both MTHFR variants reduce susceptibility of adult and childhood lymphoid leukemia but not myeloid leukemia (Weisberg et al. 1998). Low-function variants of *MTHFR* result in enhanced thymidine pools and more efficient DNA synthesis and repair capabilities. This is associated with increased availability of the *MTHFR* substrate, 5,10-methylenetetrahydrofolate (Weisberg et al. 1998). This dramatically reduces the double-strand break chromosomal damage and DNA hypomethylation/dysmethylation observed in proto-oncogenes or tumor-suppressor genes described in pediatric leukemias (Krajinovic et al. 2004; Siegel et al. 2012). In a stratified analysis of molecular cytogenetic subgroups, a protective association in carriers of the *MTHFR 677C>T* variant was found for leukemias with MLL translocation and hyperdiploidy. The *MTHFR 1298A>C* variant was associated with hyperdiploid leukemias, whereas *TEL-AML1* leukemias showed no association with either one of the variants (Krajinovic et al. 2004).

NAD(P)H dehydrogenase, quinone 1 (NQ01), is a homodimeric flavoprotein that catalyzes two-electron reduction of a broad range of substrates, mostly quinones and nitrogen oxides (Siegel et al. 2012; Riley and Workman 1992). The main function of NQO1 is to reduce the formation of reactive oxygen species by decreasing one-electron reduction and the associated redox cycling. This has been shown to play an important role in the activation of some anticancer drugs and cancer prevention (Wiemels et al. 1999; Faig et al. 2000). In cancer, NQO1 is expressed at high levels in many solid tumors including the lung, breast, and pancreas (Ernster 1987; Begleiter et al. 1992; 1997). The *NQO1*2* polymorphism has little or no activity. The *NQO1*2*, with one allele, has approximately half of the normal enzyme activity, whereas those with two *2 alleles are *NQO1 null* (Siegel and Ross 2000; Siegel et al. 2012). The *NQO1* polymorphism may influence response to therapy in chronic lymphoblastic leukemia, as patients having *NQO1 *1/*2* or **2/*2* genotypes may have low levels of p53 and may respond poorly to drug therapy. One study by Wiemels et al. in the United Kingdom reported an association between MLL gene rearrangement in infant leukemia and a low-function *NQO1* genotype (Wiemels et al. 1999). The same finding was confirmed in a study in Germany from 1993 to 1997, supporting the idea of a specific causal mechanism in infant leukemias that involves genotoxic exposures in utero (Meinert et al. 2000).

Genome-Wide Association Studies

Genome-wide association studies (GWAS) describe the genotyping of thousands of markers across the genome, which can be customized by adding new regions of interest together with current gene candidates (Buffler et al. 2005). However, GWAS studies are associated with high costs. DNA samples must be of very high quality, and amplified DNA cannot be used. Moreover, multiple comparison problems must be considered. A proposed approach for using GWAS studies in childhood ALL

should include three separate stages of genotyping, with the numbers representing a realistic plan of childhood ALL:

Stage 1: "Discovery," 300 K to 1 M single-nucleotide polymorphisms (SNPs) (n ~ 800–1,000 cases).
Stage 2: Genotyping within *stage 2* samples; replication 1, 15–50 K SNPs (n~ 1,200–1,500 cases) of smaller number of SNPs representing positive results from *stage 1*.
Stage 3: Positive results from *stage 2* would be genotype in *stage 3* samples; replication 2, n~1,200–1,500 cases.

Those that survive the three stages may be considered high-priority regions and can be followed for marker mapping, sequencing, and laboratory studies to identify potential causal regions (Kraft and Cox 2008).

Prevention and the Role of the Precautionary Principle in Childhood AL

Identifying the causes of childhood leukemia would lead to the establishment of effective preventive measurements in children who are at high risk of developing this disease, reducing the incidence rates, mortality rates, the costs of medical care, and other consequences associated with childhood leukemia worldwide.

In this chapter, we have reviewed the known risk factors and explored some other possible risk factors for the development of AL in children; however, the etiology and the pathophysiologic mechanisms remain unresolved.

The precautionary principle has been proposed as an anticipatory, proactive approach that is very close to the notion of prevention in public health before causal scientific evidence has been established (Eichbaum et al. 1999; Antó et al. 2000; Colborn et al. 1993; Tickner 2003). Indeed, in scientific literature there are some clear examples for the application of precautionary principles for a variety of diseases (Wynder 1994). In the case of childhood leukemia, precautionary principles should be considered to prevent irreversible damage, even though definitive scientific evidence on what causes childhood leukemia has not yet been found (Mezei et al. 2014).

The precautionary principle was initially applied in environmental policies in some European countries during the years 1960–1970 (Myhr 2010). Internationally it was implemented in 1992, aimed at not postponing measures to prevent degradation in the presence of possible damage to the environment (Comisión Mundial de Ética del Conocimiento Científico y la Tecnología (COMEST) 2005; Stijkel and Reijnders 1995).

In the field of AL in children, the precautionary principle has been proposed, taking into account that ELF-MFs have been considered as a possible human carcinogen and because in various meta-analyses, ELF-MF exposure has been

linked to the risk of developing AL in children when levels of exposure are higher than 3 milligauss (mG) (Ahlbom et al. 2000; Greenland et al. 2000; Schüz et al. 2007; Kheifets et al. 2010).

The proposed precautionary measures for reducing the exposure to ELF-MF are as follows: (1) increase the distance between the power line and the residence; (2) configure the wires on the poles in ways that reduce exposure; and (3) consider placing the distribution and subtransmission lines underground. All options have been considered as cost-effective measures for reducing the level of exposure to ELF-MF (Florig 1994; Jamieson and Wartenberg 2001). On the other hand, there are some issues in applying precautionary measures (Resnik 2004). For example, in 2005 Wiedemann and Schuz, who provided a questionnaire asking participants about the idea of precautionary measures (no precaution, exposure minimization, special protection of sensitive areas, and regulation of exposure limits) to avoid the exposure to ELF-MF, concluded that the implementation of precautionary measures could provoke concern and fear that it could affect the well-being of the general population (Wiedemann and Schütz 2005).

Conclusion

Innovative methods are required for knowing, describing, and dealing with uncertainty in childhood AL. We have evidence that genetic factors are related to the risk of AL. Environmental exposure is less clear, but there are many studies searching for a relationship between genetic susceptibility and environmental factors that hold promise for the identification of causal factors in AL. However, we need to implement a new framework for the etiology of AL, as we believe that solving key elements of this puzzle may lead to prevention of the development of AL in children.

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References

Ahlbom A, et al. A pooled analysis of magnetic fields and childhood leukaemia. Br J Cancer. 2000;83(5):692–8.

Allergy UK British Allergy Foundation. Allergy UK. *Allergy medications*. 2012. Available at: http://www.allergyuk.org/what-is-an-allergy/what-is-an-allergy. Accessed 20 Oct 2014.

Alter BP. Cancer in Fanconi anemia, 1927–2001. Cancer. 2003;97(2):425–40.
Alter BP, et al. Malignancies and survival patterns in the National Cancer Institute inherited bone marrow failure syndromes cohort study. Br J Haematol. 2010;150(2):179–88.
American College of Radiology. ACR practice guideline for imaging pregnant or potentially pregnant adolescents and women with ionizing radiation. Reston: American College of Radiology; 2008.
Annegers JF, Johnson CC. Studying parental occupation and childhood cancer. Epidemiology (Cambridge, Mass). 1992;3(1):1–2.
Antó JM, Sunyer J, Kogevinas M. Environment and health: the long journey of environmental epidemiology at the turn of the millennium. J Epidemiol Biostat. 2000;5(1):49–60.
Bailey HD, et al. Maternal dietary intake of folate and vitamins B6 and B12 during pregnancy and the risk of childhood acute lymphoblastic leukemia. Nutr Cancer. 2012;64(7):1122–30.
Begleiter A, Robotham E, Leith MK. Role of NAD(P)H:(quinone acceptor) oxidoreductase (DT-diaphorase) in activation of mitomycin C under hypoxia. Mol Pharmacol. 1992;41(4): 677–82.
Begleiter A, et al. Induction of DT-diaphorase in cancer chemoprevention and chemotherapy. Oncol Res. 1997;9(6–7):371–82.
Bhatia S. Disparities in cancer outcomes: lessons learned from children with cancer. Pediatr Blood Cancer. 2011;56(6):994–1002.
Bhatti P, et al. Increased frequency of chromosome translocations associated with diagnostic x-ray examinations. Radiat Res. 2008;170(2):149–55.
Bollag G, et al. Loss of NF1 results in activation of the Ras signaling pathway and leads to aberrant growth in haematopoietic cells. Nat Genet. 1996;12(2):144–8.
Brain JD, et al. Childhood leukemia: electric and magnetic fields as possible risk factors. Environ Health Perspect. 2003;111(7):962–70.
Brent RL. Carcinogenic risks of prenatal ionizing radiation. Semin Fetal Neonatal Med. 2014;19(3):203–13.
Buffler PA, et al. Environmental and genetic risk factors for childhood leukemia: appraising the evidence. Cancer Investig. 2005;23(1):60–75.
Butturini A, et al. Hematologic abnormalities in Fanconi anemia: an International Fanconi Anemia Registry study. Blood. 1994;84(5):1650–5.
Byrd JC, et al. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). Blood. 2002;100(13):4325–36.
Calvente I, et al. Exposure to electromagnetic fields (non-ionizing radiation) and its relationship with childhood leukemia: a systematic review. Sci Total Environ. 2010;408(16):3062–9.
Carroll WL, Raetz EA. Clinical and laboratory biology of childhood acute lymphoblastic leukemia. J Pediatr. 2012;160(1):10–8.
Carroll WL, et al. Pediatric acute lymphoblastic leukemia. Hematology Am Soc Hematol Educ Program. 2003:102–31. Review. PubMed PMID: 14633779.
Chang JS, Wiemels JL, Buffler PA. Allergies and childhood leukemia. Blood Cells Mol Dis. 2009;42(2):99–104.
Chen CL, et al. Higher frequency of glutathione S-transferase deletions in black children with acute lymphoblastic leukemia. Blood. 1997;89(5):1701–7.
Chokkalingam AP, Buffler P a. Genetic susceptibility to childhood leukaemia. Radiat Prot Dosim. 2008;132(2):119–29.
Chokkalingam AP, et al. Haplotypes of DNA repair and cell cycle control genes, X-ray exposure, and risk of childhood acute lymphoblastic leukemia. Cancer Causes Control: CCC. 2011;22(12):1721–30.
Chokkalingam AP, et al. Variation in xenobiotic transport and metabolism genes, household chemical exposures, and risk of childhood acute lymphoblastic leukemia. Cancer Causes Control: CCC. 2012;23(8):1367–75.

Choong K, et al. Juvenile myelomonocytic leukemia and Noonan syndrome. J Pediatr Hematol Oncol. 1999;21(6):523–7.
Colborn T, vom Saal FS, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect. 1993;101(5):378–84.
Coles BF, Kadlubar FF. Detoxification of electrophilic compounds by glutathione S-transferase catalysis: determinants of individual response to chemical carcinogens and chemotherapeutic drugs? BioFactors (Oxford, England). 2003;17(1–4):115–30.
Collins-Underwood JR, Mullighan CG. Genetic alterations targeting lymphoid development in acute lymphoblastic leukemia. In: Dyer MA, editor. Cancer and development. San Diego: Academic; 2011. p. 384.
Colt JS, Blair A. Parental occupational exposures and risk of childhood cancer. Environ Health Perspect. 1998;106(Suppl):909–25.
Comisión Mundial de Ética del Conocimiento Científico y la Tecnología (COMEST). Informe del Grupo de Expertos sobre el principio precautorio. Paris: UNESCO; 2005.
Committee to Assess Health Risks from Exposure to Low Levels of Ionizing Radiation. Health risks from exposure to low levels of ionizing radiation: BEIR VII phase 2. Washington, DC: The National Academies Press; 2006.
Does M, et al. Exposure to electrical contact currents and the risk of childhood leukemia. Radiat Res. 2011;175(3):390–6.
Eichbaum W, et al. In: Raffensperger C, Tickner J, editors. Protecting public health and the environment: implementing the precautionary principle. 1st ed. Washington, DC: Island Press; 1999.
Ernster L. DT-diaphorase: a historical review. Chem Scr. 1987;27(A):1–13.
Fabia J, Thuy TD. Occupation of father at time of birth of children dying of malignant diseases. Br J Prev Soc Med. 1974;28(2):98–100.
Faig M, et al. Structures of recombinant human and mouse NAD(P)H:quinone oxidoreductases: species comparison and structural changes with substrate binding and release. Proc Natl Acad Sci U S A. 2000;97(7):3177–82.
Farioli A, et al. Tobacco smoke and risk of childhood acute lymphoblastic leukemia: findings from the SETIL case-control study. Cancer Causes Control: CCC. 2014;25(6):683–92.
Feychting M, Svensson D, Ahlbom A. Exposure to motor vehicle exhaust and childhood cancer. Scand J Work Environ Health. 1998;24(1):8–11.
Fletcher RH, Fletcher SW, Fletcher GS. Clinical epidemiology: the essentials. 5th ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2014.
Flores-Lujano J, et al. Breastfeeding and early infection in the aetiology of childhood leukaemia in Down syndrome. Br J Cancer. 2009;101(5):860–4.
Flores-Lujano, J. et al. Clinical epidemiology of acute lymphoblastic leukemia – from the molecules to the clinic. In: Mejia-Arangure JM, editor. Clinical epidemiology of acute lymphoblastic leukemia – from the molecules to the clinic. Rijeka, Croatia: InTech; 2013. p. 342.
Florig HK. Safe, fair, affordable, practical and predictable: multiple objectives in EMF policy. Paper presented at: Annual Meeting of the National Council on Radiation Protection and Measurement; April 6–7, 1994; Arlington, Va.
Fonatsch C. The role of chromosome 21 in hematology and oncology. Genes, Chromosome Cancer. 2010;49(6):497–508.
German J. Bloom syndrome: a mendelian prototype of somatic mutational disease. Medicine. 1993;72(6):393–406.
Gobba F, et al. Extremely low frequency-magnetic fields (ELF-EMF) occupational exposure and natural killer activity in peripheral blood lymphocytes. Sci Total Environ. 2009;407(3): 1218–23.
Greaves MF. Speculations on the cause of childhood acute lymphoblastic leukemia. Leuk: Off J Leuk Soc Am Leuk Res Fund UK. 1988;2(2):120–5.
Greaves M. Pre-natal origins of childhood leukemia. Rev Clin Exp Hematol. 2003;7(3):233–45.
Greenberg P, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood. 1997;89(6):2079–88.

Greenland S, et al. A pooled analysis of magnetic fields, wire codes, and childhood leukemia. Childhood Leukemia-EMF Study Group. Epidemiology (Cambridge, Mass). 2000;11(6): 624–34.

Greenlee RT, et al. Cancer statistics, 2000. CA Cancer J Clin. 2000;50(1):7–33.

Grimwade D, et al. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. Blood. 1998;92(7):2322–33.

Gurney JG, Davis S, et al. Childhood cancer occurrence in relation to power line configurations: a study of potential selection bias in case-control studies. Epidemiology (Cambridge, Mass). 1995a;6(1):31–5.

Gurney JG, Severson RK, et al. Incidence of cancer in children in the United States. Sex-, race-, and 1-year age-specific rates by histologic type. Cancer. 1995b;75(8):2186–95.

Hakulinen T, Salonen T, Teppo L. Cancer in the offspring of fathers in hydrocarbon-related occupations. Br J Prev Soc Med. 1976;30(2):138–40.

Harbron RW. Cancer risks from low dose exposure to ionising radiation – is the linear no-threshold model still relevant? Radiography. 2012;18(1):28–33.

Hasle H. Pattern of malignant disorders in individuals with Down's syndrome. Lancet Oncol. 2001;2(7):429–36.

Hatch EE, et al. Do confounding or selection factors of residential wiring codes and magnetic fields distort findings of electromagnetic fields studies? Epidemiology (Cambridge, Mass). 2000;11(2):189–98.

Hitzler JK, Zipursky A. Origins of leukaemia in children with Down syndrome. Nat Rev Cancer. 2005;5(1):11–20.

Hoffmann L, et al. Therapy-related acute promyelocytic leukemia with t(15;17) (q22;q12) following chemotherapy with drugs targeting DNA topoisomerase II. A report of two cases and a review of the literature. Ann Oncol: Off J Eur Soc Med Oncol ESMO. 1995;6(8):781–8.

Hughes AM, et al. Allergy and risk of childhood leukaemia: results from the UKCCS. Int J Cancer J Int Cancer. 2007;121(4):819–24.

Hyakuna N, et al. Germline mutation of CBL is associated with moyamoya disease in a child with juvenile myelomonocytic leukemia and Noonan syndrome-like disorder. Pediatr Blood Cancer. 2015;62(3):542–4.

Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukaemia. Lancet. 2013;381(9881): 1943–55.

Infante-Rivard C. Diagnostic x rays, DNA repair genes and childhood acute lymphoblastic leukemia. Health Phys. 2003;85(1):60–4.

Infante-Rivard C, et al. Acute lymphoblastic leukaemia among Spanish children and mothers' occupation: a case-control study. J Epidemiol Community Health. 1991;45(1):11–5.

Infante-Rivard C, et al. Parental smoking, CYP1A1 genetic polymorphisms and childhood leukemia (Québec, Canada). Cancer Causes Control: CCC. 2000;11(6):547–53.

Izraeli S. Congenital syndromes and leukemia: clues to pathogenesis. Rev Clin Exp Hematol. 2003;7(3):246–60.

Jamieson D, Wartenberg D. The precautionary principle and electric and magnetic fields. Am J Public Health. 2001;91(9):1355–8.

Jamroziak K, et al. Functional C3435T polymorphism of MDR1 gene: an impact on genetic susceptibility and clinical outcome of childhood acute lymphoblastic leukemia. Eur J Haematol. 2004;72(5):314–21.

Jamroziak K, et al. Multi-drug transporter MDR1 gene polymorphism and prognosis in adult acute lymphoblastic leukemia. Pharmacol Rep: PR. 2005;57(6):882–8.

Jensen CD, et al. Maternal dietary risk factors in childhood acute lymphoblastic leukemia (United States). Cancer Causes Control: CCC. 2004;15(6):559–70.

Joannides M, Grimwade D. Molecular biology of therapy-related leukaemias. Clin Trans Oncol: Off Publ Feder Span Oncol Soc Natl Cancer Inst Mex. 2010;12(1):8–14.

Jones TL, et al. Selection bias from differential residential mobility as an explanation for associations of wire codes with childhood cancer. J Clin Epidemiol. 1993;46(6):545–8.

Keegan TJ, et al. Case-control study of paternal occupation and childhood leukaemia in Great Britain, 1962–2006. Br J Cancer. 2012;107(9):1652–9.
Kheifets L, Shimkhada R. Childhood leukemia and EMF: review of the epidemiologic evidence. Bioelectromagnetics Suppl. 2005;7:S51–9.
Kheifets L, Mezei G, Greenland S. Comment concerning "Childhood leukemia and residential magnetic fields: are pooled analyses more valid than the original studies?" (Bioelectromagnetics 27:1–7 [2006]). Bioelectromagnetics. 2006;27(8):674–5; discussion 675–6.
Kheifets L, et al. A pooled analysis of extremely low-frequency magnetic fields and childhood brain tumors. Am J Epidemiol. 2010a;172(7):752–61.
Kheifets L, et al. Pooled analysis of recent studies on magnetic fields and childhood leukaemia. Br J Cancer. 2010b;103(7):1128–35.
Kim YI. Methylenetetrahydrofolate reductase polymorphisms, folate, and cancer risk: a paradigm of gene-nutrient interactions in carcinogenesis. Nutr Rev. 2000;58(7):205–9.
Kinlen LJ. Epidemiological evidence for an infective basis in childhood leukaemia. Br J Cancer. 1995;71(1):1–5.
Kinzler KW, Vogelstein B. Landscaping the cancer terrain. Science (New York, NY). 1998;280(5366):1036–7.
Kotnis A, Sarin R, Mulherkar R. Genotype, phenotype and cancer: role of low penetrance genes and environment in tumour susceptibility. J Biosci. 2005;30(1):93–102.
Kraft P, Cox DG. Study designs for genome-wide association studies. Adv Genet. 2008;60: 465–504.
Krajinovic M, et al. Genetic polymorphisms of N-acetyltransferases 1 and 2 and gene-gene interaction in the susceptibility to childhood acute lymphoblastic leukemia. Cancer Epidemiol Biomark Prev: Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol. 2000;9(6):557–62.
Krajinovic M, et al. Role of MTHFR genetic polymorphisms in the susceptibility to childhood acute lymphoblastic leukemia. Blood. 2004;103(1):252–7.
Kratz CP, et al. The mutational spectrum of PTPN11 in juvenile myelomonocytic leukemia and Noonan syndrome/myeloproliferative disease. Blood. 2005;106(6):2183–5.
Kusters MAA, et al. Intrinsic defect of the immune system in children with Down syndrome: a review. Clin Exp Immunol. 2009;156(2):189–93.
Kwan ML, et al. Maternal diet and risk of childhood acute lymphoblastic leukemia. Publ Health Rep (Washington, DC). 2009;124(4):503–14.
Labuda D, et al. Rapid detection of CYP1A1, CYP2D6, and NAT variants by multiplex polymerase chain reaction and allele-specific oligonucleotide assay. Anal Biochem. 1999;275(1):84–92.
Lagroye I, et al. ELF magnetic fields: animal studies, mechanisms of action. Prog Biophys Mol Biol. 2011;107(3):369–73.
Levine EG, Bloomfield CD. Secondary myelodysplastic syndromes and leukaemias. Clin Haematol. 1986;15(4):1037–80.
Lim JY-S, et al. Genomics of racial and ethnic disparities in childhood acute lymphoblastic leukemia. Cancer. 2014;120(7):955–62.
Linabery AM, et al. The association between atopy and childhood/adolescent leukemia: a meta-analysis. Am J Epidemiol. 2010;171(7):749–64.
Ludwig W-D, et al. Classification of acute leukemias. Perspective 1 – perspective 2. In: Pui C-H, editor. Treatment of acute leukemias: new directions for clinical research. Totowa: Humana Press; 2003. p. 3–58.
Margolin J, Steuber C, Poplack D. Acute lymphocytic leukemia. In: Pizzo P, Poplack D, editors. Principles and practice of pediatric oncology. Philadelphia: JB Lippincott; 2006. p. 538–90.
McEvoy MW, Mann JR. Neurofibromatosis with leukaemia. Br Med J. 1971;3(5775):641.
McNally RJQ, Eden TOB. An infectious aetiology for childhood acute leukaemia: a review of the evidence. Br J Haematol. 2004;127(3):243–63.
Meinert R, et al. Leukemia and non-Hodgkin's lymphoma in childhood and exposure to pesticides: results of a register-based case-control study in Germany. Am J Epidemiol. 2000;151(7): 639–46; discussion 647–50.

Mejía-Arangure JM. Model for identifying the etiology of acute lymphoblastic leukemia in children. In: Mejia-Arangure JM, editor. Clinical epidemiology of acute lymphoblastic leukemia – from the molecules to the clinic. Rijeka, Croatia: InTech; 2013. p. 342.

Mejía-Aranguré JM, et al. Environmental factors contributing to the development of childhood leukemia in children with Down's syndrome. Leuk: Off J Leuk Soc Am Leuk Res Fund UK. 2003;17(9):1905–7.

Mejia-Arangure JM, et al. Magnetic fields and acute leukemia in children with Down syndrome. Epidemiology (Cambridge, Mass). 2007;18(1):158–61.

Metayer C, Milne E, et al. The childhood leukemia international consortium. Cancer Epidemiol. 2013a;37(3):336–47.

Metayer C, Zhang L, et al. Tobacco smoke exposure and the risk of childhood acute lymphoblastic and myeloid leukemias by cytogenetic subtype. Cancer Epidemiol Biomark Prev: Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol. 2013b;22(9):1600–11.

Mezei G, et al. Assessment of selection bias in the Canadian case-control study of residential magnetic field exposure and childhood leukemia. Am J Epidemiol. 2008;167(12): 1504–10.

Mezei G, et al. Epidemiology of childhood leukemia in the presence and absence of Down syndrome. Cancer Epidemiol. 2014;38(5):479–89.

Miller R. Epidemiologic conclusions from radiation toxicity studies. In: Fry R, editor. Late effects of radiation: proceedings of the colloquium held at the center for continuing education. Illinois: University of Chicago; 1969. p. 1970.

Milne E, et al. Parental prenatal smoking and risk of childhood acute lymphoblastic leukemia. Am J Epidemiol. 2012;175(1):43–53.

Moldovan G-L, D'Andrea AD. How the fanconi anemia pathway guards the genome. Annu Rev Genet. 2009;43:223–49.

Monge P, et al. Parental occupational exposure to pesticides and the risk of childhood leukemia in Costa Rica. Scand J Work Environ Health. 2007;33(4):293–303.

Morales-Sánchez A, et al. Lack of evidence for human T-lymphotropic virus type 1 and mouse mammary tumor-like virus involvement in the genesis of childhood acute lymphoblastic leukemia. Cancer Epidemiol Biomark Prev: Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol. 2013;22(11):2130–3.

Myhr AI. A precautionary approach to genetically modified organisms: challenges and Implications for policy and science. J Agric Environ Ethics. 2010;23(6):501–25.

National Research Council (US)/Committee on the Possible Effects of Electromagnetic Fields on Biologic Systems. Possible health effects of exposure to residential electric and magnetic fields. Washington, DC: National Academy Press (US); 1997.

Niemeyer CM, et al. Germline CBL mutations cause developmental abnormalities and predispose to juvenile myelomonocytic leukemia. Nat Genet. 2010;42(9):794–800.

Niihori T, et al. Functional analysis of PTPN11/SHP-2 mutants identified in Noonan syndrome and childhood leukemia. J Hum Genet. 2005;50(4):192–202.

Núñez-Enríquez JC, et al. Allergy and acute leukaemia in children with Down syndrome: a population study. Report from the Mexican inter-institutional group for the identification of the causes of childhood leukaemia. Br J Cancer. 2013;108(11):2334–8.

O'Leary LM, et al. Parental occupational exposures and risk of childhood cancer: a review. Am J Ind Med. 1991;20(1):17–35.

Patrick K, et al. Outcome of Down syndrome associated acute lymphoblastic leukaemia treated on a contemporary protocol. Br J Haematol. 2014;165(4):552–5.

Parkin DM, Kramarova E, Draper GJ, Masuyer E, Michaelis J, Neglia J, Qureshi S, Stiller CA, eds. International Incidence of Childhood Cancer vol. 2. IARC Scientific Publications no. 144. Lyon: International Agency for Research on Cancer. 1998.

Pedersen-Bjergaard J, Andersen MK, Johansson B. Balanced chromosome aberrations in leukemias following chemotherapy with DNA-topoisomerase II inhibitors. J Clin Oncol: Off J Am Soc Clin Oncol. 1998;16(5):1897–8.

Perentesis JP. Genetic predisposition and treatment-related leukemia. Med Pediatr Oncol. 2001;36(5):541–8.
Perez-Saldivar ML, et al. Father's occupational exposure to carcinogenic agents and childhood acute leukemia: a new method to assess exposure (a case-control study). BMC Cancer. 2008;8:7.
Pérez-Saldivar ML, et al. Childhood acute leukemias are frequent in Mexico City: descriptive epidemiology. BMC Cancer. 2011;11:355.
Petridou E, et al. Maternal diet and acute lymphoblastic leukemia in young children. Cancer Epidemiol Biomark Prev: Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol. 2005;14(8):1935–9.
Pizzo P. Principles and practice of pediatric oncology. 6th ed. Philadelphia: Wolters Kluwer/ Lippincott Williams & Wilkins Health; 2011.
Popp HD, Bohlander SK. Genetic instability in inherited and sporadic leukemias. Genes Chromosome Cancer. 2010;49(12):1071–81.
Poppe B, et al. Chromosomal aberrations in Bloom syndrome patients with myeloid malignancies. Cancer Genet Cytogenet. 2001;128(1):39–42.
Ratain MJ, et al. Acute nonlymphocytic leukemia following etoposide and cisplatin combination chemotherapy for advanced non-small-cell carcinoma of the lung. Blood. 1987;70(5):1412–7.
Resnik DB. The precautionary principle and medical decision making. J Med Philos. 2004;29(3):281–99.
Riley RJ, Workman P. DT-diaphorase and cancer chemotherapy. Biochem Pharmacol. 1992;43(8):1657–69.
Rosenbaum PF, Buck GM, Brecher ML. Allergy and infectious disease histories and the risk of childhood acute lymphoblastic leukaemia. Paediatr Perinat Epidemiol. 2005;19(2):152–64.
Rosenberg PS, Greene MH, Alter BP. Cancer incidence in persons with Fanconi anemia. Blood. 2003;101(3):822–6.
Ross JA, et al. Periconceptional vitamin use and leukemia risk in children with Down syndrome: a Children's Oncology Group study. Cancer. 2005;104(2):405–10.
Rothman KJ. Methodologic frontiers in environmental epidemiology. Environ Health Perspect. 1993;101(Suppl):19–21.
Rowley JD. The role of chromosome translocations in leukemogenesis. Semin Hematol. 1999;36(4 Suppl 7):59–72.
Rudant J, et al. Childhood acute leukemia, early common infections, and allergy: the ESCALE Study. Am J Epidemiol. 2010;172(9):1015–27.
Sanderson RN, et al. Population-based demographic study of karyotypes in 1709 patients with adult acute myeloid leukemia. Leukemia. 2006;20(3):444–50.
Sanz MM, German J. Bloom's syndrome. In: Pagon R, Adam M, Ardinger H, editors. GeneReviews®. Seattle: University of Washington, Seattle; 2014.
Savage SA. Genomic clues to ethnic differences in ALL. Blood. 2014;123(16):2440–2.
Savitz DA, Chen JH. Parental occupation and childhood cancer: review of epidemiologic studies. Environ Health Perspect. 1990;88:325–37.
Schmiegelow K, et al. Etiology of common childhood acute lymphoblastic leukemia: the adrenal hypothesis. Leukemia. 2008;22(12):2137–41.
Schneider M, et al. Fanconi anaemia: genetics, molecular biology, and cancer – implications for clinical management in children and adults. Clin Genet. 2015;88(1):13–24.
Schoch C, et al. Karyotype is an independent prognostic parameter in therapy-related acute myeloid leukemia (t-AML): an analysis of 93 patients with t-AML in comparison to 1091 patients with de novo AML. Leukemia. 2004;18(1):120–5.
Schüz J, et al. Atopic disease and childhood acute lymphoblastic leukemia. Int J Cancer J Int Cancer. 2003;105(2):255–60.
Schüz J, et al. Nighttime exposure to electromagnetic fields and childhood leukemia: an extended pooled analysis. Am J Epidemiol. 2007;166(3):263–9.
Seif AE. Pediatric leukemia predisposition syndromes: clues to understanding leukemogenesis. Cancer Genet. 2011;204(5):227–44.

Sermage-Faure C, et al. Childhood leukaemia close to high-voltage power lines – the Geocap study, 2002–2007. Br J Cancer. 2013;108(9):1899–906.
Shannon KM, et al. Loss of the normal NF1 allele from the bone marrow of children with type 1 neurofibromatosis and malignant myeloid disorders. N Engl J Med. 1994; 330(9):597–601.
Shimamura A, Alter BP. Pathophysiology and management of inherited bone marrow failure syndromes. Blood Rev. 2010;24(3):101–22.
Shu XO, et al. Parental exposure to medications and hydrocarbons and ras mutations in children with acute lymphoblastic leukemia: a report from the Children's Oncology Group. Cancer Epidemiol Biomark Prev: Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol. 2004;13(7):1230–5.
Siegel D, Ross D. Immunodetection of NAD(P)H:quinone oxidoreductase 1 (NQO1) in human tissues. Free Radic Biol Med. 2000;29(3–4):246–53.
Siegel D, Kepa JK, Ross D. NAD(P)H:quinone oxidoreductase 1 (NQO1) localizes to the mitotic spindle in human cells. PLoS ONE. 2012a;7(9):e44861.
Siegel R, et al. Cancer treatment and survivorship statistics, 2012. CA Cancer J Clin. 2012b;62(4):220–41.
Sinnett D, Krajinovic M, Labuda D. Genetic susceptibility to childhood acute lymphoblastic leukemia. Leuk Lymphoma. 2000;38(5–6):447–62.
Slovak ML, et al. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group Study. Blood. 2000;96(13):4075–83.
Smith M. Considerations on a possible viral etiology for B-precursor acute lymphoblastic leukemia of childhood. J Immunother. 1997;20(2):89–100.
Söderberg KC, et al. Allergic conditions and risk of hematological malignancies in adults: a cohort study. BMC Public Health. 2004;4:51.
Söderberg KC, et al. Autoimmune diseases, asthma and risk of haematological malignancies: a nationwide case-control study in Sweden. Eur J Cancer (Oxford, England). 2006;42(17): 3028–33.
Spector L, et al. Medically recorded allergies and the risk of childhood acute lymphoblastic leukaemia. Eur J Cancer (Oxford, England). 2004;40(4):579–84.
Stewart A, Kneale GW. Radiation dose effects in relation to obstetric x-rays and childhood cancers. Lancet. 1970;1(7658):1185–8.
Stewart A, et al. Preliminary communication: malignant disease in childhood and diagnostic irradiation in-utero. Lancet. 1956;2:447.
Stijkel A, Reijnders L. Implementation of the precautionary principle in standards for the workplace. Occup Environ Med. 1995;52(5):304–12.
Stiller CA, Chessells JM, Fitchett M. Neurofibromatosis and childhood leukaemia/lymphoma: a population-based UKCCSG study. Br J Cancer. 1994;70(5):969–72.
Straughen J, et al. Physical mapping of the bloom syndrome region by the identification of YAC and P1 clones from human chromosome 15 band q26.1. Genomics. 1996;35(1):118–28.
Strick R, et al. Dietary bioflavonoids induce cleavage in the MLL gene and may contribute to infant leukemia. Proc Natl Acad Sci U S A. 2000;97(9):4790–5.
Strullu M, et al. Juvenile myelomonocytic leukaemia and Noonan syndrome. J Med Genet. 2014;51(10):689–97.
Swanson J, Kheifets L. Could the geomagnetic field be an effect modifier for studies of power-frequency magnetic fields and childhood leukaemia? J Radiol Prot: Off J Soc Radiol Prot. 2012;32(4):413–8.
Tartaglia M, Zampino G, Gelb BD. Noonan syndrome: clinical aspects and molecular pathogenesis. Mol Syndromology. 2010;1(1):2–26.
Thompson JR, et al. Maternal folate supplementation in pregnancy and protection against acute lymphoblastic leukaemia in childhood: a case-control study. Lancet. 2001;358(9297):1935–40.
Tickner JA. Precaution, environmental science, and preventive public policy. New Solutions: J Environ Occup Health Policy: NS. 2003;13(3):275–82.

Tower RL, Spector LG. The epidemiology of childhood leukemia with a focus on birth weight and diet. Crit Rev Clin Lab Sci. 2007;44(3):203–42.
Urayama KY, et al. A meta-analysis of the association between day-care attendance and childhood acute lymphoblastic leukaemia. Int J Epidemiol. 2010;39(3):718–32.
Van Maele-Fabry G, et al. Childhood leukaemia and parental occupational exposure to pesticides: a systematic review and meta-analysis. Cancer Causes Control: CCC. 2010;21(6):787–809.
Vaz F, et al. Mutation of the RAD51C gene in a Fanconi anemia-like disorder. Nat Genet. 2010;42(5):406–9.
Ward G. The infective theory of acute leukemia. Br J Childhood's Dis. 1917;14:10–20.
Ward EM, et al. Priorities for development of research methods in occupational cancer. Environ Health Perspect. 2003;111(1):1–12.
Weisberg I, et al. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. Mol Genet Metab. 1998;64(3):169–72.
Wen W, et al. Parental medication use and risk of childhood acute lymphoblastic leukemia. Cancer. 2002;95(8):1786–94.
Wiedemann PM, Schütz H. The precautionary principle and risk perception: experimental studies in the EMF area. Environ Health Perspect. 2005;113(4):402–5.
Wiemels JL, et al. A lack of a functional NAD(P)H:quinone oxidoreductase allele is selectively associated with pediatric leukemias that have MLL fusions. United Kingdom Childhood Cancer Study Investigators. Cancer Res. 1999;59(16):4095–9.
Wynder EL. Invited commentary: studies in mechanism and prevention. Striking a proper balance. Am J Epidemiol. 1994;139(6):547–9.
Yan Y, et al. Association of MDR1 G2677T polymorphism and leukemia risk: evidence from a meta-analysis. Tumour Biol: J Int Soc Oncodevelopment Biol Med. 2014;35(3):2191–7.
Yohay K. Neurofibromatosis type 1 and associated malignancies. Curr Neurol Neurosci Rep. 2009;9(3):247–53.
Zack M, et al. Cancer in children of parents exposed to hydrocarbon-related industries and occupations. Am J Epidemiol. 1980;111(3):329–36.
Zvulunov A. Juvenile xanthogranuloma, neurofibromatosis, and juvenile chronic myelogenous leukemia. Arch Dermatol. 1996;132(6):712–3.

Chapter 3
HTLV-1 as a Model for Identifying the Causes of Human Leukemia

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Abstract Leukemia is a complex disease that is associated with several causes, one of which is viral infection. Human T-lymphotropic virus (HTLV) is the most studied virus and is associated with human leukemia. The epidemiology of HTLV-1 has been under investigation in several countries and is now well known. In this context Latin America has shown a high prevalence. Virus family proteins such as Tax and HBZ modulate several signaling pathways that modulate the biological activities of the cell, including cell growth and proliferation, which affect the physiology and immunology of the cell. In this chapter we analyze the most frequent mechanisms induced by HTLV-1 that affect cell proliferation and the immune response to viral infection. The effects on these processes can lead to cell transformation and

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avoidance of immune recognition of the virus in affected cells. The epidemiological, molecular, and immunological characteristics of HTLV-1 virus involved in leukemia in humans are reviewed.

Keywords HTLV-1 • Leukemia • Viral leukemia • TAX • HBZ • Immune evasion

Introduction

Several physical, chemical, and biological agents can trigger the mechanisms leading to the development of leukemia. Viral infection is one important cause (Graves 2006). This chapter outlines the role of viral infection, its epidemiology, and the mechanisms associated with the proteins of human T-lymphotropic virus (HTLV-1) that can lead to leukemia.

General Characteristics of HTLV

The discovery of HTLV-1 was published in 1980. The first report described how T cells from patients with T-cell leukemia were cultured and analyzed by reverse transcription. Viral particles were identified by electronic microscopy, and the presence of antibodies in infected patients and the ability of the virus to integrate into DNA were reported (Poiesz et al. 1980). There are four types of HTLV, but only HTLV-1 is associated with leukemia. This virus belongs to the *Retroviridae* family, *Orthoretrovirinae* subfamily, genus *Deltaretrovirus*. As species, HTLVs are classified as lymphotropic viruses (Poiesz et al. 1980).

HTLV-1 is an enveloped virus with a single-stranded RNA. Its genome is reverse transcribed, and subsequent alternative splicing gives rise to at least nine different messenger RNAs, all of which encode the viral structural and functional proteins. One of the proteins that take part in the induction of leukemia is encoded in open reading frame (ORF) IV. This protein is called Tax (transactivator of the region X) (Fig. 3.1). HBZ (basic leucine zipper), or b-zipper protein (b-ZIP), is encoded in one antisense RNA (Poiesz et al. 1980).

The possibility of viral infections causing leukemia was first proposed in the nineteenth century. However, this was not confirmed until 1908 when Ellerman and Bang demonstrated that Jaagsiekte sheep retrovirus could induce erythroleukemia in chickens. Subsequently, the discovery of new infectious agents with the ability to induce leukemia in animals rekindled the debate about the roles of viral infections as causes of leukemia (Greaves 2006). The first evidence of a link between leukemia and viral infections in primates was reported in the 1970s, when Kawakami et al. discovered the gibbon ape leukemia virus and demonstrated its association with myeloid leukemia. Subsequently, Gallo et al. identified a variant of this virus that caused leukemia in gibbon T cells. In 1972, Sarngadharan et al. measured the

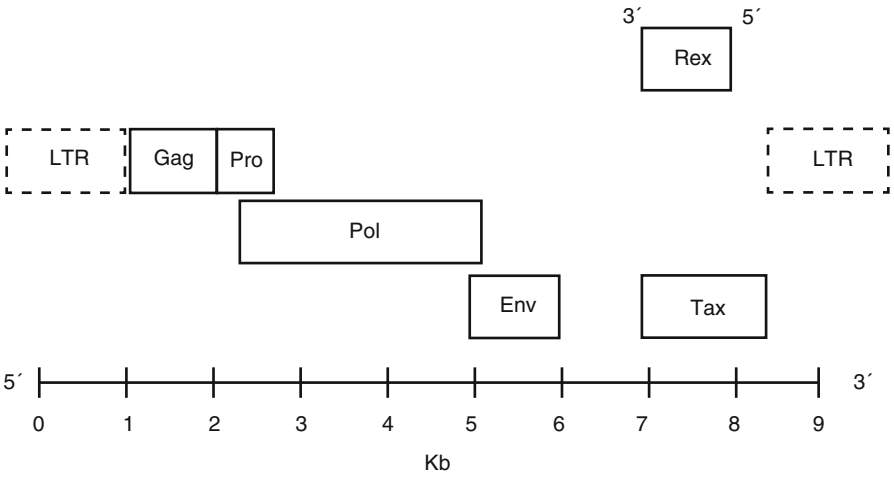

Fig. 3.1 The scheme show the nine products of transcription, which are encoded along of the viral nucleic material

activity of viral reverse transcriptase in patients with lymphoblastic acute leukemia, but the virus could not be isolated. The identification of HTLV was not possible until 1980, when stabilized T-cell cultures were obtained from patients with T-cell leukemia that exhibited reverse transcription, and the viral particles were detected by electron microscopy. The association was demonstrated according to Koch's postulates, and specific antibodies were detected in infected patients (Poiesz et al. 1980).

Four types of HTLV have been identified, but only HTLV-1 is associated with leukemia. HTLVs belong to the family *Retroviridae*, subfamily *Orthoretroviridae*, genus *Deltaretrovirus* (International Committee on Taxonomy of Viruses 2012).

HTLV-1 is an enveloped virus with a single linear RNA genome; it comprises one coding region with four ORFs flanked by a large terminal repeat region and a terminal region called *pX* (Lairmore and Franchini 2007). The proteins are encoded as follows (from 5′ to 3′): GAG, Pro-Pol precursors, ENV protein, functional proteins, expression regulatory proteins, accessory proteins, and others with unknown functions (Francesconi do Valle et al. 2001) (Fig. 3.1).

Epidemiology

HTLV-1 was the first human retrovirus to be isolated, and its association with leukemia has been clearly demonstrated. HTLV-1 is distributed throughout the world and its epidemiology has been well characterized in some countries. In China, a large cross-sectional study of 5,417 individuals detected HTLV-1 in 0.13 % of samples obtained from donors with hematological malignancies, where

a high-risk group included patients who were positive for human immunodeficiency virus (HIV), hepatitis B virus, hepatitis C virus (HCV), or *Treponema pallidum*. Most of the high-risk patients were positive for HTLV-1, and it was suggested that HTLV-1 infections may occur via coinfection. In addition, it was suggested that HTLV-1 is not endemic to China (Ma et al. 2013). In Israel, another study involving a cohort of patients who donated blood over a period of 14 years showed that 0.005 % were HTLV-1 carriers, i.e., 90 who were positive for HTLV-1, including 6 who were diagnosed with a malignancy, 3 of whom developed leukemia. Thus, according to that study, only in 0.37 % of HTLV-1 was involved with leukemia. The authors suggested that their results were high compared with previous studies because they employed a very long follow-up period (Stienlauf et al. 2013). A study in Japan based on a cohort of 272,043 blood samples obtained from a regional blood bank also detected a high prevalence of HTLV-1, where the seroprevalence was higher in females than in males (2.05 % and 1.80 %, respectively). Furthermore, the seroprevalence was higher in older patients in comparison with either males or females. The role of age in the transmission of HTLV-1 has been analyzed in the context of sexual activity and pregnancy, where it has been shown that the prevalence of HTLV-1 infection increased with the age of pregnancy, and the risk of vertical transfer from the mother to newborns also increased with age (Eshimaa et al. 2009). Intrafamilial transmission and the factors involved in the acquisition of HTLV-1 infection in pregnant women were studied in Brazil, where the prevalence was found to be 1.05 % in a group of 2,766 pregnant women. An analysis of families within this group indicated that 32.6 % showed reactivity, but there were low associations with the level of education, age, or ethnic group (Gomes Mello et al. 2014). In Spain, a study of 6,460 subjects detected a prevalence of 0.06 %, but the authors suggested that the seroprevalence is actually lower in Spain because most of the HTLV-1-positive patients came from Latin America and Africa (Treviño et al. 2012). Some studies performed in Latin America have reported high prevalence rates. For example, a study in Peru comprising 638 subjects from 27 indigenous communities detected an HTLV-1 prevalence of 1.9 %, although the prevalence was 4.1 % in one community, thereby demonstrating its high prevalence in some indigenous populations in Latin America (Alva et al. 2012). In addition, there is a frequent association between HTLV-1 and coinfection with other viruses in drug users, e.g., coinfection with HTLV-1/-2, HIV, and HCV, although the triple coinfection rate was low (0.8 %) (Prasetyo et al. 2013). This suggests that some lifestyles, such as drug use, are risk factors for the acquisition of HTLV-1 infection.

HTLV-1 and Leukemia

Despite the oncogenic activity of retrovirus being observed previously in several animal species (Gallo and Todaro 1976), the correlation between HTLV-1 and oncogenicity was not cleared in humans until 1980, when viral particles were

proved in HUT-102 and CTCL-3 cell lines derived from the lymph node and in fresh peripheral blood lymphocytes of one patient T-cell lymphoma (Reitz et al. 1981). Recent studies show that adult T-cell leukemia/lymphoma occurs in ~5 % of HTLV-1-infected individuals (Cook et al. 2014; Akinbami et al. 2014). Although such a correlation appears low, more than frequency of emergence, some molecular mechanisms which involve host and viral interaction appear to be more closely associated. For example, clonality has been more associated with other diseases such as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), although in leukemia such a relation is still not clear, and further research is necessary to discover the role of such mechanism in the development of leukemia induced by HTLV-1 (Bangham et al. 2014). In addition, adult T-cell leukemia has been more frequent in geographical regions where HTLV-1 is more frequent, and age appears to be an important factor because leukemia associated with HTLV-1 is frequent in adults but not in children. Only a very small number of isolated cases have been identified in children, e.g., a 15-year-old adolescent in Brazil who developed a lymphoma of T cells after which HTLV-1 viral infection was confirmed (Francesconi do Valle et al. 2001).

Some of the molecular mechanisms by which HTLV is able to induce leukemia are known, but other mechanisms underlying the role of viral infection are not completely clear. Of the viruses known to cause leukemia, HTLV is perhaps the most representative and the virus that is most known. Genotype 1 exhibits several viral proteins that are involved in the mechanisms that lead to cell transformation (Jun-ichirou and Matsuoka 2007). Two of these proteins, Tax and HBZ, are now discussed further.

Tax This protein is well recognized as an oncoprotein. Its role lies in the transactivation of viral transcription through its interaction with the 5′ long terminal repeat of HTLV-1 (Felber et al. 1985), although it can also transactivate transcription. Tax interacts with transcription factors such as cAMP response element-binding protein (CREB) to produce a ternary complex, which regulates the cell cycle machinery (Tie et al. 1996). Once the virus has control of the cell, several mechanisms induced by Tax lead to cell immortalization (Fig. 3.2). These processes are described as follows:

(a) p53 function is silenced through a mechanism that is independent of nuclear factor (NF)-κB (Jeang et al. 1990).
(b) Antiapoptotic proteins such as Bfl-1, a member of the BCL2 protein family, are expressed. Such proteins have been shown to contribute to the survival of HTLV-1-infected cells (Ressler et al. 1997).
(c) Tax-2 causes permanent arrest of the cell in G1, leading to failure of the G1 checkpoint, which can contribute to a nucleotide excision repair deficiency, leading to genomic instability (Tie et al. 1996).
(d) Genomic stability is reduced. Some studies have identified an association between Tax and genomic stability and have provided evidence that Tax reduces genomic stability by downregulating human polymerase β, which is involved in DNA repair and blocks the repair of cellular damage (Ressler et al. 1997).

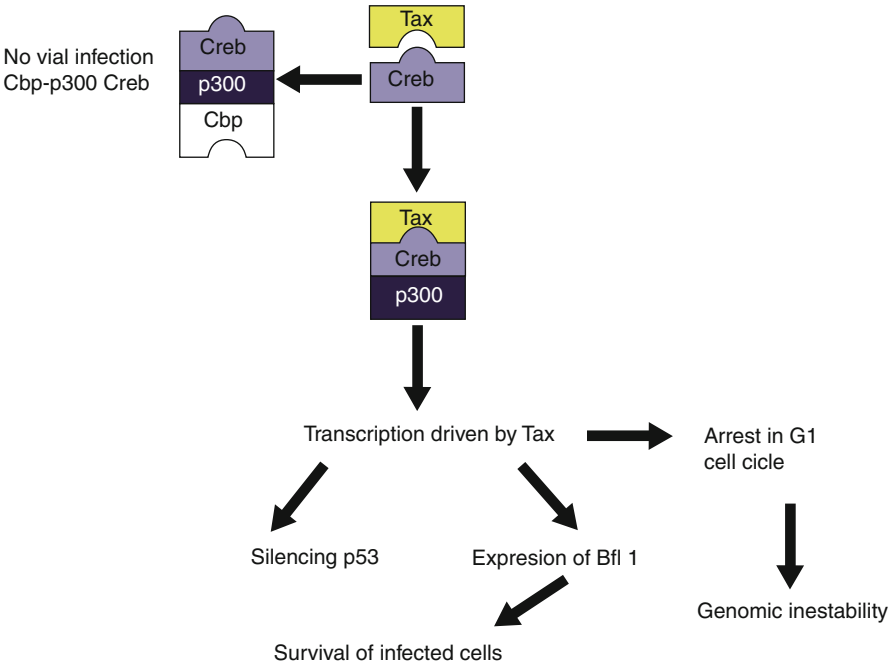

Fig. 3.2 Mechanisms by which Tax drives the cellular machinery and leads to leukemogenesis

(e) Tax can transactivate the proliferating cell nuclear antigen (PCNA) promoter and transform infected HTLV-1 cells, leading to changes in the expression of PCNA protein, which is involved in the regulation of cell proliferation and DNA replication and repair (Ressler et al. 1997). Tax also reduces the level of histones by uncoupling replication-dependent histone gene expression and DNA replication. Histones can also be acetylated by Tax recruitment of the cellular coactivator CBP/p300 (Nyborg et al. 2010).

HBZ In an in vitro model using T lymphocytes, HBZ was shown to support cell proliferation. The mechanism involves p65, a member of the NF-κB protein family. HBZ alters p65 activity by decreasing its affinity for DNA. HBZ also increases the expression of PDLIM2, which encodes a cell ubiquitin that is responsible for the degradation of p65 (Takashi et al. 2007; Tiejun et al. 2009; Turvey and Broide 2010).

Immunological Mechanisms Involved in Viral Leukemia

The innate immune response is the first line of host defense against viral infection. Once activated, the innate immune response serves two functions: (1) the production of effector molecules, which restrict the viral infection, and (2) the initiation of the acquired immune response, which leads to the complete elimination of the pathogen from the infected cells (Turvey and Broide 2010). One aspect of the innate immune

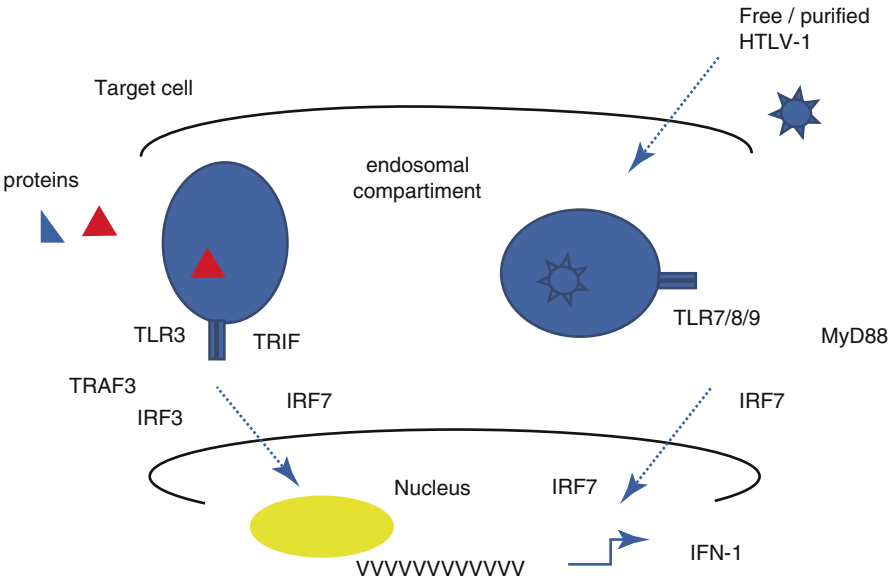

Fig. 3.3 Recognition of HTLV-1 by innate immune response. A critical component of the immune response to the viral infection involves the detection of viral PAMPS (RNAs) by PRRs, which are activated and induce activation of interferons. TLR7 localized endosomally recognizes nucleic acid and begins the way of interferon production in this mechanism take part IRF7 (interferon regulatory factor 7) and TLR3 independent way of MyD88

response is the family of molecular receptors known as pattern-recognition receptors (PRRs), which detect pathogen-associated molecular patterns (PAMPs). The interaction of PRRs with PAMPs is essential for triggering the effector mechanisms of the innate immune response (Kumar et al. 2011). During viral infection, the innate immune system recognizes viral nucleic acids (DNA or RNA, either single-stranded or double-stranded) as PAMPs and viral glycoproteins (Lester and Li 2014).

Three important classes of PRRs have been identified recently: the toll-like receptors (TLRs), the cytoplasmic proteins (NRLs) (Kawai and Akira 2011), and the retinoic-inducible gene 1-like receptors (RLRs) (Journo and Mahieux 2011). These molecules participate in different aspects of signaling that lead to the activation of transcription factors such as NF-κB. Such factors are important for the synthesis of proinflammatory cytokines, chemokines, and effector molecules such as type 1 interferons (IFNs), which contribute to the elimination of viral components and apoptosis of infected cells (Colisson et al. 2010). RLRs activate the inflammasome complex, which plays an essential role in the antiviral response (Colisson et al. 2010). Several families of viruses are associated with PRR activation, including HTLV-1, the human retrovirus associated with leukemia of T cells.

The first data on direct binding between HTLV-1 and PRRs from the innate immune system were reported in an in vitro model of infected plasmacytoid dendritic cells (pDCs) (Fig. 3.3). A strong response was observed for the production of

IFN-α, which was dependent on the TLR7 receptor (Kane et al. 2011). The addition of an inhibitor of TLR7 (oligonucleotide A151) and acidification using chloroquine contributed to the proposed binding of HTLV-1 to TLR7 (Kane et al. 2011). Other viruses that cause tumors are also related to TLR7, such as the mouse mammary tumor virus (Kane et al. 2011).

In addition, the immune response acquired involved in the late phase includes both humoral and cellular mechanisms. The effector molecules of the humoral response (antibodies) prevent the viral dissemination from the infected cells toward the cells of adjacent tissues, whereas the cytotoxic cells (CTLs) remove the infected cells by induction of apoptosis. The antibody response to the protein Tax of HTLV-1 was reported in 2002 (Levin et al. 2002). Such viral antigens induce a cross-linking of the heterogeneous ribonucleoprotein. It has been suggested that such a mechanism is involved as a form of molecular mimicry in HTLV-1 infection. In disorders such as HAM/TSP, it has been proposed that the anti-Tax produced can have an important role in the inflammation mechanisms in lesions and tissues even up to the blood-brain barrier, as well as the releasing of autoantigens. HAM/TSP disease shows a high number of immunoglobulin M antibodies with dominant reactivity to four immunodominant epitopes of the Tax protein. Other antibodies detected include envelope proteins with the ability to neutralize viral activity (Tanaka et al. 1994). CTLs with specific activity to HTLV-1 have also been reported (Bangham 2000). It has been proposed that high avidity of antibodies and the lytic efficiency of these cells might correlate with the viral load and be crucial in the outcome of the HTLV-1 infection (Kattan et al. 2009). In addition, the common antigen of HTLV-1 is recognized for CD4+ cells specific to HTLV-1 (Sakaguchi et al. 2008).

Immune Evasion

HTLV-1 uses several strategies to evade the immune response (Fig. 3.4), all of which involve blocking of cell signaling. One strategy is to interfere with the signaling pathway leading to IFN-1 production even during a strong immune response (Olière et al. 2011; Saha et al. 2010). Some studies suggest that Tax protein is taking part in the immune evasion by obstruction of the signal of transduction of IFN-γ. Other proteins of HTLV-1 also take part; for example, the HBS protein inhibits the effector activity of CD4 affecting the cytokine production of TH1, leading to an immunosuppressive effect. Other immune mechanisms that contribute to HTLV-1 pathogenesis are cell immortality and viral persistence, which allow the virus to remain within a patient for a long time, often without producing symptoms. The proviral genome expresses several proteins, of which Tax and HBZ are considered the most important to viral pathogenesis and persistence (Peloponese et al. 2006; Nyborg et al. 2010). Tax is a nuclear protein of 40 kDa that is encoded in ORF X-IV. This protein is an activator of transcription that exerts pleiotropic effects on the interactions of several signaling pathways (Jaworski et al. 2014). Tax

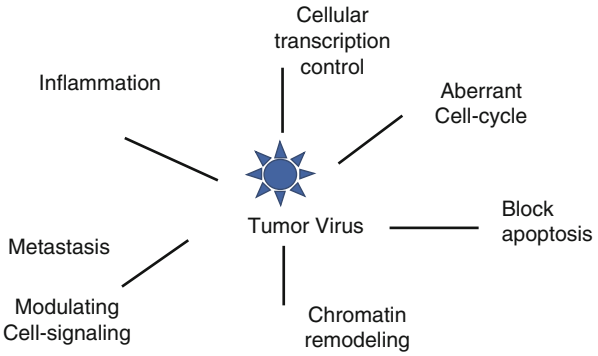

Fig. 3.4 Downregulation of pathways involved in immortalization of cells infected by oncogenic viruses. The infection of oncogenic virus leads to the immortalization of infected cells by deregulation of different pathways involved in cellular homeostasis, including immune escape by the synthesis of oncogenic proteins

is detectable primarily in the nucleus but can be detected in the cytoplasm and is one of the main oncogenic determinants of HTLV-1. This protein upregulates the transcription of NF-κB, which leads to deregulation of important genes controlling cell growth and signal transduction such as cytokines, growth factors, cytokine receptors, proto-oncogenes, and antiapoptotic proteins involved in the kinase signaling cascades. Tax also downregulates the tumor-suppressor proteins p53 and Rb (Kastan et al. 1992).

HBZ, another important protein involved in the regulation of viral transcription, acts by inhibiting and activating cellular genes (Kastan et al. 1992; Stoppa et al. 2012; Tomita et al. 2007). This protein interacts with p65 and can degrade or sequestrate c-Jun and disrupt IFN-β (Table 3.1). The dual functions of Tax and HBZ can modulate the direct or indirect signals of the PRRs, which limit the production of HTLV-1 ligands such as viral proteins and nucleic acids. The manipulation of the immune mechanisms associated with HTLV-1 is attributed to p30 and p12 proteins. These proteins are essential for the productive infection of monocyte-derived dendritic cells. The role of p12 protein in the viral cycle is not clear, although some in vitro studies have suggested that this protein participates in the maintenance of viral infection. For HTLV-1, la p12 binds to MHC class I and prevents its expression and maturation, leading to the infected cell escaping recognition (Table 3.1) (Satou and Matsuoka 2012). The viral protein p30 can also modulate innate immunity. Research using the THP-1 macrophage line has shown that p30 disturbs the signaling of TLR4. This pathway is critical to the innate immune system's response to bacterial infection, and p30 inhibits the production of cytokines normally secreted under TLR4 stimulation (Chan et al. 2013). Such disturbance of TLR4 induced by the p30 protein is mediated by the dependent interaction of inhibition of the transcription factor PU.1 (Table 3.1). p30 protein can also inhibit the proinflammatory cytokines by causing an increase in the release of interleukin-10, thereby interfering

Table 3.1 Immune system-related oncogenic mechanisms exploited by HTLV-1

Viral oncoproteins	Association with cellular event (gatekeeper)	Dysregulated signaling pathways	Reference
Tax*	Cyclic AMP, p300/CBP, MAD-1	Cell cycle, apoptosis, Ras-Erk MAPK	Boxus et al. (2009)
	MAD-2, cyclin D1, ChK1 and 2	Pathway, PI3K, NF-κB	Tomita et al. (2007)
	Signaling—interferon		Kastan et al. (1992)
	JAK/STAT		
	IRF7, IRF3,	Interference	
	TyK2, STAT2		
	Phosphorylation of complex		Nyborg et al. (2010)
	(ISGF3)		Nyborg et al. (2010)
	CCL2 secretion to attract Treg cells	Suppression of CTLs by Treg cells	Toulsa et al. (2010)
p30	TLR4 receptor (monocyte-macrophage), transcriptional activation factor, immune response to bacteria, production of cytokines	Dysregulation of macrophages and condition of immunosuppression	Datta et al (2006)
p12	MHC	Inhibition of maturation	Johnson et al. (2001)
HBZ	Inhibition of Th1	Impaired cell-mediated immunity	Miyazato et al. (2014)
	Cytokine production, expression of Foxp3	Phenotypes of CD4+ T cells altered	Satou et al. (2012)

*Most potent and studied viral oncoproteins

in the balance between the pro- and anti-inflammatory cytokine responses to bacterial infection. This may explain why some patients with adult T-cell leukemia show immunodeficiency and susceptibility to bacterial infections and suggests that p30 may be a therapeutic target (Fenizia et al. 2014).

HTLV-1 infection can also affect the acquired immune response. It has been reported that p12 CD4+ can induce protection again the cytotoxicity of natural killer cells (Datta et al. 2006). HTLV-1 primary infection of CD4+ cells can induce the downregulation of MHC-1, thus affecting effector T, memory, and regulatory cells (Fig. 3.4). Several studies have reported that HBZ induces the expression of Foxp3 by modulating transforming growth factor β signaling, which increases the expression of factors that can change the population phenotype of CD4+ cells, compromises cellular immunity, and suppresses the release of Th1 cytokines (Johnson et al. 2001).

In addition, the regulatory complex involved in the generation and migration of regulatory T cells that express Foxp3 protein has been associated with modifications of the reprogramming system of these CD4+, CD25+, and CCR4+ cells. This can lead to reduced expression of Foxp3, which is required to suppress inflammation, suggesting that HTLV-1 induces a Th1-like state in CD4+CCR4+ T cells (Figs. 3.4 and 3.5) (Miyazato and Matsuoka 2014; Sugata et al. 2012; Toulza et al. 2010). The

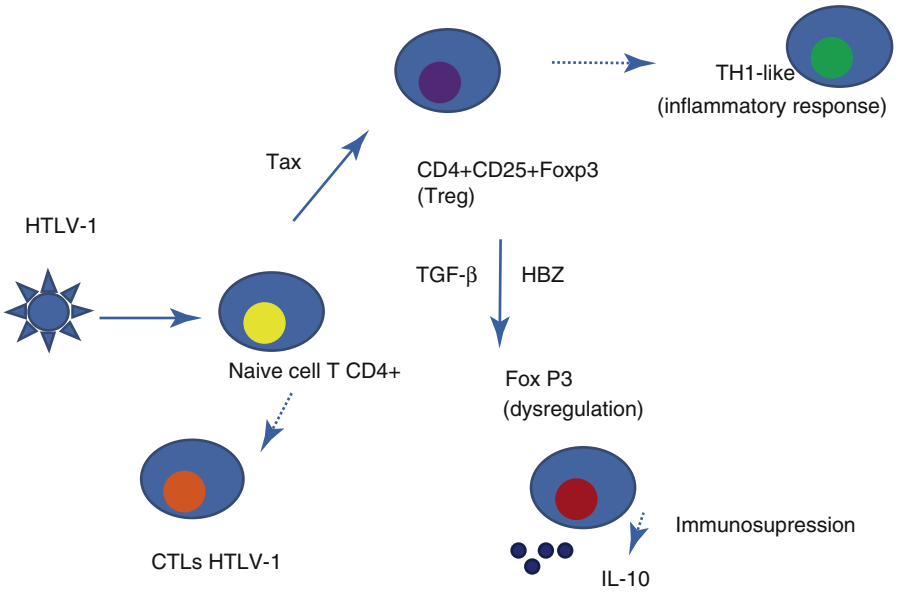

Fig. 3.5 Regulation of the immune response acquired by HTLV-1. The cellular tropism of HTLV-1 involves several types of cells. The lymphocyte CD4+ is the target of HTLV-1. HTLV-1 downregulates the acquired immune response by changing the expression of the protein FOX P3, which involves to the protein HBZ and transforming growth factor TGF-ß. For leading to immunosuppression

flexibility of differentiation in the programming of CD4+ T cells as part of the adaptive immune response has been recently associated with the pathogenesis of inflammatory diseases (Araya et al. 2014; Ishida and Ueda 2011; Miyazato and Matsuoka 2014; Murphy and Stockinger 2010; Sugata et al. 2012; Toulza et al. 2010). Moreover, the propagation of and pathological damage caused by HTLV-1 involve both the innate and adaptive immune systems. This may explain the long persistence and immune evasion by this virus.

Conclusion

The epidemiology of HTLV-1 has become clearer in the preceding years. The knowledge of geographical distribution, risk factors involved in acquiring the viral infection, and its role in human viral leukemia are important tools in the prevention and treatment of HTLV-1 viral infection and its clinical implications. In addition, it is now accepted that viral infection, specifically HTLV-1, is a cause of human leukemia. Several mechanisms triggered during the viral replication are associated with proteins such as Tax and HBZ, which can affect cell functions to maintain the survival of the infected cell. However, such effects induce molecular disorders that

alter the cell cycle, apoptosis, or immune responses and thus can lead to leukemia. Future research to extend our knowledge about the biology of these proteins is needed to determine whether they are also potential therapeutic targets.

References

Akinbami A, Durojaiye I, Dosunmu A, et al. Seroprevalence of human T-lymphotropic virus antibodies among patients with lymphoid malignancies at a tertiary center in Lagos, Nigeria. J Blood Med. 2014;5:169–74.

Alva I, Orellana R, Blas M, et al. HTLV-1 and -2 infections among 10 indigenous groups in the Peruvian Amazon. Am J Trop Med Hyg. 2012;87(5):954–6.

Araya N, Sato T, Ando H, et al. HTLV-1 induces a Th1-like state in CD4+CCR4+ T cells. J Clin Invest. 2014;124(8):3431–42.

Bangham C. The immune response to HTLV-I. Curr Opin Immunol. 2000;12(4):397–402.

Bangham C, Cook L, Melamed A. HTLV-1 clonality in adult T-cell leukaemia and non-malignant HTLV-1 infection. Semin Cancer Biol. 2014;26:89–98.

Boxus M, Willems L. Mechanisms of HTLV-1 persistence and transformation. Br J Cancer. 2009;101(9):1497–501.

Chan C, Siu Y, Kok K, et al. p21-activated kinases facilitate Tax-mediated transcriptional activation of the human T-cell leukemia virus type 1 long terminal repeats. Retrovirology. 2013;26:10–47.

Colisson R, Barblu L, Gras C, et al. Free HTLV-1 induces TLR7-dependent innate immune response and TRAIL relocalization in killer plasmacytoid dendritic cells. Blood. 2010;115(11):2177–85.

Cook LB, Melamed A, Niederer H, et al. The role of HTLV-1 clonality, proviral structure, and genomic integration site in adult T-cell leukemia/lymphoma. Blood. 2014;123(25):3925–31.

Datta A, Sinha-Datta U, Dhillon N, et al. The HTLV-I p30 interferes with TLR4 signaling and modulates the release of pro- and anti-inflammatory cytokines from human macrophages. J Biol Chem. 2006;281(33):23414–24.

Eshimaa N, Iwata O, Iwata S, et al. Age and gender specific prevalence of HTLV-1. J Clin Virol. 2009;45:135–8.

Felber B, Paskalis H, Kleinman-Ewing C. The pX protein of HTLV-I is a transcriptional activator of its long terminal repeats. Science. 1985;229:675–9.

Fenizia C, Fiocchi M, Jones K, et al. Human T-cell leukemia/lymphoma virus type 1 p30, but not p12/p8, counteracts toll-like receptor 3 (TLR3) and TLR4 signaling in human monocytes and dendritic cells. J Virol. 2014;88(1):393–402.

Francesconi do Valle A, Gutierrez Galhardo, Celestino Leite A. Adult T-cell leukemia/lymphoma associated with HTLV-1 infection in a Brazilian adolescent. Rev Inst Med Trop S Paulo. 2001;43(5):283–6.

Gallo R, Todaro G. Oncogenic RNA viruses. Semin Oncol. 1976;8–95.

Gomes Mello M, Ferreira da Conceição A, Bispo Sousa S, et al. HTLV-1 in pregnant women from the Southern Bahia, Brazil: a neglected condition despite the high prevalence. Virol J. 2014;11:28.

Greaves M. Infection, immune responses and the aetiology of childhood leukaemia. Nat Rev Cancer. 2006;6(3):193–203.

International Committee on Taxonomy of Viruses (ICTV). Virus taxonomy: classification and nomenclature of viruses. [Online]. 2012 [cited 2014 Nov 5]. Available from http://www.ictvonline.org/.

Ishida T, Ueda R. Immunopathogenesis of lymphoma: focus on CCR4. Cancer Sci. 2011; 102(1):44–50.

Jaworski E, Narayanan A, Van Duyne R. Human T-lymphotropic virus type 1-infected cells secrete exosomes that contain Tax protein. J Biol Chem. 2014;289(32):22284–305.
Jeang K, Widen G, Semmes J, et al. HTLV trans-activator protein, tax, is a trans-repressor of the human beta-polymerase gene. Science. 1990;1990(247):1082–4.
Johnson J, Nicot C, Fullen J, et al. Free major histocompatibility complex class I heavy chain is preferentially targeted for degradation by human T-cell leukemia/lymphotropic virus type 1 p12(I) protein. J Virol. 2001;75(13):6086–94.
Journo C, Mahieux R. HTLV-1 and innate immunity. Viruses. 2011;3(8):1374–94.
Jun-ichirou Y, Matsuoka M. Human T-cell leukemia virus type I induces adult T-cell leukemia: from clinical aspects to molecular mechanisms. Cancer Control. 2007;14(2):133–40.
Kane M, Case L, Wang C, et al. Innate immune sensing of retroviral infection via Toll-like receptor 7 occurs upon viral entry. Immunity. 2011;35(1):135–45.
Kastan M, Zhan Q, el-Deiry W, et al. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. Cell. 1992;71(4):587–97.
Kattan T, MacNamara A, Rowan A, et al. The avidity and lytic efficiency of the CTL response to HTLV-1. J Immunol. 2009;182(9):5723–9.
Kawai T, Akira S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity. 2011;34:637–50.
Kumar H, Kawai T, Akira S. Pathogen recognition by the innate immune system. Int Rev Immunol. 2011;30(1):16–34.
Lairmore MD, Franchini G. Chapter 56: Human T-cell leukemia virus types 1 and 2. In: Knipe DM, Howley PM, editors. Fields virology. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 2072–105.
Lester S, Li K. Toll-like receptors in antiviral innate immunity. J Mol Biol. 2014;426(6): 1246–64.
Levin M, Lee S, Kalume F, Stuart JM, et al. Autoimmunity due to molecular mimicry as a cause of neurological disease. Nat Med. 2002;8(5):509–13.
Ma Y, Zheng S, Wang N, et al. Epidemiological analysis of HTLV-1 and HTLV-2 infection among different populations in central China. PLoS One. 2013;8(6):e66795.
Miyazato P, Matsuoka M. Human T-cell leukemia virus type 1 and Foxp3 expression: viral strategy in vivo. Int Immunol. 2014;26(8):419–25.
Murphy K, Stockinger B. Effector T cell plasticity: flexibility in the face of changing circumstances. Nat Immunol. 2010;11(8):674–80.
Nyborg J, Egan D, Sharma N. The HTLV-1 Tax protein: revealing mechanisms of transcriptional activation through histone acetylation and nucleosome disassembly. Biochim Biophys Acta. 2010;1799:266–74.
Olière S, Douville R, Sze A, et al. Modulation of innate immune responses during human T-cell leukemia virus (HTLV-1) pathogenesis. Cytokine Growth Factor Rev. 2011;22(4):197–210.
Peloponese J, Yeung M, Jeang K. Modulation of nuclear factor-kappa B by human T cell leukemia virus type 1 Tax protein: implications for oncogenesis and inflammation. Immunol Res. 2006;34(1):1–12.
Poiesz B, Ruscetti F, Gazdar A, et al. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc Natl Acad Sci U S A. 1980;77(12):7415–9.
Prasetyo A, Dirgahayu P, Sari Y, et al. Molecular epidemiology of HIV, HBV, HCV, and HTLV-1/2 in drug abuser inmates in central Javan prisons, Indonesia. J Infect Dev Ctries. 2013;7(6):453–67.
Reitz Jr MS, Poiesz BJ, Ruscetti FW, Gallo R. Characterization and distribution of nucleic acid sequences of a novel type C retrovirus isolated from neoplastic human T lymphocytes. Proc Natl Acad Sci U S A. 1981;78(3):1887–91.
Ressler S, Morris G, Marriott J. Human T-cell leukemia virus type 1 Tax transactivates the human. J Virol. 1997;71:1181–90.
Saha A, Kaul R, Murakami M, et al. Tumor viruses and cancer biology: modulating signaling pathways for therapeutic intervention. Cancer Biol Ther. 2010;10(10):961–78.

Sakaguchi S, Yamaguchi T, Nomura T, et al. Regulatory T cells and immune tolerance. Cell. 2008;133(5):775–87.

Satou Y, Matsuoka M. Molecular and cellular mechanism of leukemogenesis of ATL: emergent evidence of a significant role for HBZ in HTLV-1-induced pathogenesis. Leuk Res Treat. 2012;2012:213653.

Stienlauf S, Yahalom V, Shinar E, et al. Malignant diseases and mortality in blood donors infected with human T-lymphotropic virus type 1 in Israel. Int J Infect Dis. 2013;17(11):e1022–4.

Stoppa G, Rumiato E, Saggioro D. Ras signaling contributes to survival of human T-cell leukemia/lymphoma virus type 1 (HTLV-1) Tax-positive T-cells. Apoptosis. 2012;17(3):219–28.

Sugata K, Satou Y, et al. HTLV-1 bZIP factor impairs cell-mediated immunity by suppressing production of Th1 cytokines. Blood. 2012;119(2):434–44.

Takashi T, Michael J, Tsuneyasu K. PDLIM2-mediated termination of transcription factor NF-κB activation by intranuclear sequestration and degradation of the p65 subunit. Nat Immunol. 2007;8:584–91.

Tanaka Y, Tanaka R, Terada E, et al. Induction of antibody responses that neutralize human T-cell leukemia virus type I infection in vitro and *in vivo* by peptide immunization. J Virol. 1994;68(10):6323–31.

Tie F, Adya N, Greene G, et al. Interaction of the human T-lymphotropic virus type 1 Tax dimer with CREB and the viral 21-base-pair repeat. J Virol. 1996;70(12):8368–74.

Tiejun Z, Jun-ichirou Y, Yorifumi S, et al. Human T-cell leukemia virus type 1 bZIP factor selectively suppresses the classical pathway of NF-κ. Blood. 2009;113(12):2755–64.

Tomita M, Semenza G, Michiels C, et al. Activation of hypoxia-inducible factor 1 in human T-cell leukaemia virus type 1-infected cell lines and primary adult T-cell leukaemia cells. Biochem J. 2007;406(2):317–23.

Toulza F, Nosaka K, Tanaka Y, et al. Human T-lymphotropic virus type 1-induced CC chemokine ligand 22 maintains a high frequency of functional FoxP3+ regulatory T cells. J Immunol. 2010;185(1):183–9.

Treviño A, Aguilera A, Caballero E, HTLV Spanish Study Group, et al. Trends in the prevalence and distribution of HTLV-1 and HTLV-2 infections in Spain. Virol J. 2012;9:71.

Turvey SE, Broide DH. Innate immunity. J Allergy Clin Immunol. 2010;125 Suppl 2:S24–32.

Chapter 4
Etiology of Leukemia in Children with Down Syndrome

Ana C. Xavier, Yubin Ge, and Jeffrey W. Taub

Abstract Down syndrome (DS) or trisomy 21 is the most common congenital genetic abnormality in the United States, and affected individuals have a unique predisposition to develop acute leukemias early in life. It is estimated that children with DS have a 40- and 150-fold increased risk of developing acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), respectively. The increase in leukemia risk is likely caused by endogenous alterations of genetic factors, including imbalances in chromosome 21-localized genes and altered biochemical pathways in DS cells. The hallmark features of DS-AML include the early development of a precursor disorder known as transient abnormal myelopoiesis (TAM), which clinically resembles AML but is transient in nature, and the presence of *GATA1* (Xp11.23) mutations, which are detectable in the majority of TAM and DS-AML cases. On the other hand, DS-ALL leukemogenesis is linked to alterations in the *CRLF2* gene and associated mutations affecting pathways involving either the *JAK2* or *RAS* genes. In this chapter we review current concepts of mechanisms leading to mutagenesis and leukemia in DS.

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Keywords Down syndrome • Acute lymphoblastic leukemia • Acute myeloid leukemia • Myeloproliferative disorders • GATA1 transcription factor

Introduction

Acute leukemia is the most common type of cancer in children. Although the etiology of acute leukemias remains largely unknown, there is supporting evidence in the literature suggesting that leukemogenesis is a multi-step process in which various genetic hits may be involved. Remarkable relationships exist between chromosome 21 and predisposition to leukemia, leukemogenesis, and response to therapy. Hallmark features of pediatric acute leukemias frequently involve quantitative and/or qualitative changes involving chromosome 21.

Down syndrome (DS; trisomy 21) is a disorder characterized by the constitutional presence of an extra copy of chromosome 21, and such individuals carry a significantly higher predisposition to develop leukemia, especially early in life. A progressively better understanding of the processes involved in malignant transformation in DS cells is providing additional opportunities to answer fundamental questions that still remain in relation to leukemogenesis and response to cancer therapy in patients without DS.

DS is the most common birth defect in the United States and is one of the most studied genetic conditions (Parker et al. 2010). John Langdon Down first described this disorder in 1866 in a group of children displaying common phenotypic features and cognitive impairments (Down 1866). It was only in 1959 that the presence of an extra copy of chromosome 21 was detected as constitutionally present in patients with DS (Lejeune et al. 1959). The first description of leukemia occurring in a child with DS was published in 1930 (Cannon 1930). Since then, it has become evident that individuals with DS have a striking predisposition to develop acute leukemia early in life and that the elevated risk can extend for several decades (Scholl et al. 1982; Hasle et al. 2000). Interestingly, the increased risk of malignancy seems to be limited to the development of leukemias only, since solid tumors occur significantly less frequently in children and adults with DS in comparison with individuals without DS (Xavier et al. 2009). In terms of leukemia risk, it is estimated that children with DS have a 40- and 150-fold increased risk of developing acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), respectively, in comparison with children without DS (Fong and Brodeur 1987; Hasle 2001), making them a significant proportion of patients enrolled in clinical trials. In fact, children with DS represent approximately 2 % and 15 % of the pool of pediatric patients with ALL and AML, respectively (Zeller et al. 2005; Ragab et al. 1991). The increased risks of both types of acute leukemia in the same individual seem to be independent (Hellebostad et al. 2005). Interestingly, there is a 500-fold increased risk of children with DS developing the rare subtype of AML, acute megakaryocytic leukemia (AMkL; French-American-British [FAB] classification M7) (Zipursky et al. 1994). AMkL cases correspond to less than 2 % of adult patients with AML

and up to 10 % of pediatric AML cases (Athale et al. 2001). In contrast, AMkL is the most common subtype of AML in patients with DS (Zeller et al. 2005; Al-Ahmari et al. 2006; Rao et al. 2006; Kudo et al. 2007; Reinhardt et al. 2005; O'Brien et al. 2008; Ravindranath et al. 1992; Gamis et al. 2003). This unique increase in leukemia risk is likely the result of endogenous genetic factors including chromosome 21-localized genes and altered biochemical pathways in DS cells that may drive leukemogenesis.

Down Syndrome–Acute Lymphoblastic Leukemia

Essentially there are no clinical distinctions between patients with DS-ALL and those without it. The peak incidence of ALL in children without DS is between 2 and 5 years, with the majority of cases actually being diagnosed in children aged 2–3 years. Thereafter the incidence steadily decreases, being much less common among children older than 10 years (Howlader et al. 2013a, b). The age of presentation for children with DS-ALL is similar (Robison et al. 1984; Pui et al. 1993; Chessells et al. 2001; Whitlock et al. 2005) or slightly older (Ragab et al. 1991; Dordelmann et al. 1998). Strikingly, ALL has been unreported among children with DS younger than 1 year. No cases of infant DS-ALL were registered among 653 DS cases treated in various collaborative group clinical trials (Ponti di Legno Study Group) between 1995 and 2004 (Buitenkamp et al. 2014). Similarly, no cases of infant DS-ALL were present in other large treatment cohorts (Whitlock et al. 2005; Lundin et al. 2014; Arico et al. 2008). The reasons for this apparent protection against infant ALL in DS remain unknown.

Features including gender, race, initial white blood cell (WBC) count, lymphadenopathy, and hepatosplenomegaly are not significantly different between DS and children without DS with ALL at presentation (Ragab et al. 1991; Pui et al. 1993; Chessells et al. 2001; Dordelmann et al. 1998). However, DS children with ALL have a lower frequency of central nervous system involvement at presentation and less commonly present with an anterior mediastinal mass (Pui et al. 1993; Bassal et al. 2005), although these findings have not been consistent among different DS-ALL cohorts (Zeller et al. 2005; Pui et al. 1993; Chessells et al. 2001; Dordelmann et al. 1998).

There are some noticeable differences in regard to common pediatric prognostic features that may be secondary to different pathogenesis processes. ALL of the T-cell phenotype is a very aggressive malignancy derived from T-cell progenitor cells, accounting for about 15 % of the pediatric leukemia cases in children without DS (Pizzo and Poplack 2011), and historically has an inferior outcome in comparison with acute leukemias of the B-cell phenotype. For reasons that remain unknown, T-cell ALL occurs rarely among DS children. In fact, several cohorts of patients with DS-ALL covering a large time span reported no cases of T-cell ALL at all (Zeller et al. 2005). Lower frequencies of common cytogenetic abnormalities are also seen among children with DS-ALL. They have a lower incidence of the hyper-

diploid karyotype, the *ETV6-RUNX1* t(12;21) fusion protein (Zeller et al. 2005; Pui et al. 1993; Lundin et al. 2014), or other genetic alterations such as t(9;22) (q34;q11) (*BCR/ABL* fusion gene), *MLL* rearrangements, and t(1;19) (*TCF3-PBX1* fusion gene) (Pui et al. 1993; Chessells 2001; Forestier et al. 2008).

Another important differentiation is that DS children with ALL have an inferior outcome and a greater incidence of treatment-related mortality compared with children without DS with ALL. Early reports showed that despite having similar age and WBC count at diagnosis, patients with DS-ALL had significantly lower remission rates, higher mortality rates during induction, and decreased long-term overall survival (Robison et al. 1984; Kalwinsky et al. 1990; Levitt et al. 1990). These differences have since been confirmed by multiple different trials (Ragab et al. 1991; Pui et al. 1993; Chessells et al. 2001; Whitlock et al. 2005; Dordelmann et al. 1998; Bassal et al. 2005; Rajantie and Siimes 2003). Intensification of therapy may be beneficial in improving event-free survival (EFS), although patients with DS-ALL continue to face excessive treatment-related morbidity and mortality (Ragab et al. 1991; Buitenkamp et al. 2014; Patrick et al. 2014), with higher rates of severe mucositis and infections, owing to more severe and prolonged myelosuppression (Buitenkamp et al. 2014; Rabin et al. 2012). Systemic toxicity may be intrinsically related to the constitutional presence of an extra copy of chromosome 21, with subsequent differences in pharmacokinetics of drugs or pharmacodynamic effects in the tissues (Garre et al. 1987; Buitenkamp et al. 2010). For instance, DS children poorly tolerated treatment with the antifolate agent, methotrexate. The reduced folate carrier gene is localized to chromosome 21 (*SLC19A1*, 21q22), and its increased expression in various DS tissues may result in increased intracellular methotrexate transport and consequent increased cellular toxicity. These unique distinctions suggest that besides the linkage to leukemogenesis, trisomy 21 is also linked to metabolism of chemotherapy drugs, toxicity, and response to therapy (Xavier et al. 2009).

The "Two-Hit" Model of DS-ALL Leukemogenesis

The exact mechanisms by which an additional copy of chromosome 21 predisposes to leukemia remain unknown. Carcinogenesis is a complex process that results in essential alterations in cell physiology, usually driven by mutations, genomic instability, epigenetic events, etc. DNA can be modified spontaneously in nature or after environmental exposure to mutagenic factors, such as viruses, radiation, or chemicals. Mutagenesis in certain types of cancer is greatly influenced by environmental factors, such as tobacco exposure and lung cancer (Doll and Peto 1978), or virus oncogenicity in bladder cancer (Parada et al. 1982).

In terms of leukemogenesis, it has been proposed that pediatric ALL results from at least two independent and sequential genetic mutations or events (Greaves 1988). The "two-hit" model of leukemogenesis was postulated by Mel Greaves: a preleukemic clone would arise in utero during the expansion of the B-cell precursor

compartment ("first hit"), creating a "preleukemia state," and a second mutation ("second hit") would potentially occur after birth, likely resulting from environmental exposures such as infections, or even inherited susceptibility (Greaves 2002). This model is well accepted for ALL cases characterized by the presence of chromosomal translocations that result in functional leukemia fusion genes (e.g., *ETV6-RUNX1* fusion genes) (Greaves 2002).

Trisomy 21 Taking this model into account for DS-ALL, it is possible that the "first hit" is actually the presence of an extra copy of chromosome 21. A trisomic state would lead to gene dosage imbalances that promote changes in physiological cell processes or lead to deleterious mutations. In fact, some studies using DS mouse models have suggested that the presence of trisomic genes can induce the development of heart defects (Liu et al. 2011) and promote cognitive behavior changes. Liver and marrow of DS human fetuses collected at early gestational ages display expansion of the erythroid and megakaryocytic (Yu et al. 2010) compartments and changes in lymphopoiesis without the presence of additional mutations, suggesting that the abnormal fetal hematopoiesis is likely driven by the presence of an extra copy of chromosome 21 (Chou et al. 2008; Tunstall-Pedoe et al. 2008; Roberts et al. 2013) (Fig. 4.1).

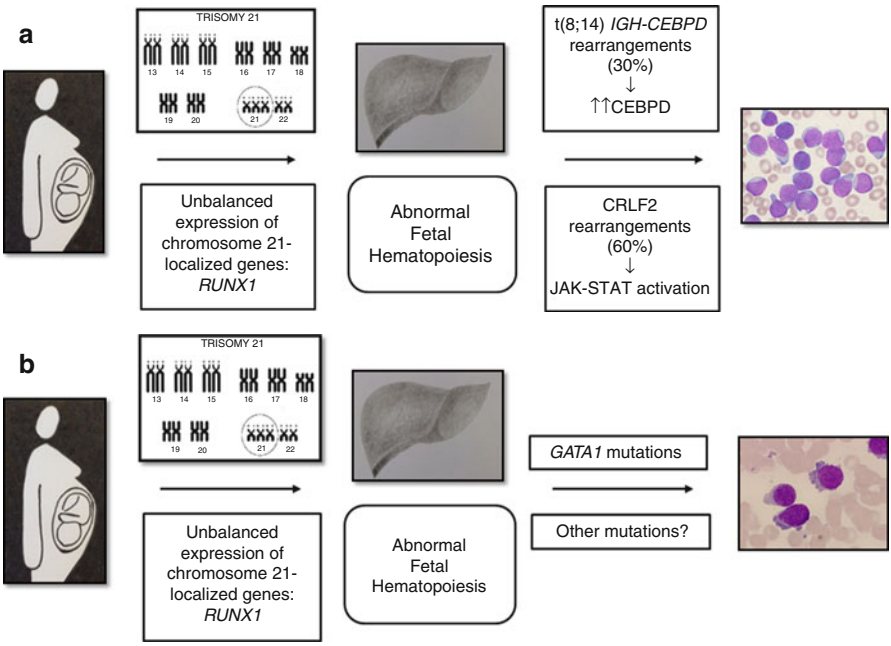

Fig. 4.1 Gene dosage imbalances caused by the presence of an extra copy of chromosome 21 in Down syndrome babies would lead to abnormal fetal hematopoiesis. Consequently, altered physiological cell processes would promote gene rearrangements and deleterious mutations, leading to the development of acute lymphoblastic leukemia (**a**) or acute myeloid leukemia (**b**)

Underlining the importance of chromosome 21 in the etiology of leukemias is the fact that somatic quantitative or qualitative changes in chromosome 21 are commonly found in non-DS B-precursor ALL patients. For instance, the t(12;21) (p13;q22) chromosomal translocation that leads to the *ETV6-RUNX1* fusion gene is present in about 20–30 % of the non-DS-ALL pediatric cases (Pui et al. 2008). Among these patients a large proportion exhibit secondary aberrations, with the most frequent being the presence of an extra copy of chromosome 21 (Loncarevic et al. 1999; Ma et al. 2001). High hyperdiploid karyotype (defined as 51–65 chromosomes per cell), which occurs in 20–25 % of the non-DS B-precursor ALL cases (Paulsson and Johansson 2009), almost uniformly have three to four copies of the chromosome 21.

Of note, children with DS-ALL may have similar cytogenetic abnormalities such as t(12;21) (p13;q22) or high hyperdiploid karyotype, although these changes are found in a much smaller proportion of cases (Zeller et al. 2005; Pui et al. 1993; Dordelmann et al. 1998; Buitenkamp et al. 2014; Arico et al. 2008; Bassal et al. 2005; Chessells 2001; Forestier et al. 2008; Lanza et al. 1997; Maloney et al. 2010). Interestingly, array comparative genome hybridization analyses of DS-ALL samples without *ETV6-RUNX1* fusion showed cytogenetic changes similar to those found in non-DS *ETV6-RUNX1* positive ALL samples (Lo et al. 2008). Other established genetic alterations such as t(9;22) (q34;q11) (*BCR/ABL* fusion gene), *MLL* rearrangements, and t(1;19) (*TCF3-PBX1* fusion gene) are also found in DS-ALL, but at a lower frequency (Pui et al. 1993; Chessells 2001; Forestier et al. 2008; Kalwinsky et al. 1990). This suggests that constitutional trisomy 21 may promote an apparent protection against common recurrent genetic abnormalities in ALL that involve chromosomal translocations, with consequent reduction of DS children presenting with infant leukemia (*MLL*-rearranged ALL), Ph+ ALL (*BCR-ABL*), and T-cell ALL (multiple different translocations involved) (Zeller et al. 2005; Buitenkamp et al. 2014). These differences stress the heterogeneity of childhood leukemia and the complexity of leukemogenesis in different groups of patients.

Chromosome 21-Localized Genes Several chromosome 21-localized genes can potentially play a role in leukemogenesis in an unbalanced state. *RUNX1* (alternative names *AML1*; core-binding factor, runt domain α-subunit 2, CBFA2) is the gene more frequently implicated in leukemia. *RUNX1* is part of the *RUNX* gene family (*RUNX2* and *RUNX3*) of transcription factors (TFs) that bind DNA via a Runt domain and a β-subunit encoded by the *CBFB* (core-binding factor, β-subunit, CBFB) gene. *RUNX1* plays key regulatory roles during hematopoiesis via regulation of various hematopoietic genes (Cohen 2009) which, when altered, result in leukemia. *RUNX1* is frequently found translocated in patients with AML (*AML1-ETO*, *AML1-MDS1-EAI1*, *AML1-FOG2*) (Helbling et al. 2004; McNeil et al. 1999; Chan et al. 2005) and ALL (*ETV6-RUNX1*) (Hong et al. 2008), and the chimeric proteins that result from the fusion induce leukemogenesis.

Nonsense/missense or deletion mutations involving *RUNX1*—that lead to a gene "haploinsufficient state"—have been found to be causative of an autosomal dominant disorder known as familiar platelet disorder with associated myeloid

malignancy (FPD/AML). FPD/AML is characterized by platelet dysfunction, altered megakaryopoiesis, and elevated risk of developing AML (Song et al. 1999). In this condition the simple inactivation of one allele is sufficient to predispose to leukemia (Song et al. 1999). In mouse models, *RUNX1* haploinsufficiency has been shown to alter hematopoiesis (Mukouyama et al. 2000). Similarly, Preudhomme et al. (2000) found an elevated incidence of missense mutations or deletions in the Runt domain, likely resulting in nonfunctional AML1 protein, in multiple different cases of hematological disorders characterized by abnormalities of *RUNX1*, including acquired trisomy 21 and tetrasomy 21 (Mukouyama et al. 2000; Roumier et al. 2003). On the other hand, overexpression of *RUNX1* in a cell model (NIH3T3 cells) induced neoplastic transformation (Kurokawa et al. 1996), suggesting that a higher *RUNX1* gene dosage can induce leukemia per se. *RUNX1* gene amplification either via multiple copies of chromosome 21 or via high-level amplification (intrachromosomal amplification or extra chromosomes) has been reported in pediatric ALL. These cases usually have corresponding increases in AML1 transcripts that are equivalent to the number of the amplified *RUNX1* gene (Busson-Le Coniat et al. 2001). Interestingly, patients with B-precursor ALL and intrachromosomal amplification of chromosomal 21 (iAMP21) have a very poor prognosis. Genomic characterization of cases harboring iAMP21 showed recurrent abnormalities in other genes such as *IKZF1*, *CDKN2A/B*, *PAX5*, *ETV6*, and *RB1*, likely secondary to chromosome 21 rearrangements (Rand et al. 2011).

Subsequent Genetic Changes Once perturbed hematopoiesis is established, multiple additional genetic abnormalities can take place ("second hit"?). Specific cytogenetic changes have been observed in DS-ALL. Otherwise rare in non-DS-ALL (<3 %), up to 30 % of the patients with DS-ALL have translocations involving chromosomes 8 and 14 [t(8;14) (q11;q32)] (*IGH-CEBPD* fusion gene) (Forestier et al. 2008; Moore et al. 2003; Lundin et al. 2009). CCAAT/enhancer-binding protein δ (CEBPD) TF is part of the CEBP family of TFs composed of at least six multifunctional basic leucine zipper (bZIP) members, which play important roles in cellular differentiation, particularly hematopoietic tissues, hepatocytes, and adipocytes. The regulation of these genes is extremely complex and involves hormones, cytokines, nutrients, toxins, etc. (Ramji and Foka 2002), and all members of this family have been implicated in leukemias or solid tumors (Ramji and Foka 2002; Nerlov 2007). The fusion of *IGH* (immunoglobulin G heavy-chain locus; IGHG1) to *CEBPD* leads to activation of *CEBPD* and overexpression of the gene by mechanisms that remain unclear.

More recently, genomic abnormalities of cytokine receptor-like factor 2 (CRLF2) have been detected in approximately 60 % of DS-ALL cases and seem to be a unique feature of DS-ALL because of the rarity of non-DS cases with analogous abnormalities (Mullighan et al. 2009). Similar to the *IGH-CEBPD* scenario, *CRLF2* (Xp22/Yp11) rearrangements can result from either (1) an intrachromosomal deletion of PAR1 (pseudoautosomal region 1) leading to *P2RY8-CRLF2* fusion or (2) a translocation with the IGH locus at 14q32 [(X;14)(p22;q32)/t(Y;14)(p11;q32)] (Mullighan et al. 2009). Both aberrations lead to overexpression of CEBPD. Prior

to the CEBPD findings, Malinge et al. had detected a novel *JAK2* (9p24) mutation in a sample from a child with B-precursor DS-ALL that involved a 5-amino-acid deletion within the JH2 pseudokinase domain (JAK2DeltaIREED) (Malinge et al. 2007). *JAK2* is a tyrosine kinase that phosphorylates cytoplasmic targets essential for signaling of hematopoietic and growth factor receptors (Kralovics et al. 2005). Interestingly, expression of JAK2DeltaIREED in Ba/F3 cells led to constitutive activation of the JAK-STAT pathway and growth factor-independent cell proliferation (Malinge et al. 2007). Subsequently, the presence of *JAK2* mutations was found in a large proportion of patients with DS-ALL (Bercovich et al. 2008; Kearney 2009). *CRLF2* alterations were found to be associated with activating *JAK2* mutations and constitutive JAK-STAT activation, which likely contribute to DS-ALL leukemogenesis (Mullighan et al. 2009; Russell et al. 2009; Hertzberg et al. 2010). Interestingly, in patients not displaying abnormalities in the *JAK2* gene, driver mutations in *RAS* (*KRAS* and *NRAS*) were found in a high proportion of cases (Nikolaev et al. 2014). Additional analysis revealed that both *RAS* and *JAK2* drove subclonal expansions primarily initiated by *CRLF2* rearrangements, and/or mutations in chromatin remodelers and lymphocyte differentiation factors, providing new insights in the understanding of DS leukemogenesis (Nikolaev et al. 2014). Another way of altering expression of *CRLF2* is through gain of chromosome X, a common abnormality among patients with DS-ALL (38 % in DS-ALL cases versus 20 % of non-DS-ALL cases) (Zeller et al. 2005; Forestier et al. 2008; Baker et al. 2003). All DS cases displaying extra copies of chromosome X also had overexpression of CRLF2 (Mullighan et al. 2009; Hertzberg et al. 2010), suggesting that CRLF2 alterations are indeed important in DS-ALL generation.

The Environment The "two-hit" model of leukemias suggests that the postnatal genetic changes needed for leukemia development may be caused by an abnormal immune response to environmental factors, such as delayed infections (Greaves 2002). Lack of exposure to infections early in life would lead to poor immune system modulation and potentially result in leukemia (Greaves 1997, 2002). Among children with DS-ALL, the Children's Oncology Group (COG) found a negative association between acute leukemia and any infection in the first 2 years of life, supporting the idea that early infection may be protective against leukemia in DS children as well (Canfield et al. 2004). Conversely, a study conducted in Mexico City showed a nonsignificant association between early infections and DS-ALL (Flores-Lujano et al. 2009). This study also did not find breastfeeding to be protective of leukemia in DS (Flores-Lujano et al. 2009). Preconception, in utero, and postnatal medical test irradiation exposure was also studied by COG, and no positive association was found with DS-ALL (Linabery et al. 2006). Preconception vitamin supplementation was found to be protective against DS-ALL (Ross et al. 2005), as well as certain maternal conditions such as vaginal bleeding (Ognjanovic et al. 2009), while maternal exposure to professional pest exterminators, pesticides, and any chemicals was positively associated with DS-ALL (Alderton et al. 2006). Larger epidemiology studies are necessary to confirm or exclude environmental factors in the etiology of DS-ALL.

Down Syndrome: Acute Myeloid Leukemia (DS-AML)

Myeloid Proliferation Related to Down Syndrome

AMkL is the most common FAB subtype (M7) of patients with DS-AML, with a frequency ranging from 40 to 100 % of the DS-AML cases in different clinical trials (Zeller et al. 2005; Al-Ahmari et al. 2006; Rao et al. 2006; Kudo et al. 2007; Reinhardt et al. 2005; O'Brien et al. 2008; Ravindranath et al. 1992; Gamis et al. 2003). Zipursky et al. (1994) estimated that DS children have a 500-fold increased risk of developing AMkL compared with children without DS, once more highlighting the unique relationship between trisomy 21 and leukemogenesis for a specific leukemia phenotype (Zipursky et al. 1994). In contrast, AMkL is estimated to represent approximately 10 % of pediatric AML cases and 2 % of adult AML cases (Athale et al. 2001; Tallman et al. 2000).

The differences between DS and non-DS cases are not only restricted to differences in subtype of myeloid leukemia. Multiple pediatric oncology cooperative group clinical trials have reported that patients with DS-AML have remarkably high EFS rates (~80–100 %) when treated with cytarabine/anthracycline-based chemotherapy (Zeller et al. 2005; Al-Ahmari et al. 2006; Rao et al. 2006; Kudo et al. 2007; O'Brien et al. 2008; Ravindranath et al. 1992; Creutzig et al. 2005). In contrast, AMkL in children without DS is associated with a relatively poor prognosis, with EFS of less than 40 % (O'Brien et al. 2013).

Interestingly, up to 10 % of newborns with DS will present with a condition known as transient abnormal myelopoiesis (TAM). This disorder, previously called "transient leukemia," is characterized by circulating blast cells in the peripheral blood with AMkL morphology and immunophenotype. TAM resolves spontaneously without chemotherapy in a high proportion of patients (Zipursky 2003). However, a subset of patients with high-risk features (e.g., hyperleukocytosis, hepatic failure) requires therapy and has a guarded prognosis (Massey et al. 2006). TAM is considered a precursor of DS-AML, as approximately 30 % of patients with DS-TAM will subsequently develop AML or, more commonly, AMkL following clinical resolution of TAM (Zipursky 2003). Hence, patients with DS-TAM represent a subgroup of individuals with one of the highest predicted predispositions to develop acute leukemia.

Prior to the diagnosis of AML, DS patients may develop signs of myelodysplasia, characterized by progressive anemia and thrombocytopenia, dysplastic erythroid cells, and megakaryocytes in the bone marrow. The myelodysplastic phase frequently precedes the development of AML (Zipursky 2003). Both myelodysplastic syndrome (MDS) and AML are often referred as the "myeloid leukemia associated with DS" (ML-DS). TAM and ML-DS are now considered separately from the other subtypes of AML by the World Health Organization classification and are designated as Myeloid Proliferation related to DS (MP-DS) (Swerdlow et al. 2008).

GATA1 *Gene and DS-Acute Myeloid Leukemogenesis*

The GATA1 Gene. The *GATA1* gene (GATA-binding protein 1; Xp11.23) encodes a zinc finger DNA-binding transcriptional factor expressed in erythroid, megakaryocyte, mast, and eosinophil lineages, which detains critical roles during normal hematopoiesis. The GATA1 N-terminal region has transactivation activity and its C-terminal domain binds DNA or other factors (Calligaris et al. 1995). GATA1 protein forms essential activating or repressing complexes with other partner proteins, such as FOG1 (friend of GATA1), CBP (CREB-binding protein), and Med1 (mediator complex subunit 1), to control and promote differentiation of erythroid and megakaryocytic cells (Crispino et al. 1999; Blobel et al. 1998; Stumpf et al. 2006; Crispino 2005). Enforced expression of GATA1 in primitive myeloid cell lines or hematopoietic stem cells induced megakaryocytic/erythroid differentiation, and loss of self-renewal activity (Visvader et al. 1995; Iwasaki et al. 2003; Yamaguchi et al. 1998; Ferreira et al. 2007). On the other hand, inactivation of *GATA1* in a mouse model caused death of male mice during gestation from severe anemia resulting from erythroid development arrest and nonlethal anemia in female mice that exhibited a heterozygous state due to random inactivation of the X chromosome (Fujiwara et al. 1996).

There are two GATA1 isoforms that result from alternative translation initiation sites (Calligaris et al. 1995). The *GATA1* gene encodes a 1.8-kb mRNA that can be translated in a 47-kDa protein or a shorter 40-kDa protein, known as GATA1s. GATA1s is translated from a downstream initiation site and lacks the N-terminal transactivation domain. GATA1 and GATA1s share identical binding activity but differ in their transactivation capacity (Calligaris et al. 1995). The two isoforms have been shown to be present in mouse embryo tissues (Calligaris et al. 1995), and have been associated with diseases. Nonsense mutations leading to truncated GATA1 proteins have been found not only in mammals but also in a set of "bloodless" zebrafish mutants characterized by a severe reduction in blood cell progenitors and circulating blood cells (Lyons et al. 2002). Loss of GATA1 has also been shown to alter erythropoiesis into myelopoiesis (Galloway et al. 2005). In humans, germline GATA1 mutations have been associated with hematopoietic disorders. Patients with X-linked thrombocytopenia (Nichols et al. 2000; Freson et al. 2001), X-linked thrombocytopenia with β-thalassemia (Yu et al. 2002) or X-linked anemia with or without neutropenia and/or platelet abnormalities (Hollanda et al. 2006), and X-linked gray platelet syndrome (Tubman et al. 2007) show various degrees of anemia, thrombocytopenia, and dyserythropoiesis that result from abnormal interactions between GATA1 and partner proteins, depending on the location of the *GATA1* mutation (Ciovacco et al. 2008). Germline mutations leading to the formation of GATA1s have also been described (Hollanda et al. 2006). Those patients presented with anemia, neutropenia, or platelet disorders; however, no leukemia cases have been described, suggesting that, although altered, GATA1s can sustain erythropoiesis.

In 2002, Wechsler et al. (2002) analyzed several samples from individuals with AML for the presence of *GATA1* mutations. Mutations were detected uniformly and exclusively only in DS-AMkL samples. Each of the mutations altered the reading frame and introduced a premature stop codon in the N-terminal transactivation domain, leading to GATA1s production (Wechsler et al. 2002). Subsequent studies showed the uniform presence of acquired *GATA1* mutations in nearly all TAM and DS-AMkL cases (Hitzler et al. 2003; Mundschau et al. 2003; Rainis et al. 2003). The exclusive detection of somatic mutations in the X-linked chromosome gene *GATA1* in DS-AMkL cases is a unique association between a gene mutation in a homogeneous subgroup of leukemia patients, which is linked to altered hematopoiesis and the downstream development of leukemia.

The "Mutator Phenotype". There is no obvious relationship linking a X-linked chromosome gene mutation with chromosome 21, yet one must exist to account for the finding of GATA1 mutations only in the DS population (including individuals with mosaicism of chromosome 21), suggesting the possibility that trisomy 21 induces a "mutator phenotype." It has been well described that trisomy 21 alters fetal liver hematopoiesis, promoting expansion of erythroid and megakaryocytic compartments (Chou et al. 2008; Tunstall-Pedoe et al. 2008; Roberts et al. 2013; Hoeller et al. 2014). There is also supporting evidence that *GATA1* mutations arise during fetal development, as *GATA1* mutations have been retrospectively detected in Guthrie newborn screening cards from patients with DS-AMkL (Ahmed et al. 2004) and have been detected in DS fetal livers as early as 21 weeks of gestational age (Taub et al. 2004). However, the exact mechanism of mutagenesis in DS is not completely understood.

Multiple studies have demonstrated evidence of DNA repair defects in DS cells. DS lymphocytes showed lower baseline DNA repair, and exhibited increased sensitivity to phytohemagglutinin stimulation, N-methyl-N′-nitro-N-nitrosoguanidine, and γ-irradiation, indicating an increased sensitivity to DNA oxidation, methylation, and strand breaks (Agarwal et al. 1970; Ankathil et al. 1997; Morawiec et al. 2008; Lavin et al. 1989). While more than one DNA repair pathway might be affected by the DS phenotype, base excision repair (BER) deficiency is a compelling candidate because it repairs these types of DNA damage.

By analyzing all published studies in which sequence data on *GATA1* mutations was available, Cabelof et al. (2009) began to elucidate possible mechanisms by which these sequence alterations arise. Mutational analysis revealed a predominance of small insertion/deletion, duplication, and base substitution mutations including G:C>T:A, G:C>A:T, and A:T>G:C. This mutational spectrum suggests that oxidative stress and aberrant folate metabolism secondary to genes on chromosome 21 (e.g., superoxide dismutase [SOD] and cystathionine-β-synthase [CBS]) may be linked to the generation of *GATA1* mutations. Both CBS and SOD transcripts are significantly overexpressed in DS-AMkL blasts compared with non-DS-AML (median 12- and 4-fold, respectively) (Taub et al. 1999). CBS overexpression has been associated with a functional folate deficiency (Li et al. 2005) and may result in increased uracil incorporation into DNA, thus providing another mechanism for

generation of mutations in DS. As the rate-limiting enzyme in the BER pathway, loss of β-pol (DNA polymerase β) could result in increased susceptibility to the mutagenic effects of unrepaired endogenous damage caused by high levels of uracil incorporation.

The relationship between two key BER gene products involved in the repair of uracil in DNA, uracil DNA glycosylase (UDG) and β-pol, and DS phenotype was evaluated in DS tissues (Cabelof et al. 2009). UDG is a monofunctional glycosylase that excises uracil from DNA to initiate BER. Loss of UDG in *Escherichia coli* and in mouse models induces mutations characterized predominantly by the G:C>A:T transition, similar to what was observed in DS (Fix and Glickman 1987). DS samples exhibited 75 % lower *UDG* expression than the non-DS (Cabelof et al. 2009). Hence, DS may predispose to mutagenesis through a uracil intermediate as a result of reduced *UDG* expression. Interestingly, DS samples (TAM and AMkL together) showed a 90 % reduction in *β-pol* expression compared with non-DS-AMkL samples. This finding is striking, as 50 % reduction in *β-pol* expression predisposed mice to develop cancer (Cabelof et al. 2006). Germline *β-pol* polymorphisms, leading to slower catalytic rates, cause increased double-strand breaks, chromosomal aberrations, and cellular transformation (Yamtich et al. 2012). Furthermore, DNA repair capacity evaluated in DS and non-DS patient samples provided evidence that the BER pathway was compromised in DS tissues (Cabelof et al. 2009), suggesting that inability to repair DNA damage may also play critical roles in the unique susceptibility of DS children to develop leukemia.

The generation of GATA1s as an end result of the mutations may provide a selective growth advantage allowing for the survival of preleukemic clones, which may ultimately lead to the development of TAM and AMkL in DS. In fact, the induction of GATA1s expression in mice led to hyperproliferation of a unique, previously unrecognized yolk sac and fetal liver progenitor, which the authors proposed to account for the transient nature of TAM and the restriction of DS-AMkL to the first years of life (Li et al. 2005). *GATA1* knockdown in a DS-AMkL cell model resulting in lower GATA1s protein levels promoted cell differentiation towards the megakaryocytic lineage, repressed cell proliferation, and increased basal apoptosis and susceptibility to various chemotherapy drugs, accompanied by downregulation of Bcl-2 and altered expression of genes related to cell death, proliferation, and differentiation (Xavier et al. 2011).

Another important aspect is the fetal liver environment. It is possible that the initial genetic hits that drive leukemogenesis depend on interactions with local stroma. Miyauchi and Kawaguchi (2014) showed that fetal liver stromal cells, but not fetal bone marrow, supported the growth of TAM blast progenitors, mainly through humoral factors. They found high concentrations of hematopoietic growth factors in culture supernatants of the fetal liver stromal cells, suggesting that a unique hematopoietic microenvironment may be the key to sustain the growth of leukemia cells.

Footsteps to Leukemia. *GATA1* mutations and GATA1s represent early or initiating "genetic hits" in a multi-step process of leukemogenesis. Whole-genome and/or whole-exome sequencing of samples from individuals with DS with TAM showed

only the exclusive presence of GATA1 mutations (Yoshida et al. 2013). The natural history of patients with TAM is the spontaneous clinical regression in the majority of cases with support of care alone (Zipursky 2003). The mechanisms behind TAM involution remain unknown. However, a proportion of DS children will, after a period of latency that can last a few years, develop MDS/AMkL that will require treatment with multi-drug chemotherapy. What drives the full development of leukemia is not completely understood, and the presence of a mutated GATA1 protein is unlikely the only driving force in leukemogenesis. This has been shown in studies using DS mouse models in which the introduction of GATA1s resulted in increased megakaryopoiesis, abnormalities in the liver and bone marrow, or anemia, but did not result in leukemia (Alford et al. 2010; Carmichael et al. 2009).

In addition, genomic profile performed on samples from patients with DS-AMkL have revealed mutations in other target genes, including genes involved in epigenetic regulation, common signaling pathways, and multiple cohesion components, in addition to the presence of GATA1 mutations (Yoshida et al. 2013). *KIT*, *FLT3*, *JAK2 JAK3*, and *MPL* gene mutations have been identified DS TAM or AMkL samples (De Vita et al. 2007; Norton et al. 2007). More recently, and using DS TAM/AMkL exome sequencing and genome-wide single nucleotide polymorphism (SNP) microarray, Nikolaev et al. found that 40 % of TAM cases and all AMkL cases showed mutations/deletions other than GATA1 in genes proven as transformation drivers in non-DS leukemia (*EZH2*, *APC*, *FLT3*, *JAK1*, *PARK2-PACRG*, *EXT1*, *DLEC1*, *SMC3*). Two clonal expansions with different GATA1 mutations were found in a TAM sample, one clone with an additional driver mutation and a second clone that gave rise to AMkL after accumulation mutations in seven other genes (Nikolaev et al. 2013). These findings suggested that *GATA1* mutations alone are sufficient for clonal expansion, and that the presence of additional mutations at the TAM stage do not predict AMkL progression. The authors postulated that leukemia progression requires a "third-hit driver," putative driver mutations resulting in aberrant activation of WNT, JAK-STAT, or MAPK-PI3K pathways and consequent overexpression of MYC (Nikolaev et al. 2013). The presence of multiple subclones with varying leukemia-initiating potential and self-renewal capacity was also suggested in a xenograft model of TAM: during serial transplantation of TAM-derived cells, divergent subclones with another *GATA1* mutation and various copy number alterations emerged (Saida et al. 2013). Epigenetic changes can also contribute to leukemogenesis in DS. Early genome-wide DNA methylation changes were detected in DS fetal liver mononuclear cells prior to the presence of GATA1 mutations. These changes were characterized by loss of DNA methylation at genes associated with development disorders. Gain of methylation was detected in DS TAM/AMkL samples, affecting different sets of genes involved in hematopoiesis and the regulation of cell growth and proliferation (Malinge et al. 2013).

In summary, the mechanism of leukemogenesis in DS children is probably multifactorial and involves chromosome 21-localized genes as well as genes localized to other chromosomes. Studying leukemia in DS children is a paradigm to further improve our understanding of the role of genetic disorders associated with a

predisposition to develop cancer and the role of specific genes associated with cancer predisposition. Future work identifying the mechanisms underlying *GATA1* mutagenesis and leukemogenesis in DS will shed important light on why DS children have a significantly higher risk of developing acute leukemia in comparison with children without DS.

References

Agarwal SS, Blumberg BS, Gerstley BJ, London WT, Sutnick AI, Loeb LA. DNA polymerase activity as an index of lymphocyte stimulation: studies in Down's syndrome. J Clin Invest. 1970;49(1):161–9.

Ahmed M, Sternberg A, Hall G, et al. Natural history of GATA1 mutations in Down syndrome. Blood. 2004;103(7):2480–9.

Al-Ahmari A, Shah N, Sung L, Zipursky A, Hitzler J. Long-term results of an ultra low-dose cytarabine-based regimen for the treatment of acute megakaryoblastic leukaemia in children with Down syndrome. Br J Haematol. 2006;133(6):646–8.

Alderton LE, Spector LG, Blair CK, et al. Child and maternal household chemical exposure and the risk of acute leukemia in children with Down's syndrome: a report from the Children's Oncology Group. Am J Epidemiol. 2006;164(3):212–21.

Alford KA, Slender A, Vanes L, et al. Perturbed hematopoiesis in the Tc1 mouse model of Down syndrome. Blood. 2010;115(14):2928–37.

Ankathil R, Kusumakumary P, Nair MK. Increased levels of mutagen-induced chromosome breakage in Down syndrome children with malignancy. Cancer Genet Cytogenet. 1997; 99(2):126–8.

Arico M, Ziino O, Valsecchi MG, et al. Acute lymphoblastic leukemia and Down syndrome: presenting features and treatment outcome in the experience of the Italian Association of Pediatric Hematology and Oncology (AIEOP). Cancer. 2008;113(3):515–21.

Athale UH, Razzouk BI, Raimondi SC, et al. Biology and outcome of childhood acute megakaryoblastic leukemia: a single institution's experience. Blood. 2001;97(12):3727–32.

Baker JM, Coppes MJ, Roland B. A case of Down syndrome with acute lymphoblastic leukemia and isochromosome Xp. Cancer Genet Cytogenet. 2003;147(1):75–7.

Bassal M, La MK, Whitlock JA, et al. Lymphoblast biology and outcome among children with Down syndrome and ALL treated on CCG-1952. Pediatr Blood Cancer. 2005;44(1):21–8.

Bercovich D, Ganmore I, Scott LM, et al. Mutations of JAK2 in acute lymphoblastic leukaemias associated with Down's syndrome. Lancet. 2008;372(9648):1484–92.

Blobel GA, Nakajima T, Eckner R, Montminy M, Orkin SH. CREB-binding protein cooperates with transcription factor GATA-1 and is required for erythroid differentiation. Proc Natl Acad Sci U S A. 1998;95(5):2061–6.

Buitenkamp TD, Mathot RA, de Haas V, Pieters R, Zwaan CM. Methotrexate-induced side effects are not due to differences in pharmacokinetics in children with Down syndrome and acute lymphoblastic leukemia. Haematologica. 2010;95(7):1106–13.

Buitenkamp TD, Izraeli S, Zimmermann M, et al. Acute lymphoblastic leukemia in children with Down syndrome: a retrospective analysis from the Ponte di Legno study group. Blood. 2014;123(1):70–7.

Busson-Le Coniat M, Nguyen Khac F, Daniel MT, Bernard OA, Berger R. Chromosome 21 abnormalities with AML1 amplification in acute lymphoblastic leukemia. Genes Chromosomes Cancer. 2001;32(3):244–9.

Cabelof DC, Ikeno Y, Nyska A, et al. Haploinsufficiency in DNA polymerase beta increases cancer risk with age and alters mortality rate. Cancer Res. 2006;66(15):7460–5.

Cabelof DC, Patel HV, Chen Q, et al. Mutational spectrum at GATA1 provides insights into mutagenesis and leukemogenesis in Down syndrome. Blood. 2009;114(13):2753–63.
Calligaris R, Bottardi S, Cogoi S, Apezteguia I, Santoro C. Alternative translation initiation site usage results in two functionally distinct forms of the GATA-1 transcription factor. Proc Natl Acad Sci U S A. 1995;92(25):11598–602.
Canfield KN, Spector LG, Robison LL, et al. Childhood and maternal infections and risk of acute leukaemia in children with Down syndrome: a report from the Children's Oncology Group. Br J Cancer. 2004;91(11):1866–72.
Cannon HE. Acute lymphatic leukemia: report of a case in an eleventh month Mongolian idiot. New Orleans Med Surg J. 1930;82:872–3.
Carmichael CL, Majewski IJ, Alexander WS, et al. Hematopoietic defects in the Ts1Cje mouse model of Down syndrome. Blood. 2009;113(9):1929–37.
Chan EM, Comer EM, Brown FC, et al. AML1-FOG2 fusion protein in myelodysplasia. Blood. 2005;105(11):4523–6.
Chessells JM. Pitfalls in the diagnosis of childhood leukaemia. Br J Haematol. 2001; 114(3):506–11.
Chessells JM, Harrison G, Richards SM, et al. Down's syndrome and acute lymphoblastic leukaemia: clinical features and response to treatment. Arch Dis Child. 2001;85(4):321–5.
Chou ST, Opalinska JB, Yao Y, et al. Trisomy 21 enhances human fetal erythro-megakaryocytic development. Blood. 2008;112(12):4503–6.
Ciovacco WA, Raskind WH, Kacena MA. Human phenotypes associated with GATA-1 mutations. Gene. 2008;427(1–2):1–6.
Cohen Jr MM. Perspectives on RUNX genes: an update. Am J Med Genet A. 2009; 149A(12):2629–46.
Creutzig U, Reinhardt D, Diekamp S, Dworzak M, Stary J, Zimmermann M. AML patients with Down syndrome have a high cure rate with AML-BFM therapy with reduced dose intensity. Leukemia. 2005;19(8):1355–60.
Crispino JD. GATA1 in normal and malignant hematopoiesis. Semin Cell Dev Biol. 2005;16(1):137–47.
Crispino JD, Lodish MB, MacKay JP, Orkin SH. Use of altered specificity mutants to probe a specific protein-protein interaction in differentiation: the GATA-1:FOG complex. Mol Cell. 1999;3(2):219–28.
De Vita S, Mulligan C, McElwaine S, et al. Loss-of-function JAK3 mutations in TMD and AMKL of Down syndrome. Br J Haematol. 2007;137(4):337–41.
Doll R, Peto R. Cigarette smoking and bronchial carcinoma: dose and time relationships among regular smokers and lifelong non-smokers. J Epidemiol Community Health. 1978;32(4): 303–13.
Dordelmann M, Schrappe M, Reiter A, et al. Down's syndrome in childhood acute lymphoblastic leukemia: clinical characteristics and treatment outcome in four consecutive BFM trials. Berlin-Frankfurt-Munster Group. Leukemia. 1998;12(5):645–51.
Down JHL. Observations on an ethnic classification of idiots. Clin Lect Rep: Lond Hosp. 1866;3:259–62.
Ferreira R, Wai A, Shimizu R, et al. Dynamic regulation of Gata factor levels is more important than their identity. Blood. 2007;109(12):5481–90.
Fix DF, Glickman BW. Asymmetric cytosine deamination revealed by spontaneous mutational specificity in an Ung- strain of Escherichia coli. Mol Gen Genet. 1987;209(1):78–82.
Flores-Lujano J, Perez-Saldivar ML, Fuentes-Panana EM, et al. Breastfeeding and early infection in the aetiology of childhood leukaemia in Down syndrome. Br J Cancer. 2009;101(5):860–4.
Fong CT, Brodeur GM. Down's syndrome and leukemia: epidemiology, genetics, cytogenetics and mechanisms of leukemogenesis. Cancer Genet Cytogenet. 1987;28(1):55–76.
Forestier E, Izraeli S, Beverloo B, et al. Cytogenetic features of acute lymphoblastic and myeloid leukemias in pediatric patients with Down syndrome: an iBFM-SG study. Blood. 2008;111(3):1575–83.

Freson K, Devriendt K, Matthijs G, et al. Platelet characteristics in patients with X-linked macrothrombocytopenia because of a novel GATA1 mutation. Blood. 2001;98(1):85–92.
Fujiwara Y, Browne CP, Cunniff K, Goff SC, Orkin SH. Arrested development of embryonic red cell precursors in mouse embryos lacking transcription factor GATA-1. Proc Natl Acad Sci U S A. 1996;93(22):12355–8.
Galloway JL, Wingert RA, Thisse C, Thisse B, Zon LI. Loss of gata1 but not gata2 converts erythropoiesis to myelopoiesis in zebrafish embryos. Dev Cell. 2005;8(1):109–16.
Gamis AS, Woods WG, Alonzo TA, et al. Increased age at diagnosis has a significantly negative effect on outcome in children with Down syndrome and acute myeloid leukemia: a report from the Children's Cancer Group Study 2891. J Clin Oncol. 2003;21(18):3415–22.
Garre ML, Relling MV, Kalwinsky D, et al. Pharmacokinetics and toxicity of methotrexate in children with Down syndrome and acute lymphocytic leukemia. J Pediatr. 1987; 111(4):606–12.
Greaves MF. Speculations on the cause of childhood acute lymphoblastic leukemia. Leukemia. 1988;2(2):120–5.
Greaves MF. Aetiology of acute leukaemia. Lancet. 1997;349(9048):344–9.
Greaves M. Childhood leukaemia. BMJ. 2002;324(7332):283–7.
Hasle H. Pattern of malignant disorders in individuals with Down's syndrome. Lancet Oncol. 2001;2(7):429–36.
Hasle H, Clemmensen IH, Mikkelsen M. Risks of leukaemia and solid tumours in individuals with Down's syndrome. Lancet. 2000;355(9199):165–9.
Helbling D, Mueller BU, Timchenko NA, et al. The leukemic fusion gene AML1-MDS1-EVI1 suppresses CEBPA in acute myeloid leukemia by activation of Calreticulin. Proc Natl Acad Sci U S A. 2004;101(36):13312–7.
Hellebostad M, Carpenter E, Hasle H, Mitchell C, Vyas P. GATA1 mutation analysis demonstrates two distinct primary leukemias in a child with down syndrome; implications for leukemogenesis. J Pediatr Hematol Oncol. 2005;27(7):408–9.
Hertzberg L, Vendramini E, Ganmore I, et al. Down syndrome acute lymphoblastic leukemia, a highly heterogeneous disease in which aberrant expression of CRLF2 is associated with mutated JAK2: a report from the International BFM Study Group. Blood. 2010; 115(5):1006–17.
Hitzler JK, Cheung J, Li Y, Scherer SW, Zipursky A. GATA1 mutations in transient leukemia and acute megakaryoblastic leukemia of Down syndrome. Blood. 2003;101(11):4301–4.
Hoeller S, Bihl MP, Tzankov A, et al. Morphologic and GATA1 sequencing analysis of hematopoiesis in fetuses with trisomy 21. Hum Pathol. 2014;45(5):1003–9.
Hollanda LM, Lima CS, Cunha AF, et al. An inherited mutation leading to production of only the short isoform of GATA-1 is associated with impaired erythropoiesis. Nat Genet. 2006;38(7):807–12.
Hong D, Gupta R, Ancliff P, et al. Initiating and cancer-propagating cells in TEL-AML1-associated childhood leukemia. Science. 2008;319(5861):336–9.
Howlader N, Noone A, Krapcho M, et al. Childhood cancer. SEER cancer statistics review, 1075–2010. Bethersda: National Cancer Institute; 2013a. http://seer.cancer.gob/csr/1975_2010, based on Nov 2012 SEER data submission, posted to the SEER web site, Apr, Section 28.
Howlader N, Noone A, Krapcho M et al. Childhood cancer by the International Classification of Childhood Cancer. SEER Cancer Statistics Review, 1075–2010. Bethesda: National Cancer Institute; 2013b. http://seer.cancer.gov/csr/1975_2010, based on Nov 2012 SEER data submission, posted to the SEER web site, Apr, Section 29
Iwasaki H, Mizuno S, Wells RA, Cantor AB, Watanabe S, Akashi K. GATA-1 converts lymphoid and myelomonocytic progenitors into the megakaryocyte/erythrocyte lineages. Immunity. 2003;19(3):451–62.
Kalwinsky DK, Raimondi SC, Bunin NJ, et al. Clinical and biological characteristics of acute lymphocytic leukemia in children with Down syndrome. Am J Med Genet Suppl. 1990;7:267–71.

Kearney L, Gonzalez De Castro D, Yeung J, et al. Specific JAK2 mutation (JAK2R683) and multiple gene deletions in Down syndrome acute lymphoblastic leukemia. Blood. 2009; 113(3):646–8.

Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med. 2005;352(17):1779–90.

Kudo K, Kojima S, Tabuchi K, et al. Prospective study of a pirarubicin, intermediate-dose cytarabine, and etoposide regimen in children with Down syndrome and acute myeloid leukemia: the Japanese Childhood AML Cooperative Study Group. J Clin Oncol. 2007;25(34):5442–7.

Kurokawa M, Tanaka T, Tanaka K, et al. Overexpression of the AML1 proto-oncoprotein in NIH3T3 cells leads to neoplastic transformation depending on the DNA-binding and transactivational potencies. Oncogene. 1996;12(4):883–92.

Lanza C, Volpe G, Basso G, et al. The common TEL/AML1 rearrangement does not represent a frequent event in acute lymphoblastic leukaemia occurring in children with Down syndrome. Leukemia. 1997;11(6):820–1.

Lavin MF, Bates P, Le Poidevin P, Chen PC. Normal inhibition of DNA synthesis following gamma-irradiation of radiosensitive cell lines from patients with Down's syndrome and Alzheimer's disease. Mutat Res. 1989;218(1):41–7.

Lejeune LG, Gautier M, Turpin R. Les chromosomes humains en culture de tissues. C R Acad Bulg Sci. 1959;248:602–3.

Levitt GA, Stiller CA, Chessells JM. Prognosis of Down's syndrome with acute leukaemia. Arch Dis Child. 1990;65(2):212–6.

Li Z, Godinho FJ, Klusmann JH, Garriga-Canut M, Yu C, Orkin SH. Developmental stage-selective effect of somatically mutated leukemogenic transcription factor GATA1. Nat Genet. 2005;37(6):613–9.

Linabery AM, Olshan AF, Gamis AS, et al. Exposure to medical test irradiation and acute leukemia among children with Down syndrome: a report from the Children's Oncology Group. Pediatrics. 2006;118(5):e1499–508.

Liu C, Morishima M, Yu T, et al. Genetic analysis of Down syndrome-associated heart defects in mice. Hum Genet. 2011;130(5):623–32.

Lo KC, Chalker J, Strehl S, et al. Array comparative genome hybridization analysis of acute lymphoblastic leukaemia and acute megakaryoblastic leukaemia in patients with Down syndrome. Br J Haematol. 2008;142(6):934–45.

Loncarevic IF, Roitzheim B, Ritterbach J, et al. Trisomy 21 is a recurrent secondary aberration in childhood acute lymphoblastic leukemia with TEL/AML1 gene fusion. Genes Chromosomes Cancer. 1999;24(3):272–7.

Lundin C, Heldrup J, Ahlgren T, Olofsson T, Johansson B. B-cell precursor t(8;14)(q11;q32)-positive acute lymphoblastic leukemia in children is strongly associated with Down syndrome or with a concomitant Philadelphia chromosome. Eur J Haematol. 2009;82(1):46–53.

Lundin C, Forestier E, Klarskov Andersen M, et al. Clinical and genetic features of pediatric acute lymphoblastic leukemia in Down syndrome in the Nordic countries. J Hematol Oncol. 2014;7(1):32.

Lyons SE, Lawson ND, Lei L, Bennett PE, Weinstein BM, Liu PP. A nonsense mutation in zebrafish gata1 causes the bloodless phenotype in Vlad tepes. Proc Natl Acad Sci U S A. 2002;99(8):5454–9.

Ma SK, Wan TS, Cheuk AT, et al. Characterization of additional genetic events in childhood acute lymphoblastic leukemia with TEL/AML1 gene fusion: a molecular cytogenetics study. Leukemia. 2001;15(9):1442–7.

Malinge S, Ben-Abdelali R, Settegrana C, et al. Novel activating JAK2 mutation in a patient with Down syndrome and B-cell precursor acute lymphoblastic leukemia. Blood. 2007;109(5):2202–4.

Malinge S, Chlon T, Dore LC, et al. Development of acute megakaryoblastic leukemia in Down syndrome is associated with sequential epigenetic changes. Blood. 2013;122(14):e33–43.

Maloney KW, Carroll WL, Carroll AJ, et al. Down syndrome childhood acute lymphoblastic leukemia has a unique spectrum of sentinel cytogenetic lesions that influences treatment outcome: a report from the Children's Oncology Group. Blood. 2010;116(7):1045–50.

Massey GV, Zipursky A, Chang MN, et al. A prospective study of the natural history of transient leukemia (TL) in neonates with Down syndrome (DS): children's Oncology Group (COG) study POG-9481. Blood. 2006;107(12):4606–13.

McNeil S, Zeng C, Harrington KS, et al. The t(8;21) chromosomal translocation in acute myelogenous leukemia modifies intranuclear targeting of the AML1/CBFalpha2 transcription factor. Proc Natl Acad Sci U S A. 1999;96(26):14882–7.

Miyauchi J, Kawaguchi H. Fetal liver stromal cells support blast growth in transient abnormal myelopoiesis in Down syndrome through GM-CSF. J Cell Biochem. 2014;115(6):1176–86.

Moore S, Suttle J, Bain S, Story C, Rice M. Acute lymphoblastic leukemia characterized by t(8;14)(q11.2;q32). Cancer Genet Cytogenet. 2003;141(1):1–4.

Morawiec Z, Janik K, Kowalski M, et al. DNA damage and repair in children with Down's syndrome. Mutat Res. 2008;637(1–2):118–23.

Mukouyama Y, Chiba N, Hara T, et al. The AML1 transcription factor functions to develop and maintain hematogenic precursor cells in the embryonic aorta-gonad-mesonephros region. Dev Biol. 2000;220(1):27–36.

Mullighan CG, Collins-Underwood JR, Phillips LA, et al. Rearrangement of CRLF2 in B-progenitor- and Down syndrome-associated acute lymphoblastic leukemia. Nat Genet. 2009;41(11):1243–6.

Mundschau G, Gurbuxani S, Gamis AS, Greene ME, Arceci RJ, Crispino JD. Mutagenesis of GATA1 is an initiating event in Down syndrome leukemogenesis. Blood. 2003;101(11):4298–300.

Nerlov C. The C/EBP family of transcription factors: a paradigm for interaction between gene expression and proliferation control. Trends Cell Biol. 2007;17(7):318–24.

Nichols KE, Crispino JD, Poncz M, et al. Familial dyserythropoietic anaemia and thrombocytopenia due to an inherited mutation in GATA1. Nat Genet. 2000;24(3):266–70.

Nikolaev SI, Santoni F, Vannier A, et al. Exome sequencing identifies putative drivers of progression of transient myeloproliferative disorder to AMKL in infants with Down syndrome. Blood. 2013;122(4):554–61.

Nikolaev SI, Garieri M, Santoni F, et al. Frequent cases of RAS-mutated Down syndrome acute lymphoblastic leukaemia lack JAK2 mutations. Nat Commun. 2014;5:4654.

Norton A, Fisher C, Liu H, et al. Analysis of JAK3, JAK2, and C-MPL mutations in transient myeloproliferative disorder and myeloid leukemia of Down syndrome blasts in children with Down syndrome. Blood. 2007;110(3):1077–9.

O'Brien MM, Taub JW, Chang MN, et al. Cardiomyopathy in children with Down syndrome treated for acute myeloid leukemia: a report from the Children's Oncology Group Study POG 9421. J Clin Oncol. 2008;26(3):414–20.

O'Brien MM, Cao X, Pounds S, et al. Prognostic features in acute megakaryoblastic leukemia in children without Down syndrome: a report from the AML02 multicenter trial and the Children's Oncology Group Study POG 9421. Leukemia. 2013;27(3):731–4.

Ognjanovic S, Puumala S, Spector LG, et al. Maternal health conditions during pregnancy and acute leukemia in children with down syndrome: a Children's Oncology Group study. Pediatr Blood Cancer. 2009;52(5):602–8.

Parada LF, Tabin CJ, Shih C, Weinberg RA. Human EJ bladder carcinoma oncogene is homologue of Harvey sarcoma virus ras gene. Nature. 1982;297(5866):474–8.

Parker SE, Mai CT, Canfield MA, et al. Updated national birth prevalence estimates for selected birth defects in the United States, 2004–2006. Birth Defects Res A Clin Mol Teratol. 2010;88(12):1008–16.

Patrick K, Wade R, Goulden N, et al. Outcome of Down syndrome associated acute lymphoblastic leukaemia treated on a contemporary protocol. Br J Haematol. 2014;165(4):552–5.

Paulsson K, Johansson B. High hyperdiploid childhood acute lymphoblastic leukemia. Genes Chromosomes Cancer. 2009;48(8):637–60.
Pizzo P, De Poplack. Principles and practice of pediatric oncology. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2011.
Preudhomme C, Warot-Loze D, Roumier C, et al. High incidence of biallelic point mutations in the Runt domain of the AML1/PEBP2 alpha B gene in Mo acute myeloid leukemia and in myeloid malignancies with acquired trisomy 21. Blood. 2000;96(8):2862–9.
Pui CH, Raimondi SC, Borowitz MJ, et al. Immunophenotypes and karyotypes of leukemic cells in children with Down syndrome and acute lymphoblastic leukemia. J Clin Oncol. 1993;11(7):1361–7.
Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. Lancet. 2008;371(9617): 1030–43.
Rabin KR, Smith J, Kozinetz CA. Myelosuppression and infectious complications in children with Down syndrome and acute lymphoblastic leukemia. Pediatr Blood Cancer. 2012;58(4):633–5.
Ragab AH, Abdel-Mageed A, Shuster JJ, et al. Clinical characteristics and treatment outcome of children with acute lymphocytic leukemia and Down's syndrome. A Pediatric Oncology Group study. Cancer. 1991;67(4):1057–63.
Rainis L, Bercovich D, Strehl S, et al. Mutations in exon 2 of GATA1 are early events in megakaryocytic malignancies associated with trisomy 21. Blood. 2003;102(3):981–6.
Rajantie J, Siimes MA. Long-term prognosis of children with Down's syndrome and leukaemia: a 34-year nation-wide experience. J Intellect Disabil Res. 2003;47(Pt 8):617–21.
Ramji DP, Foka P. CCAAT/enhancer-binding proteins: structure, function and regulation. Biochem J. 2002;365(Pt 3):561–75.
Rand V, Parker H, Russell LJ, et al. Genomic characterization implicates iAMP21 as a likely primary genetic event in childhood B-cell precursor acute lymphoblastic leukemia. Blood. 2011;117(25):6848–55.
Rao A, Hills RK, Stiller C, et al. Treatment for myeloid leukaemia of Down syndrome: population-based experience in the UK and results from the Medical Research Council AML 10 and AML 12 trials. Br J Haematol. 2006;132(5):576–83.
Ravindranath Y, Abella E, Krischer JP, et al. Acute myeloid leukemia (AML) in Down's syndrome is highly responsive to chemotherapy: experience on Pediatric Oncology Group AML Study 8498. Blood. 1992;80(9):2210–4.
Reinhardt D, Diekamp S, Langebrake C, et al. Acute megakaryoblastic leukemia in children and adolescents, excluding Down's syndrome: improved outcome with intensified induction treatment. Leukemia. 2005;19(8):1495–6.
Roberts I, O'Connor D, Roy A, Cowan G, Vyas P. The impact of trisomy 21 on foetal haematopoiesis. Blood Cells Mol Dis. 2013;51(4):277–81.
Robison LL, Nesbit Jr ME, Sather HN, et al. Down syndrome and acute leukemia in children: a 10-year retrospective survey from Childrens Cancer Study Group. J Pediatr. 1984;105(2): 235–42.
Ross JA, Blair CK, Olshan AF, et al. Periconceptional vitamin use and leukemia risk in children with Down syndrome: a Children's Oncology Group study. Cancer. 2005;104(2):405–10.
Roumier C, Fenaux P, Lafage M, Imbert M, Eclache V, Preudhomme C. New mechanisms of AML1 gene alteration in hematological malignancies. Leukemia. 2003;17(1):9–16.
Russell LJ, Capasso M, Vater I, et al. Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukemia. Blood. 2009;114(13):2688–98.
Saida S, Watanabe K, Sato-Otsubo A, et al. Clonal selection in xenografted TAM recapitulates the evolutionary process of myeloid leukemia in Down syndrome. Blood. 2013;121(21):4377–87.
Scholl T, Stein Z, Hansen H. Leukemia and other cancers, anomalies and infections as causes of death in Down's syndrome in the United States during 1976. Dev Med Child Neurol. 1982;24(6):817–29.

Song WJ, Sullivan MG, Legare RD, et al. Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. Nat Genet. 1999;23(2):166–75.

Stumpf M, Waskow C, Krotschel M, et al. The mediator complex functions as a coactivator for GATA-1 in erythropoiesis via subunit Med1/TRAP220. Proc Natl Acad Sci U S A. 2006;103(49):18504–9.

Swerdlow S, Camps E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW. WHO classification of tumors of haematopoietic and lymphoid tissues. 4th ed. Lyon: IARC; 2008.

Tallman MS, Neuberg D, Bennett JM, et al. Acute megakaryocytic leukemia: the Eastern Cooperative Oncology Group experience. Blood. 2000;96(7):2405–11.

Taub JW, Huang X, Matherly LH, et al. Expression of chromosome 21-localized genes in acute myeloid leukemia: differences between Down syndrome and non-Down syndrome blast cells and relationship to in vitro sensitivity to cytosine arabinoside and daunorubicin. Blood. 1999;94(4):1393–400.

Taub JW, Mundschau G, Ge Y, et al. Prenatal origin of GATA1 mutations may be an initiating step in the development of megakaryocytic leukemia in Down syndrome. Blood. 2004; 104(5):1588–9.

Tubman VN, Levine JE, Campagna DR, et al. X-linked gray platelet syndrome due to a GATA1 Arg216Gln mutation. Blood. 2007;109(8):3297–9.

Tunstall-Pedoe O, Roy A, Karadimitris A, et al. Abnormalities in the myeloid progenitor compartment in Down syndrome fetal liver precede acquisition of GATA1 mutations. Blood. 2008;112(12):4507–11.

Visvader JE, Crossley M, Hill J, Orkin SH, Adams JM. The C-terminal zinc finger of GATA-1 or GATA-2 is sufficient to induce megakaryocytic differentiation of an early myeloid cell line. Mol Cell Biol. 1995;15(2):634–41.

Wechsler J, Greene M, McDevitt MA, et al. Acquired mutations in GATA1 in the megakaryoblastic leukemia of Down syndrome. Nat Genet. 2002;32(1):148–52.

Whitlock JA, Sather HN, Gaynon P, et al. Clinical characteristics and outcome of children with Down syndrome and acute lymphoblastic leukemia: a Children's Cancer Group study. Blood. 2005;106(13):4043–9.

Xavier AC, Ge Y, Taub JW. Down syndrome and malignancies: a unique clinical relationship: a paper from the 2008 William Beaumont Hospital Symposium on Molecular Pathology. J Mol Diagn. 2009;11(5):371–80.

Xavier AC, Edwards H, Dombkowski AA, et al. A unique role of GATA1s in Down syndrome acute megakaryocytic leukemia biology and therapy. PLoS One. 2011;6(11):e27486.

Yamaguchi Y, Zon LI, Ackerman SJ, Yamamoto M, Suda T. Forced GATA-1 expression in the murine myeloid cell line M1: induction of c-Mpl expression and megakaryocytic/erythroid differentiation. Blood. 1998;91(2):450–7.

Yamtich J, Nemec AA, Keh A, Sweasy JB. A germline polymorphism of DNA polymerase beta induces genomic instability and cellular transformation. PLoS Genet. 2012;8(11):e1003052.

Yoshida K, Toki T, Okuno Y, et al. The landscape of somatic mutations in Down syndrome-related myeloid disorders. Nat Genet. 2013;45(11):1293–9.

Yu C, Niakan KK, Matsushita M, Stamatoyannopoulos G, Orkin SH, Raskind WH. X-linked thrombocytopenia with thalassemia from a mutation in the amino finger of GATA-1 affecting DNA binding rather than FOG-1 interaction. Blood. 2002;100(6):2040–5.

Yu T, Liu C, Belichenko P, et al. Effects of individual segmental trisomies of human chromosome 21 syntenic regions on hippocampal long-term potentiation and cognitive behaviors in mice. Brain Res. 2010;1366:162–71.

Zeller B, Gustafsson G, Forestier E, et al. Acute leukaemia in children with Down syndrome: a population-based Nordic study. Br J Haematol. 2005;128(6):797–804.

Zipursky A. Transient leukaemia – a benign form of leukaemia in newborn infants with trisomy 21. Br J Haematol. 2003;120(6):930–8.

Zipursky A, Thorner P, De Harven E, Christensen H, Doyle J. Myelodysplasia and acute megakaryoblastic leukemia in Down's syndrome. Leuk Res. 1994;18(3):163–71.

Chapter 5
Origin of Leukemia in Children with Down Syndrome

Johann K. Hitzler

Abstract Children with Down syndrome (DS) are more likely to develop acute myeloid (AML) or acute lymphoblastic leukemia (ALL). AML in children with DS is initiated during fetal hematopoiesis by somatic mutations of GATA1. Leukemic blasts of ALL in DS contain rearrangements of CRLF2 in more than half of all patients. DS is associated with distinct changes of cell subsets during fetal liver hematopoiesis, of folate/one-carbon metabolism and of cell signaling involving NFAT, TGF and WNT pathways. Possible genetic mechanisms of the increased risk for leukemia in DS include gene dosage imbalance of candidate genes and epigenetic dysregulation of gene expression. Fewer data are available regarding the role of non-cell-autonomous risk factors, such as abnormal immune function and exposure to environmental carcinogens, during the development of leukemia in children with DS.

Keywords Down syndrome • GATA1 • CRLF2 • Fetal liver hematopoiesis • Folate metabolism • NFAT signaling • Down syndrome critical region • Down syndrome candidate genes • DNA methylation • Histone marks • Carcinogens

Introduction

Leukemia presents with specific phenotypic features and disease mechanisms in children with constitutional trisomy 21 (Down syndrome [DS], OMIM 190685 [OMIM]). The overall incidence of acute leukemia in children with DS is increased 10- to 20-fold (Hasle 2001; Hasle et al. 2000). Acute myeloid leukemia (AML) in young children with DS is 150-fold and acute lymphoblastic leukemia (ALL) 40-fold more common compared with the general pediatric population (Hasle et al. 2000). At the same time, solid tumors of both childhood and adulthood occur with significantly lower incidence in individuals with DS (Hasle 2001; Hasle et al. 2000; Nizetic and Groet 2012).

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In addition to this specific distribution of malignancies in people with DS, the clinical presentation of leukemia in children with DS is unique (Roberts and Izraeli 2014). AML occurs at a younger median age in those with DS (1.8 versus 7.5 years; Lange et al. 1998). Overt AML frequently follows a prodrome of myelodysplasia, lasting weeks to months, and a neonatal transient accumulation of abnormal megakaryoblasts (termed transient myeloproliferative disorder [TMD], transient leukemia [TL], transient abnormal myelopoiesis [TAM]), which spontaneously resolves within months in the majority of cases (Klusmann et al. 2008; Muramatsu et al. 2008; Roy et al. 2012a). A subset of 20–30 % of children with TL, however, go on to develop DS-AML typically within the first 4 years of life (Creutzig et al. 2006; Gamis et al. 2003; Sorrell et al. 2012). Blasts of AML in DS typically are defined by a megakaryoblastic lineage phenotype and somatic mutations of the gene coding for the hematopoietic transcription factor *GATA1* (Roberts and Izraeli 2014; Roy et al. 2012a; Hitzler and Zipursky 2005; Roberts et al. 2013). Treatment response is excellent for the majority of patients (event-free survival is approximately 80 %) (Sorrell et al. 2012; Creutzig et al. 2005; Kudo et al. 2007; Taub et al. 2014), at least in part because of the hypersensitivity of blasts to a number of chemotherapeutic agents including cytarabine, anthracyclines, and epipodophyllotoxins (Frost et al. 2000; Taub and Ge 2005; Taub et al. 1996; Zwaan et al. 2002). As a result, DS-AML is considered both mechanistically and nosologically a distinct form of leukemia, termed myeloid leukemia of Down syndrome (DS-ML) in the recent World Health Organization classification (Hasle et al. 2003).

In contrast, ALL in children with DS has a similar age distribution and predominance of the B-lineage blast phenotype in comparison with the overall pediatric population (Maloney 2011; Whitlock 2006). The spectrum of cytogenetic features of ALL in DS (DS-ALL), however, shows a lower prevalence both of common prognostically favorable markers, such as high hyperdiploidy and *ETV6-RUNX1* fusions, and unfavorable fusions such as *BCR-ABL1* (Buitenkamp et al. 2014; Forestier et al. 2008; Maloney et al. 2010). Strikingly, T-ALL and infant ALL (<1 year of age) are very rare and virtually absent, respectively, in children with DS (Buitenkamp et al. 2014). Although a pathognomonic disease mechanism of DS-ALL is at present not evident, in approximately 60 % of cases DS-ALL blasts contain translocations and interstitial deletions that result in rearrangement and increased expression of the *CRLF2* gene (Buitenkamp et al. 2014; Mullighan et al. 2009a). In addition, approximately half of these cases (25 % of all DS-ALL) harbor activating mutations of *JAK2* and less frequently of *JAK1* (Buitenkamp et al. 2014; Mullighan et al. 2009a; Bercovich et al. 2008). In contrast to the overall pediatric population, in which either marker is associated with an adverse prognosis of ALL (Cario et al. 2010; Mullighan et al. 2009b), both markers are prognostically neutral in DS-ALL. Adverse effects of treatment such as infection, mucositis, and hyperglycemia are more frequent in children with DS (Whitlock 2006; Bassal et al. 2005) and have been attributed to agents such as glucocorticoids and methotrexate (Whitlock 2006). Survival outcomes of ALL are inferior in children with DS (8-year overall survival of 74 % and event-free survival of 64 %, compared with 89 % and 81 %, respectively, in patients without DS) (Buitenkamp et al. 2014), attributable to both a higher rate of relapse and significantly increased treatment-related mortality (Maloney 2011; Buitenkamp et al. 2014; O'Connor et al. 2014). Fatal infections during all phases of ALL therapy,

including maintenance therapy, remain a significant barrier to success and highlight the role of host factors during the treatment of ALL in DS (O'Connor et al. 2014).

Although the incidence of acute leukemia, both AML and ALL, is increased 10- to 20-fold and children with DS have access both to clinical trials and a curative standard of care, extremely scarce reports of secondary malignancies (Hasle et al. 2000) suggest that second malignancies are particularly rare in children with leukemia and DS.

Given these striking clinical observations concerning acute leukemia in children with DS, substantial attention has focused on the one shared variable: trisomy 21. This chapter aims to summarize hypotheses and observations put forth to explain why leukemia is more frequent in children with DS.

Observations Regarding the Mechanisms of DS-ML and DS-ALL

Requirements for AML in DS

Fetal Hematopoiesis

The first signs of the process that eventually culminates in DS-ML (Fig. 5.1) are detectable during fetal liver hematopoiesis, based on the appearance of cells harboring

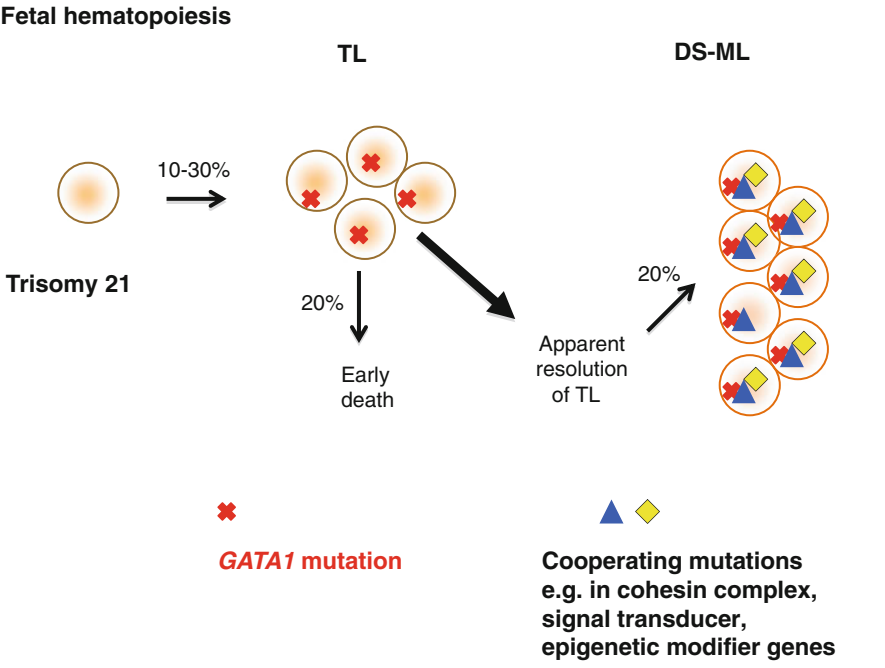

Fig. 5.1 Stepwise development of acute myeloid leukemia in Down syndrome. *GATA1* mutations in fetal hematopoietic cells with trisomy 21 result in transient leukemia (TL). After apparent resolution of TL, additional mutations cooperating with mutant GATA1 result in transformation to myelodysplastic syndrome and AML in a subset

an acquired mutation of the gene encoding the hematopoietic transcription factor *GATA1* (Taub et al. 2004). After birth, approximately 10 % of newborns with DS are diagnosed with an accumulation of blasts in the blood displaying megakaryoblastic, erythroid, and myeloid lineage markers in a disorder variably called TL (Zipursky 2003), TMD (Gamis and Smith 2012), and TAM (Roberts et al. 2013; Roberts and Izraeli 2014). The liver, the site of the immediately preceding developmental stage of fetal liver hematopoiesis, is the most commonly involved organ, with enlargement due to cellular infiltration—or residual hematopoiesis, depending on one's point of view—and fibrosis attributed to fibrogenic mediators such as platelet-derived growth factor (Ogawa et al. 2008) secreted by the population of abnormal megakaryoblasts. TL, therefore, is a disorder of fetal hematopoiesis in the context of cellular trisomy 21.

Trisomy 21 (Constitutional, Mosaicism, Somatic)

The karyotype of TL blasts almost universally shows trisomy 21, with rare exceptions (Schifferli et al. 2015), for which subchromosomal gain of genes encoded on chromosome 21 need to be investigated, confirming the rule rather than questioning it. In most cases trisomy 21 is constitutional (Down syndrome (OMIM)). Trisomy 21, however, may only be present in a proportion of tissues including the hematopoietic one in infants, with TL lacking the typical features of DS (trisomy 21 mosaicism) (Zipursky 2003; Gamis et al. 2011). In fact, trisomy 21 may be confined only to a proportion of hematopoietic cells, including normal cells and the TL blast population, or exclusively to the population of TL blasts (Apollonsky et al. 2008; Ono et al. 2015; Tsai et al. 2011). Trisomy may involve the entire chromosome 21 or a specific part (segmental trisomy 21) (Korbel et al. 2009; Korenberg et al. 1994). The functional impact of trisomy 21 on fetal hematopoiesis is discussed below. In sum, the developmental stage of fetal hematopoiesis and trisomy 21 constitute the first two classical conditions for the in the development of DS-ML.

Acquired *GATA1* Mutations

Acquired, somatic mutations of the X-linked gene encoding the hematopoietic transcription factor *GATA1* are the third of the events cooperating in the development of DS-AML (Wechsler et al. 2002). The mutations consist of short insertions/deletions or point mutations in exon 2, rarely exon 3, and corresponding splice sites, which introduce a premature stop codon, frame shift, or splice site mutation (Alford et al. 2011). The result is the translation of a truncated mutant protein (GATA1s), which lacks 83 amino-terminal amino acid residues and the encoded putative transactivation domain and interaction site with proteins such as RUNX1 (Elagib et al. 2003) and E2F (Klusmann et al. 2010a). Expression of GATA1s *in vivo* is associated with proliferation of megakaryocyte precursors during a fetal stage of hematopoiesis, but in this system did not interfere with normal adult murine blood cell formation (Li et al. 2005). This experimental finding, together

with the observation that corresponding *GATA1* mutations if occurring in the germ line and non-fetal hematopoietic cells are associated with anemia and neutropenia in males (Hollanda et al. 2006) or Diamond-Blackfan anemia (Sankaran et al. 2012), highlights that GATA1s has a specific functional impact during fetal hematopoiesis that results in TL. Of note, expression of *Gata1s* in a murine model resulted in the transient expansion of fetal megakaryoblastic progenitors with high proliferative capacity even in the absence of trisomy 21 (Li et al. 2005), suggesting that a fetal liver hematopoietic progenitor is the likely cell of origin for TL and that a degree of overlap exists between proliferative and survival stimuli provided to it by trisomy 21 and GATA1s.

TL blasts may be oligoclonal with regard to *GATA1* mutations (Ahmed et al. 2004; Saida et al. 2013), but the DS-ML clones in the majority of cases are monoclonal (Alford et al. 2011). *GATA1* mutations are concordant within the same individual between the stage of TL and DS-AML (Hitzler et al. 2003; Rainis et al. 2003; Yoshida et al. 2013). These observations are consistent with a model in which DS-ML or, more precisely, the initial myelodysplastic syndrome (defined by lack of differentiated blood cells in the peripheral blood, presence of morphologically abnormal differentiated precursors in the bone marrow, and fewer than <20 % blasts in the bone marrow) and then overt DS-ML arise from a subclone of TL. Additional mutations, which chronologically rank as fourth or fifth events, are expected to function as progression events that propel the *GATA1* mutant TL clone to fully transformed DS-ML (Fig. 5.1). The onset of DS-ML and, thus, the timing of these progression events appears limited to the first 4 years of life (Hasle et al. 2000; Gamis et al. 2003), defining the maximal time interval for which the preleukemic TL clone(s) may persist in the environment of postnatal bone marrow hematopoiesis.

Progression Events

The existence of an identifiable preleukemic disorder (TL) and clonally linked, fully transformed AML (DS-ML) in children with DS provides the unique opportunity of identifying those genetic events that are associated with the progression of TL to DS-ML, with a view to functional validation. Cytogenetic analysis had suggested that somatic trisomy 8 (Massey et al. 2006) and other cytogenetic abnormalities (Klusmann et al. 2008; Forestier et al. 2008) could be associated with progression to DS-AML. Mutational screening of DS-ML blasts (Malinge et al. 2008) and co-expression of *Gata1* and candidate progression genes in a murine model of DS-ML (Malinge et al. 2012) led to the identification of activating mutations of the genes encoding the signal transducer *JAK3* and thrombopoietin receptor *MPL*. Subsequent studies, however, showed that these events were infrequent in DS-AML (Kiyoi et al. 2007; Norton et al. 2007).

Another approach was the direct comparison of TL and DS-ML blasts, preferably from the same individual. Early expression studies (Lightfoot et al. 2004; McElwaine et al. 2004) were hampered by interindividual variability (lack of pairs) and impurity of analyzed cell fractions.

Recently, exome sequencing of sample pairs of TL and DS-ML blasts from the same individuals revealed that, as expected, *GATA1* mutations are the predominant and mostly sole mutation detectable in TL blasts (Yoshida et al. 2013; Nikolaev et al. 2013), arguing against genomic instability as a mechanism. In contrast, blasts of DS-ML harbored an average of six mutations. Mutations of cohesin complex genes such as *SMC1A*, *STAG2*, and *RAD21* were found in 57 % of cases of DS-ML followed by activating mutations in genes encoding signal transducers (e.g., *JAK1-3*, *MPL*, and *SH2B3*; 35 % of cases) and epigenetic modifiers (*EZH2*; 33 % of cases) (Yoshida et al. 2013). WNT, JAK-STAT, and MAPK/PI3K pathways were targeted by mutations in DS-ML blasts (Nikolaev et al. 2013). Mutations of cohesin complex genes had previously been described in blasts of non-DS-AML (Nikolaev et al. 2013; Welch et al. 2012).

These data support a disease model in which initiation of AML is enhanced in DS by the presence and expansion of target cells during fetal hematopoiesis and the proliferative effects of mutant GATA1 protein, whereas progression from preleukemia (TL) to AML in DS may rely on generic mechanisms that are also operative in non-DS-AML.

Requirements for ALL in DS

In contrast to AML, which is a distinct form of leukemia based on age distribution, blast immunophenotype, disease mechanism (*GATA1* mutation), drug sensitivity, prognosis, and treatment approach, a similarly distinct nature of ALL in children with DS is less obvious. A large retrospective study confirmed inferior survival outcomes of DS-ALL and pointed to both an increased relapse rate and a higher risk of treatment-related mortality as the main barriers to success (Buitenkamp et al. 2014).

Approximately 10 % of children with high-risk B-precursor ALL have a gene rearrangement involving the *CRLF2* gene (cytokine receptor-like factor 2), which consists either of a translocation into the immunoglobulin heavy-chain locus or an interstitial deletion of within the pseudo-autosomal region of X or Y (Mullighan et al. 2009a; Harvey et al. 2010). In both cases the expected result is the increased expression of CRLF2 and constitutive activation of JAK2/STAT5 signaling, which in experimental systems endows growth factor-independent growth *in vitro* (Bercovich et al. 2008). In ALL of children with DS, the corresponding *CRLF2* gene rearrangements are found in as many as 60 % of cases (Buitenkamp et al. 2014; Mullighan et al. 2009a) but do not have an unfavorable prognostic impact. About half of the cases of DS-ALL with *CRLF2* gene rearrangements have additional activating mutations of *JAK2* and, much less frequently, *JAK1* (Mullighan et al. 2009a; Bercovich et al. 2008), resulting in constitutive JAK2/STAT5 signaling. Again, in contrast to non-DS-ALL, the presence of *JAK2* mutations is not associated with unfavorable prognosis in DS-ALL but offers a target for therapeutic intervention (Roberts et al. 2014; Maude et al. 2012) that may lack the toxicity of standard chemotherapy for DS-ALL. Investigators also compared gene expression of DS-ALL blasts with that in defined cytogenetic subgroups of non-DS B-precursor ALL and discovered a hetero-

geneity of gene expression between DS-ALL samples that, in contrast to DS-AML, did not lend support to a unifying disease mechanism in DS-ALL (Hertzberg et al. 2010). Recent findings of distinct epigenetic gene regulation in ALL blasts with trisomy 21 (Lane et al. 2014), however, suggest that such differences exist.

The observation that both AML and ALL are significantly more common in children with DS suggests that regulation of normal blood cell formation may differ between children with and without DS in ways that predispose the former to leukemic transformation.

Developmental Hematopoietic Abnormalities Associated with Trisomy 21

Expression of mutant GATA1 protein (functionally equivalent to GATA1s) was sufficient to induce the expansion of a megakaryoblastic progenitor with high proliferative capacity during fetal liver hematopoiesis, but had no corresponding impact on adult blood cell formation (Li et al. 2005). This observation suggests that transforming events, at least in DS-AML, are specific for a developmental stage. Whether blood cell formation in general is different in individuals with and without DS has been studied by analysis of hematopoietic stem and progenitor cells (HSPC) derived from human fetal liver, differentiated embryonic and induced pluripotent stem cells of individuals with DS, and murine models of human trisomy 21.

Human Fetal Liver Hematopoiesis and Trisomy 21

Based on immunophenotype and colony formation *in vitro*, the hematopoietic progenitors in the second trimester fetal liver with trisomy 21 show a marked expansion of megakaryocyte-erythroid progenitors (MEP) (Chou et al. 2008; Roy et al. 2012b; Tunstall-Pedoe et al. 2008) and hematopoietic stem cells (HSC) (Roy et al. 2012b), whereas the number of common myeloid progenitors and granulocyte-macrophage progenitors (GMP) is decreased (Chou et al. 2008; Roy et al. 2012b; Tunstall-Pedoe et al. 2008). Megkaryoblastic and erythroid output was increased in colony-forming assays *in vitro* and xenograft recipients *in vivo* (Chou et al. 2008), illustrating an apparent bias of trisomic human fetal liver hematopoiesis in favor of megakaryo- and erythropoiesis. Among lymphoid progenitors, the number of lymphoid-primed multi-potential progenitors and early lymphoid progenitors was maintained, while the number of committed B-lymphoid progenitors was reduced tenfold (Roy et al. 2012b). Interestingly, the expression of genes that have a role in human hematopoiesis and are encoded on chromosome 21, such as *ERG*, *ETS*, *RUNX1*, and *SON*, was not different in HSC derived from trisomy and non-trisomic fetal livers (Chou et al. 2008). Analysis of induced human pluripotent stem cells with trisomy 21 (Maclean et al. 2012) confirmed the increased colony-forming potential of trisomic hematopoietic progenitors.

Murine Models of Hematopoiesis in DS

A series of mouse models of human DS has been established (Alford et al. 2010), which differ by the number of trisomic genes that are orthologous to those encoded on human chromosome 21 (HSA21). Ts65Dn (Kirsammer et al. 2008), Tc1 (Alford et al. 2010), and Ts1Cje (Carmichael et al. 2009) mice all show macrocytic red cells consistent with findings in human DS (Starc 1992). However, only Ts65Dn mice develop a myeloproliferative disorder over time, which is not linked to fetal hematopoiesis (Kirsammer et al. 2008). Furthermore, the number of MEP is smaller and of GMP increased, opposite to the findings in trisomic human fetal liver hematopoiesis (Chou et al. 2008). Co-expression of Gata1s in Tc1 mice (Alford et al. 2010) or of a *Gata1* allele containing a point mutation in TsCje mice (Carmichael et al. 2009) did not result in a TMD (or AML) phenotype. The same result was observed in double transgenic mice expressing human *ERG* and murine Gata1s (Birger et al. 2013), although this model reproduced the expansion of MEP and decrease of GMP found in trisomic human fetal liver hematopoietic progenitors. Interestingly, *Runx1* was not necessary for the hematopoietic phenotype of Ts65Dn mice (Kirsammer et al. 2008).

In summary, the hematopoietic stem and progenitor compartments, at least at the fetal stage of blood cell development, appear to be structured differently in human DS, resulting in the expansion of progenitors with megakaryocytic and erythroid differentiation potential. At the same time the number of committed B-lymphocytic progenitors is decreased.

Hematopoiesis in DS, therefore, may result in a larger supply of target cells such as MEP and HSC for transformation to preleukemic TL and further amplification by *GATA1s*. The observed amplification of a progenitor population such as MEP, however, does not rule out an impact of trisomy 21 on other, upstream multipotential progenitors or even HSC, which could explain the expression of a combination of megakaryoblastic, erythroid, myeloid, and lymphoid markers on TL blasts. While this model is plausible for TL and DS-ML, it is unclear whether ALL in DS is derived from an abnormally expanded corresponding lymphoid progenitor or HSC population. What is apparent is that the cellular targets of the process resulting in AML and ALL are only available in individuals with DS during the first 4 years and 30 years of life, respectively (Hasle et al. 2000).

Cellular Pathways Favoring the Development of Leukemia in DS

The search for the causes of the increased risk for leukemia in children with Down syndrome early on focused on cellular pathways that could enhance the cancer cell phenotype. Cell-autonomous consequences of trisomy 21 that would stimulate cell division and renewal, impair differentiation, and extend cell survival, therefore, would provide suitable candidate pathways. Alternatively, leukemia could result

from non-cell-autonomous effects of trisomy 21 that may increase support for nascent cancer cells or decrease the effectiveness of anticancer surveillance.

A number of basic cellular responses have been analyzed in DS cells. Apoptosis was found to be increased in fetal cortical neurons with trisomy 21 and associated with greater accumulation of intracellular reactive oxygen species compared with euploid controls (Busciglio and Yankner 1995). Serum levels of the angiogenesis inhibitor endostatin, a cleavage product of collagen XVIII encoded on chromosome 21, were high in people with DS (Zorick et al. 2001). These observations, however, are more useful to explain a protection against solid tumors than the increased risk for leukemia in children with DS, although they may also hold clues as to why secondary malignancies including leukemias are very rare in people with DS (Hasle et al. 2000).

A number of cellular pathways have been investigated as risk factors for leukemia in DS, and we focus here on a few conceptually intriguing ones.

Homocysteine, Folate, and One-Carbon Metabolism

The enzyme cystathionine β-synthase (CBS) is encoded on human chromosome 21, shows approximately 50 % increased activity in individuals with DS (Pogribna et al. 2001), and has an impact on all three metabolic branches. Increased *CBS* transcripts were also documented in DS-AML blasts (Ge et al. 2003; Taub et al. 1999, 2000). Increased activity of CBS is expected to remove homocysteine from the methionine cycle, thus depriving methionine synthase of its substrate and establishing a methyl trap by promoting accumulation of 5-methyltetrahydrofolate (Pogribna et al. 2001) (Fig. 5.2). At the same time reduced methionine synthase activity decreases the conversion of 5-methyltetrahydrofolate to tetrahydrofolate, the metabolically active form of folate, which is required for *de novo* nucleotide synthesis, in effect generating a functional intracellular folate deficiency in DS even in the presence of normal serum folate and vitamin B12 levels (Pogribna et al. 2001). Interestingly, despite the increased synthesis of cysteine, plasma levels of glutathione are low in children with DS, consistent with the increased generation of hydrogen peroxide mediated by increased expression of superoxide CuZn dismutase (SOD), which like CBS is also encoded on chromosome 21. It is intriguing to speculate that both generation and reduction of reactive oxygen species are enhanced in cells with trisomy 21 and that the balance and net effect of both reactions may vary in different tissues and developmental stages.

The concept of functional folate deficiency in DS matches well with several clinical observations. Children with DS frequently show macrocytosis of their red blood cells (Starc 1992), a feature also observed in folate-deficient individuals and incidentally in all murine models of DS. Use of folate antagonists such as methotrexate is complicated by a higher frequency and severity of adverse effects, for example, breakdown of skin and mucosal membranes, in children with DS-ALL who are treated with this agent (Bassal et al. 2005; Garre et al. 1987). This effect could be

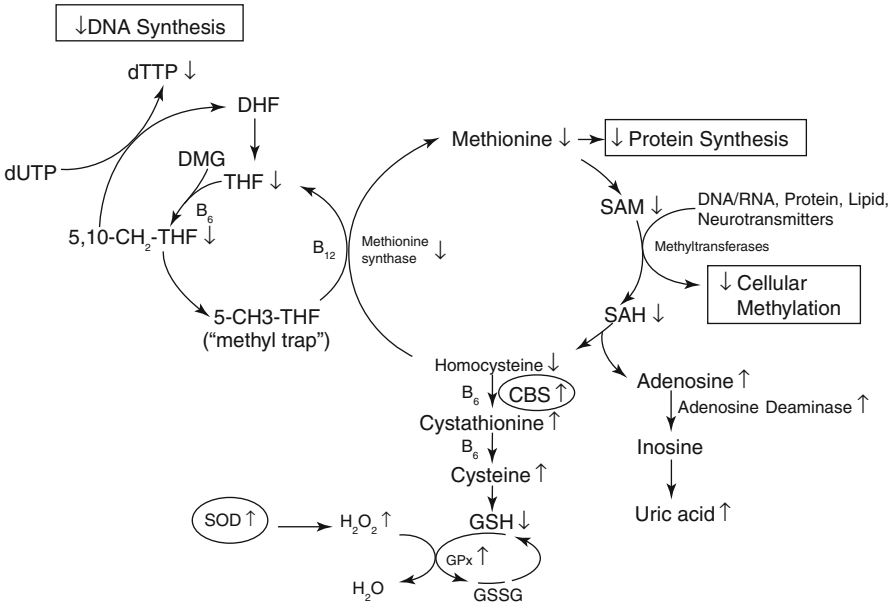

Fig. 5.2 Homocysteine, folate, and one-carbon metabolism (From Pogribna et al. 2001)

explained by the functional folate deficiency of DS described above or by increased expression of the membrane-bound transport protein used by methotrexate (reduced folate carrier, RFC), which is encoded on human chromosome 21. The absence of increased sensitivity of blasts of DS-ALL to methotrexate (Zwaan et al. 2002), however, argues against the latter explanation. In principle, a state of functional folate deficiency could exert a leukemia-inducing effect through increased risk of DNA strand breaks or be mediated by abnormal methylation reactions that alter epigenetic regulation of gene expression. Hypomethylation of DNA was found to be predominant in hematopoietic fetal liver cells with trisomy 21 (Malinge et al. 2013). In contrast, lymphocyte DNA of children with DS was found to be hypermethylated compared with euploid sibling controls (Pogribna et al. 2001) despite the decreased level of methyl donors such as S-adenosylmethionine in the plasma of children with DS. Intriguing as they are, these observations caution against simplistic interpretation of abnormal folate and homocysteine metabolism as a cause of cancer with specificity for hematopoietic cells in DS.

Nuclear Factor of Activated T Cells (NFAT) Signaling

The challenge for any candidate pathway in trying to explain the higher risk for leukemia in DS is the need to accommodate a decreased risk for solid tumors at the same time. Abnormal VEGF-calcineurin-NFAT signaling is a particularly intriguing

concept in his regard. Specifically, two negative regulators of calcineurin signaling, DSCR1 and DYRK1A, are encoded within the Down syndrome critical region (DSCR) on human chromosome 21. Increased expression of DYRK1A may favor the abnormal proliferation of megakaryocytes consistent with the predominant lineage phenotype of TL and DS-ML blasts, while increased DSCR1 activity interferes with the progression of solid tumors by suppressing tumor angiogenesis.

Vascular endothelial growth factor (VEGF) is a critical factor in physiological and tumor angiogenesis (Ryeom et al. 2008) that is capable of activating calcineurin-NFAT signaling. Binding of VEGF to one of its receptors results in increased intracellular calcium, which activates the serine-threonine phosphatase calcineurin. Calcineurin dephosphorylates cytoplasmic NFAT proteins, thus allowing their translocation into the nucleus, where they bind partner proteins to form NFAT transcription complexes. The net result is the activation of NFAT target genes, which include the proangiogenic genes *COX2*, E-selectin, and tissue factor (Ryeom et al. 2008; Arron et al. 2006). On the other hand, rephosphorylation of NFAT proteins triggers their export from the nucleus and inactivation of the pathway, with DYRK1A functioning as the priming kinase (for GSK3) (Arron et al. 2006). DSCR1 inhibits NFAT signaling by inhibition of calcineurin. Both DSCR1 and DYRK1A act synergistically to block calcineurin-NFAT signaling (Arron et al. 2006). Accordingly, tumor angiogenesis is suppressed in experimental animals with increased Dscr1 expression (Baek et al. 2009), and VEGF-mediated proliferation of endothelial cell is inhibited by both Dscr1 and even more potently by the combination of Dscr1 and DYRK1A (Baek et al. 2009). In addition to DSCR1 and DYRK1A, two other antiangiogenic proteins are encoded on chromosome 21, collagen XVIII, the precursor to the angiogenesis inhibitor endostatin (Ryeom et al. 2009), and ADAMTS1, a matrix metalloproteinase that regulates the antiangiogenic function of thrombospondin-1 (Ryeom et al. 2009). Suppression of tumor angiogenesis and inhibition of tumor progression by impaired calcineurin-NFAT signaling, therefore, is an attractive candidate mechanism underlying the lower incidence of solid tumors in DS.

At the same time, however, increased levels of DYRK1A and DSCR1 due to cellular trisomy 21 may also account for the increased incidence of leukemia in DS, at least of those with a megakaryoblastic lineage phenotype. In a murine model that combined trisomy for 33 orthologous genes in the human DSCR (Ts1Rhr), expression of Gata1s, and an activating mutation of the thrombopoietin receptor gene *MPL*, an oligoclonal, non-transplantable form of acute megakaryoblastic leukemia (AMKL) developed, reminiscent of AML in DS (Malinge et al. 2012). *Dyrk1a* was significantly overexpressed in megakaryoblasts in this model. Suppressed expression or function of *Dyrk1a* was associated with impaired expansion of megakaryocytes in trisomic cells (with and without additional *Gata1* mutation), highlighting the role of *Dyrk1A* in the excessive expansion of trisomic megakaryoblasts. Finally, increased *Dirk1a* was associated with an increased proportion of phosphorylated (inactive) NFAT proteins in trisomic mice, consistent with DYRK1A modulating megakaryoblastic expansion through inhibition of the calcineurin-NFAT pathway (Malinge et al. 2012).

Taken together, the dual function of DYRK1A encoded on chromosome 21 as oncogene for megakaryoblastic leukemia and tumor suppressor for solid tumors (Birger and Izraeli 2012) may help us understand the paradoxical distribution of malignancies in people with DS.

TGFβ and WNT Signaling

An interesting functional study of microRNAs encoded on human chromosome 21 revealed the concerted activation of Wnt and inhibition of transforming growth factor-β (TGFβ) signaling pathways in trisomic hematopoietic cells.

MicroRNAs are short, non-coding RNAs that negatively regulate gene expression. By binding to complementary sequences in target transcripts, they repress their translation or enhance their cleavage and degradation (Carthew and Sontheimer 2009). MiR-125b, encoded on human chromosome 21, is highly expressed in blasts of human DS-ML, TL, and to a lesser degree non-DS-AMKL when compared with euploid CD34-positive hematopoietic stem and progenitor cells (Klusmann et al. 2010b). It endows murine megakaryocytic progenitors in the fetal liver with increased capacity for proliferation and self-renewal in vitro—a property that was further enhanced by expression of GATA1s, the mutation found in TL—and expands fetal liver progenitors with both megakaryocytic and erythroid lineage potential. Conversely, downregulation of miR125b decreased proliferation of human TL blasts. Interestingly, the target genes suppressed by miRNA 125b include *DICER1*, which encodes the RNAse III enzyme required for the production of mature miRNAs (Emmrich et al. 2014). This suggested that miRNA125b expressed at high levels in trisomic cells may result in global post-transcriptional blockage of miRNA processing and thus promote the development of leukemia in DS due to disordered hematopoietic cell differentiation (Klusmann et al. 2010b).

Subsequently, miR125b was shown to be transcribed in a phylogenetically conserved tricistron of miRNAs that also includes miR-99 and let-7c and is embedded in the intron of long intervening non-coding RNA host gene *LINC00478* on chromosome 21 (Emmrich et al. 2014). The tricistron most efficiently stimulated the growth of megakaryocytic cells and colonies derived from CD34-positive human cord blood when compared with the single miRNA controls. In addition, competitive repopulation experiments showed that expression of the miRNA tricistron expanded long-term repopulating hematopoietic stem cells and megakaryocytic progenitors in vivo (Emmrich et al. 2014). Analysis of miRNA target genes by expression profiling revealed that positive effectors of the TGFβ pathway were repressed at multiple levels including expression of genes encoding receptors, transmitters, and transcription factors. Since TGFβ blocks megakaryopoiesis, the authors hypothesized that expression of the chromosome 21-encoded tricistronic miR-99a, let-7c and miR-125b-2 may allow the megakaryoblasts of DS-ML to evade TGFβ1-induced apoptosis and cell-cycle arrest.

At the same time tricistronic miR-99a, let-7c and miR-125b-2 activated canonical Wnt signaling, which induces self-renewal and proliferation of hematopoietic stem cells (Luis et al. 2011).

Upon binding of Wnt ligands to membrane-bound receptor, β-catenin accumulates and translocates into the nucleus where it complexes with T-cell factor/lymphoid enhancer factor to activate the transcription of target genes. In the absence of pathway activation, β-catenin is degraded after binding to and phosphorylation by destruction complex (consisting of APC, GSK3β, and AXIN1) (Emmrich et al. 2014).

Accordingly, CD34-positive hematopoietic stem and progenitor cells transduced to express the tricistron activated Wnt signaling as evidenced by increased unphosphorylated (active) β-catenin and upregulation of Wnt downstream targets such as cyclins and BCL-9. APC and its homolog APC2 were targets of the tricistron, resulting in decreased levels of protein. In contrast to miR125b alone, hematopoietic stem cells expressing the tricistron had the capacity for long-term reconstitution in serial transplants without loss of self-renewal (Emmrich et al. 2014).

These intriguing observations of cellular pathways that are different in hematopoietic cells with trisomy 21 are complemented by long-standing efforts to identify genes on chromosome 21 that determine the phenotype of human DS.

Genetic Mechanisms Underlying the Increased Risk of Leukemia in DS

Gene-Dosage Imbalance

The observation that trisomy of human chromosome 21 (HSA21) underlies DS (Lejeune et al. 1959) leads to the model that dosage imbalance of genes encoded on chromosome 21 (Hattori et al. 2000) accounts for the phenotypic features of DS. This model quickly becomes more complex if the possibility is taken into account that only a subset of HSA21 genes may be dosage-sensitive (i.e., result in phenotypic effects if present in three copies) and that the effect of dosage-sensitive genes may depend on the combination of alleles (i.e., be allele specific) and affect the phenotype only if expression surpasses a threshold (Antonarakis et al. 2004). Potential variability of expression of HSA21 genes not only across different tissues but also during different developmental phases adds another level of complexity. In the murine Ts65Dn model of DS, for example, only approximately one-third of genes evaluated were expressed at the expected theoretical level of 1.5 that of euploid control cells, and an effect of developmental stage on the expression level was found (reviewed in Antonarakis et al. 2004). These considerations help explain phenotypic variability in DS but leave open whether the phenotype of DS can be accounted for by a shared pattern of gene activity associated with chromosome 21. Specific inactivation of genes on chromosome 21 has become available as a new experimental tool to address this question (Jiang et al. 2013).

Down Syndrome Critical Region

The hypothesis of a DSCR (or DS consensus region) (Korbel et al. 2009) postulates that expression of a defined set of genes encoded in the DSCR on HSA21, for example, *DYRK1A*, *DSCR1*, and *APP*, is sufficient to cause the phenotype of DS. Evidence gathered through mapping of organ-specific features of DS, for example, congenital heart disease, cognitive impairment, gastrointestinal malformations, and acute megakaryoblastic leukemia (and TL), to specific regions of chromosome 21 in rare individuals with DS due to trisomy only of segments of HSA21 (Korbel et al. 2009) does not support the existence of a single DSCR and casts doubt on previously favored genes encoded in the DSCR such as DSCR1, DYRK1A, and APP as functionally relevant contributors to phenotypic features of DS. Murine experiments demonstrating that expression of 33 murine orthologs of human genes encoded in the DSCR did not reproduce the craniofacial phenotype of DS support this conclusion (Olson et al. 2004). If few genes in a single critical region of HSA21 do not account for DS, it is possible that non-specific small effects of many genes perturb genetic homeostasis (developmental instability hypothesis) or that both concepts together apply in the generation of DS (Olson et al. 2004). Thus the expression pattern of all HSA21 genes and its specific impact on expression of non-HSA21 genes (Letourneau et al. 2014) need to be incorporated in the genetic model of DS.

This conceptual complexity notwithstanding, a number of genes encoded on HSA21 have been studied with a focus on the development of leukemia in DS.

Candidate Genes on Human Chromosome 21

Gene databases currently contain 794 entries encoded on human chromosome 21, including protein coding and non-coding genes (LINC and 29 miRNAs) as well as uncharacterized gene models (see http://www.ncbi.nlm.nih.gov/gene/?term=21[CHR]+AND+human[ORGN] and ftp://mirbase.org/pub/mirbase/CURRENT/genomes/hsa.gff3). Among these, *ERG*, *ETS*, and *RUNX1* have attracted particular attention (see Table 1 in Mateos et al. 2015).

Expression of *ERG*, for *ETS*-related gene, expands megakaryocyte-erythroid progenitor cells in fetal liver (Birger et al. 2013) and results in megakaryoblastic leukemia in a murine transplant model (Salek-Ardakani et al. 2009). Co-expression of ERG with Gata1s was associated with liver fibrosis in ERG/Gata1s double transgenic mice, a phenotype reminiscent of transient leukemia of DS (Birger et al. 2013). A functional role for ERG in transient leukemia of DS is further suggested by the observation that the myeloproliferative disorder on the Ts65Dn murine model of DS is prevented by the specific experimental reduction of Erg trisomy to functional disomy (Ng et al. 2010).

RUNX1 is essential for the establishment of definitive hematopoiesis (Okuda et al. 1996) and is the target of chromosomal translocations in both pediatric ALL and AML (e.g., t(12;21) and t(8;21), respectively). The myeloproliferative disorder observed in a murine model of DS (Ts65Dn), however, is not dependent on trisomy of *Runx1* (Kirsammer et al. 2008). *RUNX1* is not overexpressed in blasts of AMKL of individuals with DS compared with those of non-DS AMKL (Bourquin et al. 2006). The evidence in favor of a central pathogenic role of RUNX1 in the leukemias of DS, therefore, is not compelling at present. The intriguing potential implication of HSA21 genes *DYRK1A* and *DSCR1* as well of miRNAs encoded on human chromosome 21, such as *miR-125b*, are outlined above (see sections "Nuclear Factor of Activated T Cells (NFAT) Signaling" and "TGFβ1 and WNT Signaling"). A more extensive gene list and characterization of functional phenotypes can be found in Table 1 of Mateos et al. (2015).

It may be unlikely that a single gene or even a small group of genes alone account for the phenotype of DS in general and the increased risk of leukemia in particular. Similarly, increased genomic instability as a cause for the association of leukemia with DS appears unlikely, given the lower incidence of nearly all solid tumors of child and adulthood in DS. Observations regarding the epigenetic regulation of gene expression in cells with trisomy 21, however, are beginning to provide unexpected insights.

Epigenetic Gene Regulation in DS

A basic study of gene expression in the cellular context of trisomy 21 was recently accomplished in fetal fibroblasts of monozygotic twin fetuses discordant for trisomy 21 (Letourneau et al. 2014). Differential gene expression was organized in domains which were either up- or downregulated, conserved in a mouse model of DS, and whose organization could be attributed to the extra chromosome 21. Histone mark profiles (H3Kme3) were different in trisomic cells, confirming the role of chromatin modifications in the gene expression changes due to trisomy 21 (Letourneau et al. 2014). Which gene or genes on human chromosome 21 can accomplish this modification of the chromatin environment and the subsequent change of global gene expression in trisomic cells is unknown.

Alterations of DNA methylation profiles, which may contribute to the development of AML in DS, were studied at different stages of the process from fetal liver mononuclear cell to blasts of TL and DS-ML (Malinge et al. 2013). DNA methylation was found to be markedly decreased in trisomic fetal liver mononuclear cells compared with euploid controls and was associated with gene networks involved developmental disorders of the cardiovascular, nervous, and endocrine systems (for a detailed list see Table 1 of Malinge et al. 2013). Genes within the DSCR and neighboring regions on chromosome 21 were both hypomethylated and highly expressed in trisomic fetal liver mononuclear cells com-

pared with non-trisomic controls. In contrast, blasts of TL showed gains of methylation compared with trisomic fetal liver mononuclear cells, mostly in regions that were not previously affected by differential DNA methylation. The affected gene networks were associated with blood cell formation, cell cycle, signaling, and cell death (for a detailed list see Table 2 of Malinge et al. 2013). Interestingly, DNA methylation patterns were not significantly different between TL and DS-ML. How trisomy 21 results in hypomethylation of DNA is unclear, although the authors wondered about the impact of the increased activity of CBS and the methyl trap in trisomy 21 cells (see section "Homocysteine, Folate, and One-Carbon Metabolism").

Finally, trisomy 21-related epigenetic regulation was recently shown to play a role in the development of B-lineage ALL (Lane et al. 2014). Bone marrow cells from a mouse model of DS (Ts1Rhr) generated B-cell colonies with increased self-renewal in vitro and resulted in the development of B-lineage ALL with greater penetrance and shorter latency compared with non-trisomic controls (the models included collaboration with gain-of-function alleles of *CRLF2* and *JAK2*; loss-of-function alleles of *Pax5* and *IKZF1*; or, alternatively, with *BCR-ABL1*). Gene expression analysis searching for pathways that were specifically perturbed by trisomic genes identified targets of the polycomb repressor complex 2 (PRC2) and sites that contained the repressive mark (H3K27me3) added by PRC2. In DS-ALL cells, PRC2 target genes were found to be overexpressed due to global reduction of the repressive H3K27 marks. This effect was due to trisomy of the chromosomal region 21q22 present in the trisomic Ts1Rhr mouse model and could be reproduced in B precursors and ALL cells by overexpression of the *HMGN1* gene. *HMGN1* maps to human chromosome 21 and encodes a nucleosome remodeling protein. Thus, trisomy of a gene on chromosome 21 can promote B-lineage ALL by suppressing inhibitory epigenetic marks and in effect upregulating genes required for the development of B-lineage ALL (Lane et al. 2014).

Environmental Causes of the Increased Risk of Leukemia in DS

The causes for the increased risk of leukemia in children with DS may not only be explained by cell-autonomous mechanisms operative in hematopoietic cells with trisomy 21. It would be interesting to investigate, for example, whether the increased expression of CRLF2 (cytokine-related factor 2) as part of the receptor for thymic stromal lymphopoietin on the blasts of 50 % of cases with DS-ALL (Buitenkamp et al. 2014; Mullighan et al. 2009a) corresponds with a DS-specific bone marrow niche that provides DS-ALL blasts with this ligand. Another non-cell-autonomous (with regard to the leukemic cell population) or environmental mechanism at the level of the organism that may contribute to the increased risk for leukemia is provided by the abnormal immune system of children with DS.

Abnormal Immune Function

During treatment for ALL, children with DS have a three- to tenfold increased treatment-related mortality (Buitenkamp et al. 2014; O'Connor et al. 2014), mostly due to fatal infections. Even in the absence of the immunosuppressive and myelosuppressive effects of ALL therapy, children with DS who develop sepsis have a 30 % increased risk of case fatality compared with other patients hospitalized with a diagnosis of sepsis (Garrison et al. 2005). DS is associated with thymic hypoplasia (Kusters et al. 2009), and impaired neutrophil chemotaxis and phagocytosis in children (Ugazio et al. 1990). Immunoglobulin subclass deficiency (Loh et al. 1990), hyper- and dysgammaglobulinemia, and absence of the developmental expansion of both B and T cells become apparent during the first year of life (Kusters et al. 2009; de Hingh et al. 2005; Verstegen et al. 2010). Whether inhibition of NFAT signaling in T cells by the chromosome 21-encoded negative regulators *DSCR1* and *DYRK1A* could exacerbate this immune dysfunction is an intriguing speculation. Abnormalities of the innate and adaptive immune system in children with DS, therefore, may not only contribute to increased risk for life-threatening infection during leukemia therapy, but function as a non-cell-autonomous mechanism that could contribute to a higher incidence of leukemia due to impaired immune surveillance.

Environmental Carcinogens and Exposures

It is frequently assumed that people with DS have a decreased exposure to environmental carcinogens such as tobacco and alcohol (Rabin and Whitlock 2009). A case-control study of 27 children with DS and ALL and 58 children with DS investigated a potential association between parental tobacco and alcohol use and acute leukemia in children with DS (Mejia-Arangure et al. 2003). They found that not only maternal age but also increased passive exposure of the probands to cigarette smoke, paternal smoking, and paternal alcohol consumption prior to the pregnancy were increased among cases with DS and ALL (Mejia-Arangure et al. 2003). Preconceptional acquisition of mutations, for example DNA adducts induced by benzo-[a]-pyrene during spermatogenesis, was considered a mechanism of paternal exposure. Using a similar design, exposure to magnetic fields, determined by spot measurements as ≥6 mG, was more frequently found in children with DS who developed acute leukemia (80 % of cases had ALL, the remainder AML) compared to children with DS without leukemia (Mejia-Arangure et al. 2007).

The Children's Oncology Group investigated a series on environmental exposures in matched cases with DS and ALL ($n=97$) or DS and AML ($n=61$) and 173 children with DS in the control group. They did not find an association between leukemia in children with DS and exposure to ionizing irradiation as part of diagnostic tests (Linabery et al. 2006). Maternal exposure to professional pest

exterminations, any pesticides, and any chemical was positively associated with ALL but not AML in children with DS (Alderton et al. 2006). In addition, they observed a significant negative association between acute leukemia or ALL and any infection in the first 2 years of life in children with DS, suggesting that infections early in life may exert a protective role against leukemia in children with DS (Canfield et al. 2004). In contrast, no associations were found with maternal reproductive history (Puumala et al. 2007) and a child's regular use of multivitamins (Blair et al. 2008).

Conclusions

The lymphoblastic and myeloid leukemias of children with DS pose distinct clinical challenges. Specific disease mechanisms involving *CRLF2*/*JAK2* and *GATA1*, respectively, have been uncovered and may offer opportunities for target-specific intervention (e.g., JAK2 inhibition). In a broader sense the leukemias of children with DS have stimulated research into the mechanisms regarding how this form of aneuploidy may both promote leukemia and inhibit most solid tumors. Initial simplistic models of abnormal gene expression are being replaced by increasingly complex ones that include epigenetic gene regulation. Finally, specific responses to environmental stimuli may contribute to an increased risk of leukemia in DS.

References

Ahmed M, et al. Natural history of GATA1 mutations in Down Syndrome. Blood. 2004;103(7):2480–9.

Alderton LE, et al. Child and maternal household chemical exposure and the risk of acute leukemia in children with Down's syndrome: a report from the Children's Oncology Group. Am J Epidemiol. 2006;164(3):212–21.

Alford KA, et al. Perturbed hematopoiesis in the Tc1 mouse model of Down syndrome. Blood. 2010;115(14):2928–37.

Alford KA, et al. Analysis of GATA1 mutations in Down syndrome transient myeloproliferative disorder and myeloid leukemia. Blood. 2011;118(8):2222–38.

Antonarakis SE, et al. Chromosome 21 and down syndrome: from genomics to pathophysiology. Nat Rev Genet. 2004;5(10):725–38.

Apollonsky N, et al. Transient myeloproliferative disorder in neonates with and without Down syndrome: a tale of 2 syndromes. J Pediatr Hematol Oncol. 2008;30(11):860–4.

Arron JR, et al. NFAT dysregulation by increased dosage of DSCR1 and DYRK1A on chromosome 21. Nature. 2006;441(7093):595–600.

Baek KH, et al. Down's syndrome suppression of tumour growth and the role of the calcineurin inhibitor DSCR1. Nature. 2009;459(7250):1126–30.

Bassal M, et al. Lymphoblast biology and outcome among children with Down syndrome and ALL treated on CCG-1952. Pediatr Blood Cancer. 2005;44(1):21–8.

Bercovich D, et al. Mutations of JAK2 in acute lymphoblastic leukaemias associated with Down's syndrome. Lancet. 2008;372(9648):1484–92.

Birger Y, Izraeli S. DYRK1A in Down syndrome: an oncogene or tumor suppressor? J Clin Invest. 2012;122(3):807–10.
Birger Y, et al. Perturbation of fetal hematopoiesis in a mouse model of Down syndrome's transient myeloproliferative disorder. Blood. 2013;122(6):988–98.
Blair CK, et al. Vitamin supplement use among children with Down's syndrome and risk of leukaemia: a Children's Oncology Group (COG) study. Paediatr Perinat Epidemiol. 2008;22(3):288–95.
Bourquin J-P, et al. Identification of distinct molecular phenotypes in acute megakaryoblastic leukemia by gene expression profiling. Proc Natl Acad Sci U S A. 2006;103(9):3339–44.
Buitenkamp TD, et al. Acute lymphoblastic leukemia in children with Down syndrome: a retrospective analysis from the Ponte di Legno study group. Blood. 2014;123(1):70–7.
Busciglio J, Yankner BA. Apoptosis and increased generation of reactive oxygen species in Down's syndrome neurons in vitro. Nature. 1995;378(6559):776–9.
Canfield KN, et al. Childhood and maternal infections and risk of acute leukaemia in children with Down syndrome: a report from the Children's Oncology Group. Br J Cancer. 2004;91(11): 1866–72.
Cario G, et al. Presence of the P2RY8-CRLF2 rearrangement is associated with a poor prognosis in non-high-risk precursor B-cell acute lymphoblastic leukemia in children treated according to the ALL-BFM 2000 protocol. Blood. 2010;115(26):5393–7.
Carmichael CL, et al. Hematopoietic defects in the Ts1Cje mouse model of Down syndrome. Blood. 2009;113(9):1929–37.
Carthew RW, Sontheimer EJ. Origins and mechanisms of miRNAs and siRNAs. Cell. 2009;136(4):642–55.
Chou ST, et al. Trisomy 21 enhances human fetal erythro-megakaryocytic development. Blood. 2008;112(12):4503–6.
Creutzig U, et al. AML patients with Down syndrome have a high cure rate with AML-BFM therapy with reduced dose intensity. Leukemia. 2005;19(8):1355–60.
Creutzig U, et al. Less toxicity by optimizing chemotherapy, but not by addition of granulocyte colony-stimulating factor in children and adolescents with acute myeloid leukemia: results of AML-BFM 98. J Clin Oncol. 2006;24(27):4499–506.
de Hingh YC, et al. Intrinsic abnormalities of lymphocyte counts in children with down syndrome. J Pediatr. 2005;147(6):744–7.
Elagib KE, et al. RUNX1 and GATA-1 coexpression and cooperation in megakaryocytic differentiation. Blood. 2003;101(11):4333–41.
Emmrich S, et al. miR-99a/100~125b tricistrons regulate hematopoietic stem and progenitor cell homeostasis by shifting the balance between TGFbeta and Wnt signaling. Genes Dev. 2014;28(8):858–74.
Forestier E, et al. Cytogenetic features of acute lymphoblastic and myeloid leukemias in pediatric patients with Down syndrome: an iBFM-SG study. Blood. 2008;111(3):1575–83.
Frost BM, et al. Cellular cytotoxic drug sensitivity in children with acute leukemia and Down's syndrome: an explanation to differences in clinical outcome? Leukemia. 2000;14(5):943–4.
Gamis AS, Smith FO. Transient myeloproliferative disorder in children with Down syndrome: clarity to this enigmatic disorder. Br J Haematol. 2012;159(3):277–87.
Gamis AS, et al. Increased age at diagnosis Has a significantly negative effect on outcome in children with down syndrome and acute myeloid leukemia: a report from the Children's Cancer Group Study 2891. J Clin Oncol. 2003;21(18):3415–22.
Gamis AS, et al. Natural history of transient myeloproliferative disorder clinically diagnosed in Down syndrome neonates: a report from the Children's Oncology Group Study A2971. Blood. 2011;118(26):6752–9, quiz 6996.
Garre ML, et al. Pharmacokinetics and toxicity of methotrexate in children with Down syndrome and acute lymphocytic leukemia. J Pediatr. 1987;111(4):606–12.
Garrison MM, Jeffries H, Christakis DA. Risk of death for children with down syndrome and sepsis. J Pediatr. 2005;147(6):748–52.

Ge Y, et al. Transcriptional regulation of the cystathionine-beta -synthase gene in Down syndrome and non-Down syndrome megakaryocytic leukemia cell lines. Blood. 2003;101(4):1551–7.
Harvey RC, et al. Rearrangement of CRLF2 is associated with mutation of JAK kinases, alteration of IKZF1, Hispanic/Latino ethnicity, and a poor outcome in pediatric B-progenitor acute lymphoblastic leukemia. Blood. 2010;115(26):5312–21.
Hasle H. Pattern of malignant disorders in individuals with Down's syndrome. Lancet Oncol. 2001;2(7):429–36.
Hasle H, Clemmensen IH, Mikkelsen M. Risks of leukaemia and solid tumours in individuals with Down's syndrome. Lancet. 2000;355(9199):165–9.
Hasle H, et al. A pediatric approach to the WHO classification of myelodysplastic and myeloproliferative diseases. Leukemia. 2003;17(2):277–82.
Hattori M, et al. The DNA sequence of human chromosome 21. Nature. 2000;405(6784):311–9.
Hertzberg L, et al. Down syndrome acute lymphoblastic leukemia, a highly heterogeneous disease in which aberrant expression of CRLF2 is associated with mutated JAK2: a report from the International BFM Study Group. Blood. 2010;115(5):1006–17.
Hitzler JK, Zipursky A. Origins of leukaemia in children with Down syndrome. Nat Rev Cancer. 2005;5(1):11–20.
Hitzler JK, et al. GATA1 mutations in transient leukemia and acute megakaryoblastic leukemia of Down syndrome. Blood. 2003;101(11):4301–4.
Hollanda LM, et al. An inherited mutation leading to production of only the short isoform of GATA-1 is associated with impaired erythropoiesis. Nat Genet. 2006;38(7):807–12.
Jiang J, et al. Translating dosage compensation to trisomy 21. Nature. 2013;500(7462):296–300.
Kirsammer G, et al. Highly penetrant myeloproliferative disease in the Ts65Dn mouse model of Down syndrome. Blood. 2008;111(2):767–75.
Kiyoi H, et al. JAK3 mutations occur in acute megakaryoblastic leukemia both in Down syndrome children and non-Down syndrome adults. Leukemia. 2007;21(3):574–6.
Klusmann JH, et al. Treatment and prognostic impact of transient leukemia in neonates with Down syndrome. Blood. 2008;111(6):2991–8.
Klusmann JH, et al. Developmental stage-specific interplay of GATA1 and IGF signaling in fetal megakaryopoiesis and leukemogenesis. Genes Dev. 2010a;24(15):1659–72.
Klusmann JH, et al. miR-125b-2 is a potential oncomiR on human chromosome 21 in megakaryoblastic leukemia. Genes Dev. 2010b;24(5):478–90.
Korbel JO, et al. The genetic architecture of Down syndrome phenotypes revealed by high-resolution analysis of human segmental trisomies. Proc Natl Acad Sci. 2009;106(29): 12031–6.
Korenberg JR, et al. Down syndrome phenotypes: the consequences of chromosomal imbalance. Proc Natl Acad Sci U S A. 1994;91:4997–5001.
Kudo K, et al. Prospective study of a pirarubicin, intermediate-dose cytarabine, and etoposide regimen in children with down syndrome and acute myeloid leukemia: the Japanese Childhood AML Cooperative Study Group. J Clin Oncol. 2007;25(34):5442–7.
Kusters MA, et al. Intrinsic defect of the immune system in children with Down syndrome: a review. Clin Exp Immunol. 2009;156(2):189–93.
Lane AA, et al. Triplication of a 21q22 region contributes to B cell transformation through HMGN1 overexpression and loss of histone H3 Lys27 trimethylation. Nat Genet. 2014;46(6):618–23.
Lange BJ, et al. Distinctive demography, biology, and outcome of acute myeloid leukemia and myelodysplastic syndrome in children with Down syndrome: Children's Cancer Group Studies 2861 and 2891. Blood. 1998;91(2):608–15.
Lejeune J, Gautier M, Turpin R. Etude des chromosomes somatiques de neuf enfants mongoliens. C R Acad Sci. 1959;248:1721–2.
Letourneau A, et al. Domains of genome-wide gene expression dysregulation in Down's syndrome. Nature. 2014;508(7496):345–50.
Li Z, et al. Developmental stage-selective effect of somatically mutated leukemogenic transcription factor GATA1. Nat Genet. 2005;37(6):613–9.

Lightfoot J, et al. Distinct gene signatures of transient and acute megakaryoblastic leukemia in Down syndrome. Leukemia. 2004;18(10):1617–23.
Linabery AM, et al. Exposure to medical test irradiation and acute leukemia among children with Down syndrome: a report from the Children's Oncology Group. Pediatrics. 2006;118(5): e1499–508.
Loh RK, et al. Immunoglobulin G subclass deficiency and predisposition to infection in Down's syndrome. Pediatr Infect Dis J. 1990;9(8):547–51.
Luis TC, et al. Canonical wnt signaling regulates hematopoiesis in a dosage-dependent fashion. Cell Stem Cell. 2011;9(4):345–56.
Maclean GA, et al. Altered hematopoiesis in trisomy 21 as revealed through in vitro differentiation of isogenic human pluripotent cells. Proc Natl Acad Sci U S A. 2012;109(43):17567–72.
Malinge S, et al. Activating mutations in human acute megakaryoblastic leukemia. Blood. 2008;112(10):4220–6.
Malinge S, et al. Increased dosage of the chromosome 21 ortholog Dyrk1a promotes megakaryoblastic leukemia in a murine model of Down syndrome. J Clin Invest. 2012;122(3):948–62.
Malinge S, et al. Development of acute megakaryoblastic leukemia in Down syndrome is associated with sequential epigenetic changes. Blood. 2013;122(14):e33–43.
Maloney KW. Acute lymphoblastic leukaemia in children with Down syndrome: an updated review. Br J Haematol. 2011;155(4):420–5.
Maloney KW, et al. Down syndrome childhood acute lymphoblastic leukemia has a unique spectrum of sentinel cytogenetic lesions that influences treatment outcome: a report from the Children's Oncology Group. Blood. 2010;116(7):1045–50. p. blood-2009-07-235291.
Massey GV, et al. A prospective study of the natural history of transient leukemia (TL) in neonates with Down syndrome (DS): Children's Oncology Group (COG) study POG-9481. Blood. 2006;107(12):4606–13.
Mateos M, et al. Down syndrome and leukemia: insights into leukemogenesis and translationla targets. Transl Pediatr. 2015;4(2):76–92.
Maude SL, et al. Targeting JAK1/2 and mTOR in murine xenograft models of Ph-like acute lymphoblastic leukemia. Blood. 2012;120(17):3510–8.
McElwaine S, et al. Microarray transcript profiling distinguishes the transient from the acute type of megakaryoblastic leukaemia (M7) in Down's syndrome, revealing PRAME as a specific discriminating marker. Br J Haematol. 2004;125(6):729–42.
Mejia-Arangure JM, et al. Environmental factors contributing to the development of childhood leukemia in children with Down's syndrome. Leukemia. 2003;17(9):1905–7.
Mejia-Arangure JM, et al. Magnetic fields and acute leukemia in children with Down syndrome. Epidemiology. 2007;18(1):158–61.
Mullighan CG, et al. Rearrangement of CRLF2 in B-progenitor- and Down syndrome-associated acute lymphoblastic leukemia. Nat Genet. 2009a;41(11):1243–6.
Mullighan CG, et al. JAK mutations in high-risk childhood acute lymphoblastic leukemia. Proc Natl Acad Sci U S A. 2009b;106(23):9414–8.
Muramatsu H, et al. Risk factors for early death in neonates with Down syndrome and transient leukaemia. Br J Haematol. 2008;142(4):610–5.
Ng AP, et al. Trisomy of Erg is required for myeloproliferation in a mouse model of Down syndrome. Blood. 2010;115(19):3966–9.
Nikolaev SI, et al. Exome sequencing identifies putative drivers of progression of transient myeloproliferative disorder to AMKL in infants with Down syndrome. Blood. 2013;122(4):554–61.
Nizetic D, Groet J. Tumorigenesis in Down's syndrome: big lessons from a small chromosome. Nat Rev Cancer. 2012;12(10):721–32.
Norton A, et al. Analysis of JAK3, JAK2, and C-MPL mutations in transient myeloproliferative disorder and myeloid leukemia of Down syndrome blasts in children with Down syndrome. Blood. 2007;110(3):1077–9.
O'Connor D, et al. Infection-related mortality in children with acute lymphoblastic leukemia: a retrospective analysis of infectious deaths on UKALL 2003. Blood. 2014;124(7):1056–61.

Ogawa J, et al. Platelet-derived growth factor may be associated with fibrosis in a Down syndrome patient with transient myeloproliferative disorder. Eur J Haematol. 2008;81(1):58–64.
Okuda T, et al. AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis. Cell. 1996;84(2):321–30.
Olson LE, et al. A chromosome 21 critical region does not cause specific Down syndrome phenotypes. Science. 2004;306(5696):687–90.
OMIM. http://www.ncbi.nlm.nih.gov. #190685.
Ono R, et al. Acute megakaryoblastic leukemia with acquired trisomy 21 and GATA1 mutations in phenotypically normal children. Eur J Pediatr. 2015;174(4):525–31.
Pogribna M, et al. Homocysteine metabolism in children with Down syndrome: in vitro modulation. Am J Hum Genet. 2001;69(1):88–95.
Puumala SE, et al. Reproductive history, infertility treatment, and the risk of acute leukemia in children with down syndrome: a report from the Children's Oncology Group. Cancer. 2007;110(9):2067–74.
Rabin KR, Whitlock JA. Malignancy in children with trisomy 21. Oncologist. 2009;14(2):164–73.
Rainis L, et al. Mutations in exon 2 of GATA1 are early events in megakaryocytic malignancies associated with trisomy 21. Blood. 2003;102(3):981–6.
Roberts I, Izraeli S. Haematopoietic development and leukaemia in Down syndrome. Br J Haematol. 2014;167:587–99.
Roberts I, et al. GATA1-mutant clones are frequent and often unsuspected in babies with Down syndrome: identification of a population at risk of leukemia. Blood. 2013;122(24):3908–17.
Roberts KG, et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. N Engl J Med. 2014;371(11):1005–15.
Roy A, Roberts I, Vyas P. Biology and management of transient abnormal myelopoiesis (TAM) in children with Down syndrome. Semin Fetal Neonatal Med. 2012a;17(4):196–201.
Roy A, et al. Perturbation of fetal liver hematopoietic stem and progenitor cell development by trisomy 21. Proc Natl Acad Sci. 2012b;109(43):17579–84.
Ryeom S, et al. Targeted deletion of the calcineurin inhibitor DSCR1 suppresses tumor growth. Cancer Cell. 2008;13(5):420–31.
Ryeom S, Baek KH, Zaslavsky A. Down's syndrome: protection against cancer and the therapeutic potential of DSCR1. Future Oncol. 2009;5(8):1185–8.
Saida S, et al. Clonal selection in xenografted TAM recapitulates the evolutionary process of myeloid leukemia in Down syndrome. Blood. 2013;121(21):4377–87.
Salek-Ardakani S, et al. ERG is a megakaryocytic oncogene. Cancer Res. 2009;69(11):4665–73.
Sankaran VG, et al. Exome sequencing identifies GATA1 mutations resulting in Diamond-Blackfan anemia. J Clin Invest. 2012;122(7):2439–43.
Schifferli A, et al. Transient myeloproliferative disorder in neonates without Down syndrome: case report and review. Eur J Haematol. 2015;94(5):456–62.
Sorrell AD, et al. Favorable survival maintained in children who have myeloid leukemia associated with Down syndrome using reduced-dose chemotherapy on Children's Oncology Group trial A2971: a report from the Children's Oncology Group. Cancer. 2012;118(19):4806–14.
Starc TJ. Erythrocyte macrocytosis in infants and children with Down syndrome. J Pediatr. 1992;121(4):578–81.
Taub JW, Ge Y. Down syndrome, drug metabolism and chromosome 21. Pediatr Blood Cancer. 2005;44(1):33–9.
Taub JW, et al. Enhanced metabolism of 1-beta-D-arabinofuranosylcytosine in Down syndrome cells: a contributing factor to the superior event free survival of Down syndrome children with acute myeloid leukemia. Blood. 1996;87(8):3395–403.
Taub JW, et al. Expression of chromosome 21-localized genes in acute myeloid leukemia: differences between Down syndrome and non-Down syndrome blast cells and relationship to in vitro sensitivity to cytosine arabinoside and daunorubicin. Blood. 1999;94(4):1393–400.

Taub JW, et al. Cystathionine-{beta}-synthase cDNA transfection alters the sensitivity and metabolism of 1-{beta}-D-arabinofuranosylcytosine in CCRF-CEM leukemia cells in vitro and in vivo: a model of leukemia in down syndrome. Cancer Res. 2000;60(22):6421–6.

Taub JW, et al. Prenatal origin of GATA1 mutations may be an initiating step in the development of megakaryocytic leukemia in Down syndrome. Blood. 2004;104(5):1588–9.

Taub J, et al. Minimal residual disease (MRD) identifies Down syndrome acute myeloid leukemia (DS-AML) patients with extremely high event free survival (EFS) rates: results of the Children's Oncology Group (COG) phase III AAML0431 trial. Annual Meeting of the American Society of Hematology, abstract, Blood. 2014;124(21).

Tsai MH, et al. Transient myeloproliferative disorder and GATA1 mutation in neonates with and without Down syndrome. Indian J Pediatr. 2011;78(7):826–32.

Tunstall-Pedoe O, et al. Abnormalities in the myeloid progenitor compartment in Down syndrome fetal liver precede acquisition of GATA1 mutations. Blood. 2008;112(12):4507–11.

Ugazio AG, et al. Immunology of Down syndrome: a review. Am J Med Genet Suppl. 1990;7:204–12.

Verstegen RH, et al. Down syndrome B-lymphocyte subpopulations, intrinsic defect or decreased T-lymphocyte help. Pediatr Res. 2010;67(5):563–9.

Wechsler J, et al. Acquired mutations in GATA1 in the megakaryoblastic leukemia of Down syndrome. Nat Genet. 2002;32(1):148–52.

Welch JS, et al. The origin and evolution of mutations in acute myeloid leukemia. Cell. 2012;150(2):264–78.

Whitlock JA. Down syndrome and acute lymphoblastic leukaemia. Br J Haematol. 2006;135(5):595–602.

Yoshida K, et al. The landscape of somatic mutations in Down syndrome-related myeloid disorders. Nat Genet. 2013;45(11):1293–9.

Zipursky A. Transient leukaemia – a benign form of leukaemia in newborn infants with trisomy 21. Br J Haematol. 2003;120(6):930–8.

Zorick TS, et al. High serum endostatin levels in Down syndrome: implications for improved treatment and prevention of solid tumours. Eur J Hum Genet. 2001;9(11):811–4.

Zwaan CM, et al. Different drug sensitivity profiles of acute myeloid and lymphoblastic leukemia and normal peripheral blood mononuclear cells in children with and without Down syndrome. Blood. 2002;99(1):245–51.

Chapter 6
Model for the Origin of Acute Leukemias in Children: Interaction of Three Factors—Susceptibility, Exposure, and Window of Vulnerability

Juan Manuel Mejía-Aranguré

Abstract Various theoretical models concerning the origin of leukemias in children, especially acute lymphoblastic leukemia (ALL), attempt to explain why leukemia occurs and how it develops; the proposed model, being relatively simpler, attempts to specify the moment when a child develops acute leukemia. The causes of childhood leukemia have not yet been identified because the theoretical basis of the search has been at fault. The risk factors for acute leukemia (AL) are distinct, depending on the age at onset. It is probable that the older the child, the greater the necessity of risk factors to which the child must be exposed for the disease to develop and the less the susceptibility to AL with which the child was born. For this reason, I venture to say that the age at onset of AL is a reflection of the degree of susceptibility to the disease and of the number of factors of exposure to carcinogens that are necessary for the development of the disease. This conjecture also depends on the window of vulnerability in which the child is situated. This window of vulnerability is directly involved with the proliferation of the child's B or T cells, which cause the interaction between the degree of susceptibility and the degree of exposure to carcinogens, thereby provoking the onset of AL.

Keywords Acute leukemia • Acute lymphoblastic leukemia • Acute myeloid leukemia • Etiology • Epidemiology • Children • Environmental risk factors

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Childhood leukemias are a collection of illnesses. The etiology of each depends on its morphology, its immunophenotype, and the molecular changes that characterize it.

Theoretical models exist concerning the origin of leukemias in children, especially ALL and, more particularly, precursor B-cell leukemias. Some models attempt to explain why leukemia occurs (Greaves 2000a), whereas others explain how it develops (Taylor 1994; Greaves and Wiemels 2003; Greaves 2006a; Schmiegelow et al. 2008; Richardson 2011). The proposed model, being relatively simple, attempts to specify the moment when a child develops acute leukemia (Mejía-Aranguré 2013). The major part of this model is centered on the moment that ALL appears; however, acute myeloid leukemia (AML) serves as a point of departure to demonstrate that this type of leukemias fits the proposed model.

ALL was first described by Velpeau in 1827. That work was cited 20 years later by Virchov (Henderson 2002); since then, attempts have been made to explain the causes of leukemias in children. Both in the past and the present, theories of infectious agents have played an important role in attempts to determine the origin of childhood leukemias; it is possible that, in the coming years, these theories will continue to proffer great weight (Greaves 2006b; McNally and Eden 2004).

Despite many years of study, only two determining factors have been identified in childhood leukemias: exposure to X-rays in utero and Down syndrome (DS) (Maloney et al. 2015; Inaba et al. 2013). Although it is known that the genetic rearrangements *ETV6/RUNX1* and *MLL* appear during the intrauterine stage and that, thereafter, the children develop leukemia, it is not possible to say that these rearrangements necessarily cause the disease, but rather that they appear to be components of this infirmity.

That it has not been possible to identify the environmental causes of childhood leukemias may lead to two suppositions, the first being that environmental causes have not been identified because, perhaps, they do not exist (Greaves 2000a). Thus, leukemia results from the high division of blood cells, during which an error in such division generates a series of mutations that (together with a great deal of bad luck) could determine the onset of leukemia. The other option, with which I am more in accord, is that the causes of childhood leukemia have not yet been identified because the theoretical basis of the search has been at fault.

If the appearance of childhood leukemias were only the result of the rate of mutation of the pluripotential cells in the blood and to randomness, there would be an expectation that the frequency of childhood leukemias worldwide would vary little, the differences being ceded by the probability that a child would survive to develop leukemia. However, the worldwide frequency of childhood leukemias is highly variable. There is a higher frequency among populations having better economic resources: the highest frequency of lymphoblastic leukemias is reported among white populations and in Hispanics (Wartenberg et al. 2008; Mejía-Aranguré et al. 2011a, b). This variability in the rates of incidence of leukemias leads one to think that there may exist factors, external to the individual, that may be involved and would generate the marked difference in the incidence of leukemias found among different populations.

As mentioned, the proposed model assumes that the reason that environmental factors have not been identified as causes of leukemias relates to the fact that the search has been at fault, and not that environmental factors do not exist. Among the reasons for considering the search faulty is that the statistical power has been insufficient to identify an association between environmental factors and the development of leukemia. Therefore, some groups, such as the Childhood Leukemia International Consortium, have sought to perform analysis in collaboration; through the use of databases of different countries, the aim is to efficiently achieve sufficient power to identify the causes of childhood leukemias (Metayer et al. 2013). This approach is based on the following two important points. First, childhood leukemias are rare, affecting in general terms 1 in 2,000 children (Greaves 2006a). This frequency is extraordinarily rare when compared with those of asthma, diarrheic infections, or infections of the upper respiratory tract. Second, other exposures that generate leukemia may be very rare, or the effect with which they are associated and which produces leukemia may be very weak (this is measured by use of the odds ratio).

However, exposures that are less rare do exist; for example, passive exposure to tobacco smoke (which contains a great number of carcinogenic substances) and, in particular, exposure to substances such as benzenes and its derivatives, which have a predilection for affecting blood cells to produce leukemia. Yet, although such exposures are not rare, they cannot be identified as causes of leukemias because the results have been inconsistent (Chang 2009; Pyatt and Hays 2010). Here, I will summarize by saying that such inconsistency may be due to selection biases, the best example of this being exposure to extremely low-frequency electromagnetic fields. Kheifets et al. (2010) pointed out that one of the principal reasons why it has not been possible to determine whether such exposure is a cause of ALL, or not, is the presence of selection biases. In these studies, the controls often come from a higher socioeconomic level than do the cases; the population that comes from a lower socioeconomic level is often more exposed to high levels of magnetic fields. It is assumed that fewer controls come from the lower socioeconomic category. Thus, an artificial association between high levels of magnetic fields and ALL would be generated: if controls recruited from lower socioeconomic levels were to participate in the same proportion as that of the cases of ALL, the said association would be diluted (Mejía-Aranguré et al. 2007).

On the other hand, the lack of identification of the causes of leukemias may be due to errors in the manner whereby the variables are measured. In most of such studies, exposures were measured retrospectively, under the assumption that the sources of exposure would have remained intact or varied little over time or that people would remember precisely to what they had been exposed to in prior years. If a child developed leukemia when 1 year old, it is reasonable that the mother and father would remember what occurred during the pregnancy or during the year prior to the development of the disease with a greater degree of precision than would parents whose child developed leukemia when 15 years old (Schüz et al. 2003; Rudant et al. 2010). It is difficult to imagine that parents would remember with detail the exposures that occurred 15 years earlier during the pregnancy.

Fortunately for epidemiology, details concerning the use of tobacco, such as the age at which smoking was initiated and the times when the individual smoked more or stopped smoking, are relatively easy to remember. From such information, a reconstruction of the history of exposure can be attempted; if individuals do not have knowledge concerning some particular factor that may be associated with the development of leukemia, the assumption is made that the exposures in that population will be equal to those in the control population (Mejía-Aranguré et al. 2003). Thus, researchers expect that if a positive association between exposure and leukemia is found, then that association may be even higher than the one observed, because this is generally the way non-differential errors in measurement of exposure behave. There are those who attempt to disparage the value of case-control studies, pointing out that such studies are subject to recall bias, in that the cases often better remember their exposures than do the controls. However, on evaluation no empirical data have been found that support the presence, in particular, of recall bias in studies of the causes of childhood leukemia (Schüz et al. 2003).

On the other hand, biases originating in confounding are another reason why it is not possible to establish whether the relation between a variable and leukemia is real or is due to the presence of other factors, called confounders. It has been proposed that a factor may be considered a confounder when it is a true risk factor for leukemia, is associated with the independent variable or the risk factor being studied, and, in addition, is not an intermediate in the causal chain. An example would be determining whether the exposure of parents to tobacco smoke before conception of a child who then develops leukemia was a true risk factor for the development of the disease. A factor that may act as a confounder is the consumption of alcohol by the parents before conception of the child. Both behaviors are factors of risk for leukemia; both appear strongly correlated, as those who smoke also drink alcoholic beverages with a higher frequency than those who do not smoke. Neither of these behaviors acts as an intermediate in the causal chain; therefore, when analyzing whether the exposure of parents to tobacco smoke is a risk factor for leukemia, it is important to eliminate the possibility that the association is not due to the effect of alcohol and not in fact to the effect of tobacco smoke (Fig. 6.1).

Here the problem is that, in practice, either associations are not encountered or the associations that are found are very weak. To me, it appears that it is the manner

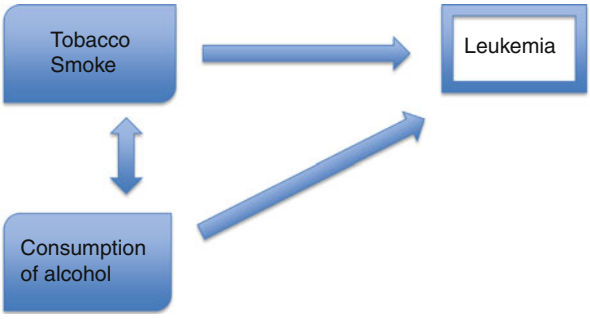

Fig. 6.1 In this example, tobacco smoke and consumption of alcohol are mentioned with respect to the father before conception

in which we search for these risk factors that determines whether identification of the cause of childhood leukemias can be achieved. In the present model, it is proposed that environmental factors only provoke cancer depending on the degree of susceptibility of the child for developing leukemia or on the ability to detoxify the effect of the carcinogenic agent. Complicating this situation even more is the possibility that the quantity of other substances that accompany the primary substance must also be taken into account. We used this approach in a study where we evaluated the association between the degree of exposure to carcinogenic substances and the development of childhood leukemias (Perez-Saldivar et al. 2008). In that study, instead of searching for a relation between only one carcinogenic factor and the development of leukemias, we investigated whether the addition of various carcinogenic factors would permit us to identify the relationship with AL. Such an association was found; AL was associated, not with one substance in particular, but with the sum of carcinogenic substances to which the child had been exposed, independent of the stage of life at which said exposure occurred.

There are substances, such as benzene, which are known to be carcinogenic and which also have a great predilection for damaging blood cells and are thought to be strongly associated with the development of leukemia, be it in adults or in children. Yet despite such strong associations, the results of studies with these substances have been contradictory or inconsistent. Although there are methodological problems that may explain such inconsistency, if benzene is a risk factor for AL, why are we not able to easily identify it as such?

I will start by considering two aspects; for this, it is necessary to begin with the theory of multiple causation. We begin with the proposition that AL is the result of one or many sufficient causes, "pies," and not that there is a unique sufficient cause, as is thought to be the case with mixed-lineage leukemia (MLL) rearrangements in leukemias of children younger than 1 year (Greaves 1999). A sufficient cause is understood as the set of component causes which, when taken together, form the cause that brings about development of the disease (Rothman 1976). Because benzene would be only a "piece of the pie," it is not possible that, by study of this substance alone, we would be able to determine whether this is what induces leukemia in the population. That is, if the theory of multiple causation is true, then using the present context, a complete pie, not just a part (here, benzene), is required to induce leukemia. If all the possible pies that may exist for a child to develop leukemia included benzene as a component, it would be easy to identify benzene as a risk factor in leukemia. However, if this factor is the component of one, or very few, of the many possible pies and, in addition, said pie is one of the least frequent in the study population, then it would be very difficult to determine that benzene induced AL in that population. If the pie was very frequent in one population, the factor might be identified in that population, but very probably would not be in other populations in which such a factor is less frequent. It is possible that this factor would need to be accompanied by one or many factors to produce AL.

It is possible that this factor would produce AL only in children having a determined susceptibility. It is also possible that all sufficient causes—each of the pies—may be very rare, and that more depends on each population, in such manner

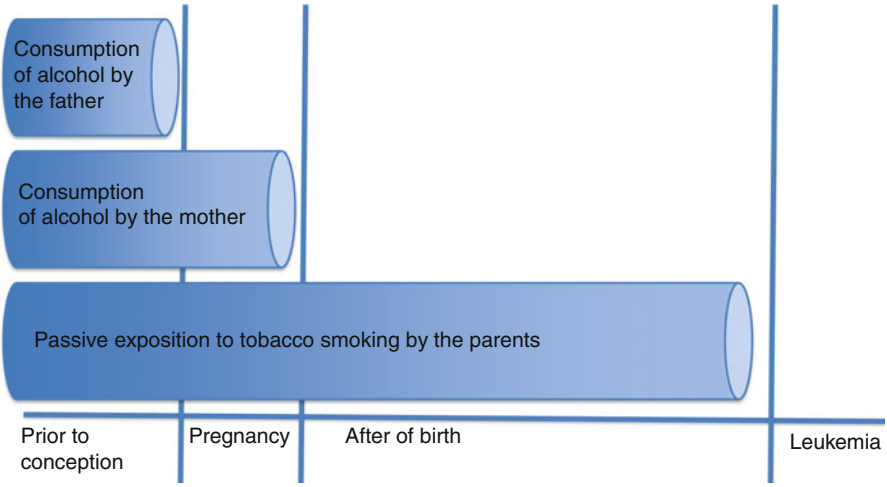

Fig. 6.2 Exposure is not unique, nor does it occur in only one moment. There are factors that have an effect only at one stage of life, as is the case of the consumption of alcohol by the father of the child with leukemia: the critical period is that prior to conception. Some factors, such as the consumption of alcohol by the mother, have an effect only prior to conception or during pregnancy. However there are factors, such as tobacco smoking by the parents, which have an effect during all stages of life. Such factors induce damage to the cells at different levels, thus favoring not only the onset of leukemic cells, but also that these continue acquiring advantages over the cells in their microenvironment, which can result in leukemia

that AL may not be a result of exposure to one risk factor, but rather to the sum of many risk factors that may occur at different time periods (Fig. 6.2). Time is a determining element in the causation of AL (and other cancers); for example, if a cell is not dividing, there cannot be mutations that lead to cancer. Cells neither divide all the time, nor with the same intensity. Just as an automobile that hits a small obstacle in the road creates a large accident, it may be, as proposed by Greaves (2006a), that it is in a moment of immunological stress, when a cell needs to divide more, that AL develops. In contrast to Greaves, I consider this to be the moment when a cell, previously damaged or not, may develop AL; that is, if there is a great proliferation of cells that can be induced to develop AL, a pluripotential cell and an intense exposure, independent of the degree of susceptibility of the individual, could induce the development of AL (Fig. 6.3). Viruses may make cells enter into states of intense proliferation such that, independent of whether the child is susceptible to AL, if there is high exposure, then AL will develop. This is more evident in the case of immunological stress in pre-B-cell ALL, but there is evidence that, in the moment when there is an exaggerated increase of cell division in a tissue, said tissue is more vulnerable to the development of cancer, as is true in the cases of retinoblastoma, Wilms' tumor, or osteosarcoma (Mejía-Aranguré et al. 2005).

However, it is probable that this increase leading to cancer may be associated to something more that makes the tissue vulnerable to cancer; in other cancers, undeveloped embryonic tissue predisposes to cancer (Greaves 2000b; Anderson

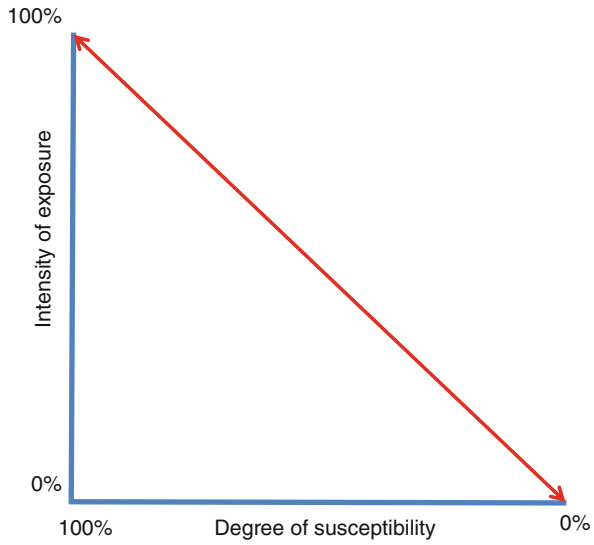

Fig. 6.3 The gradient for exposure is given as 0–100 %, where, theoretically, 0 % implies that the exposure is incapable of inducing leukemia and 100 % is the exposure level that will invariably induce acute leukemia in an individual. The degree of susceptibility also ranges from 0 % to 100 %, where 0 % refers to the risk that a child has of developing acute leukemia and 100 % is the risk of a child having the susceptibility necessary for developing leukemia. Hypothetically, an example of the latter may be those children born with the MLL/AF4 rearrangement

et al. 2000; Marshall et al. 2014). In the case of osteosarcoma, the age peak with the highest number of cases occurring at adolescence (10–14 years of age), it must be taken into account that adolescence is not the stage of life at which the bones of the child grow the most but rather in the intrauterine stage; nevertheless, osteosarcoma is not encountered in the first years of life (Bassin et al. 2006; Kansara et al. 2014). It is probable that some factors, such as viruses, may be capable of bringing cells to developmental stages at which carcinogenic agents may induce mutations.

To find these three factors together (susceptibility, exposition, and vulnerable time) is extraordinarily rare in childhood cancer, especially AL. Nevertheless, if we know which factors are more commonly parts of the pies and if we know that these factors are frequent in a specific population, this information may lead us to strategies for the prevention of AL.

Various pies have to be tested in the same population, but the various factors must be gathered together in striving to identify the causes of AL. I believe that the search for unique factors has led to the failure to determine the causes of childhood leukemias. For more than 100 years we have searched for the causes of leukemias and, to date, have made little advancement. If we do not acknowledge that we have committed an error in the manner of this search, I am discouraged as to where we are going. I do not know how many more failures will be necessary before it is recognized that the approach we have used to identify the risk factors for leukemia has not led us anywhere.

That exposure to X-rays is a risk factor for the development of leukemia is an undeniable fact, but how many cases explain this? Practically none at present. The same is true for exposure to radiation generated by the explosion of an atomic bomb (Parkin and Darby 2011). These factors are a cause of leukemia; however, their involvement in identified cases depends on the frequency and intensity of such factors in the population (Rothman 1976).

Explanation of the causes of leukemias has become more complex, because it is necessary not only to identify the factors that produce leukemias, but also to know their frequency and intensity in the population under study. In one population a specific factor could be very important, whereas that same factor may not be prominent in other populations. These are very old concepts in epidemiology, which support the idea of attributable risk and the theory of component causes/sufficient causes (Rothman 1976).

One of the challenges is to identify not only which factors provoke leukemia, but also how many are needed for leukemia to develop. If this is true, even factors that potentially may not be so important because the association does not appear to be strong or is not so prevalent in all populations may, in combination with other factors associated with the development of leukemia in a population, have an impact on avoiding a large number of cases of disease within that community.

A potential factor that may fulfill this condition is infections by various viruses, producing effects such as the reprogramming of somatic cells to induce pluripotent stem cells (iPSCs) (Ramos-Mejía et al. 2010, 2012; Bueno et al. 2012; Muñoz et al. 2012). These not only could produce an excess proliferation of these cells but also could induce a mature cell to regress to a prior state whereby the cell may proliferate, thus permitting an external carcinogen to produce leukemia. If it were possible to avoid the viral infection or to inhibit the effect of the virus to bring about regression of the cells, this could prevent a great number of cases of leukemia.

Cells affected by a genetic rearrangement, such as ETV6/RUNX1, are extraordinarily sensitive to the effect of steroids. If an immune response, such as that provoked by an infection, can destroy these cells by increasing steroids and consequently avoid the development of leukemias, it is reasonable to surmise that, in the near future, a vaccine against leukemia could be produced (Schmiegelow et al. 2008). Although this would not prevent all cases of leukemia, in populations where this rearrangement is frequent there would be a great reduction in the number of new cases of leukemias.

The exposure factors that we must study are those that may be capable of producing a mutation in hematopoietic cells; those that promote the proliferation of these cells; and those that induce the hematopoietic microenvironment to favor the increase of malignant cells over that of benign cells (see chapter by Rosana Pelayo et al.). There are factors, especially benzene and all its derivatives, which have been proposed to damage hematopoietic cells. Just as in the case of tobacco smoke, this factor (benzene) is at times associated with the development of leukemias, particularly myeloid leukemias, the latter being considered to have the most consistent association (Chang 2009; Pyatt and Hays 2010). Yet, with tobacco smoke, there is a high percentage of children who develop leukemias, even though their

parents report not having been exposed to tobacco smoke. Therefore, according to the theory of component causes/sufficient causes, there must be at least one other sufficient cause that does not have exposure to tobacco smoke as a component (Fig. 6.4).

Exposure is variable: not everyone is exposed to a carcinogenic substance, and of those exposed, not everyone is exposed to the same degree. It is possible that, in the case of tobacco smoke, the smoke is capable not only of causing a cell to mutate, but also of causing that cell to proliferate until the cell becomes cancerous. In leukemia, something similar could happen. An intense exposure may make a child not only susceptible to the disease but also more vulnerable to other exposures, which

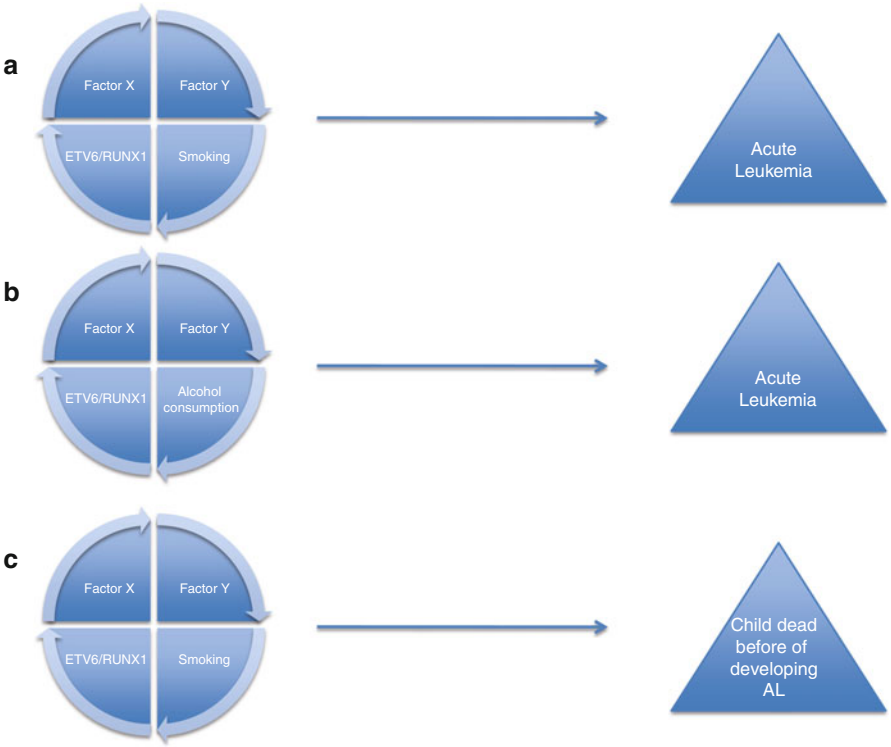

Fig. 6.4 Here, it is assumed that the components of tobacco smoke are capable of causing a child to develop leukemia, through the child's passive exposure to the smoke. Being capable of producing leukemia is part of a component cause (**a**). However, there are also children who developed leukemia but whose parents did not smoke. This implies that there is at least one other sufficient cause that does not include tobacco smoke (**b**). The inconsistency in the associations does not mean that passive exposure to tobacco smoke would not be a cause of leukemia in children, but that the frequency of this factor may be extraordinarily rare in the population, or that the factor provokes other diseases or even death, such that the child never reaches an age at which leukemia could develop (**c**)

together then cause the leukemia (Fig. 6.5). Thus, there may be children who with very little exposure to a particular factor may develop leukemias, because their prior exposure to other factors made them highly susceptible.

I remember a case of a boy whose father, when interviewed, reported there was no specific environmental factor that would have caused his son to develop leukemia. However, the father did mention to me that several weeks before, he had fumigated the house. Apparently attracted by the odor of the insecticide, the boy approached the door of the room and took a deep breath, possibly trying to better sample the smell. Upon seeing him, the father removed him from the area. In a few weeks, the boy began to present symptoms of fever and petechia; he was later diagnosed with leukemia. I am not saying that this exposure was enough to produce leukemia. However, is it possible that the boy had had a very high susceptibility to the disease and that an exposure, even as small as this one, brought about the development of leukemia? We do not know. But perhaps in many cases it would be worth the effort to investigate exposure to factors which occurred chronologically close to the onset

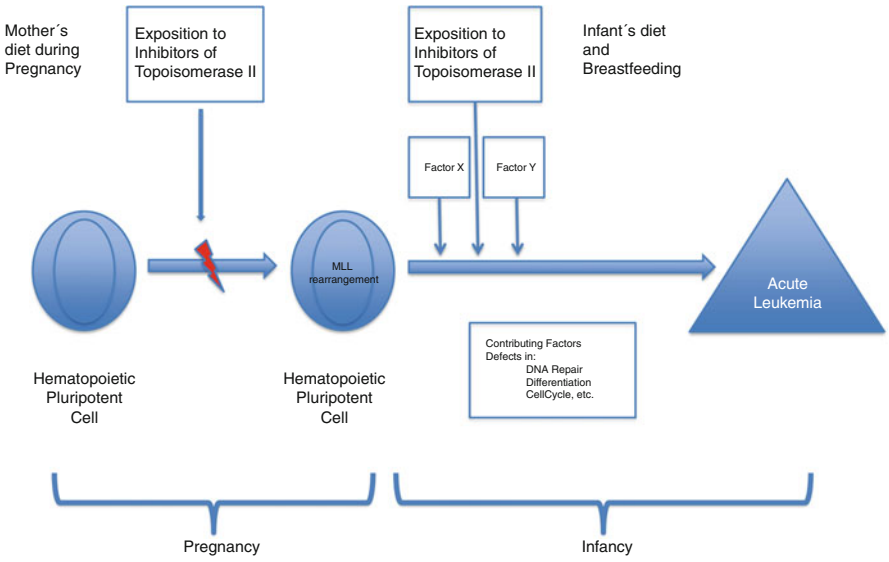

Fig. 6.5 Hematopoietic stem cells are exposed to an environment that at times is capable of generating mutations. In the drawing, the inhibitors of topoisomerase II are shown. When taken by the mother during pregnancy, they have been associated with the risk that the child will develop leukemia (Pendleton et al. 2014). However, it is more probable that not all children will develop susceptibility in this manner, but rather that there exist factors that influence susceptibility in children, in particular by affecting the ability to metabolize this type of substance, or in the way by which this type of mutation is allowed to persist, finally resulting in the child's becoming susceptible to leukemia, with a rearrangement in the *MLL* gene being generated. If this same exposure in maintained during the pregnancy (or perhaps it may be speculated that even if this substance were present in the child's diet), it could be that such exposure may cause the child to develop leukemia. However, contributing factors, such as defects in DNA repair, differentiation, or the cell cycle, would be necessary

of the disease; such information may reveal cases of those who are most susceptible to the disease. This seems similar to what happened in cases of children who, because they had symptoms of uncontrollable fever, received pharmaceuticals, such as diprone, and who were later diagnosed with leukemia. It is not that I think the leukemia could not have already been present. Had it not been present, perhaps only one factor could have unleashed the ultimate step that led to the development of leukemia. In the model of the development of leukemia proposed by Marshall et al. (2014), the assumption is made that there is a mutation from the earliest moment in the formation of blood cells, when these cells are found in the fetal liver; that, in the postnatal stage, other mutations occur that do not kill the cells; and, thereafter, still other mutations permit the leukemia to manifest.

I now turn to the topic of chronic exposures. Some children may have very little susceptibility to leukemia; therefore, a much longer time of exposure would be required for such a child first to become susceptible; thereafter it would be necessary for different mutations to accumulate in order for the leukemia to originate (Fig. 6.6). In the case of the survivors of the atomic bombing of Hiroshima and Nagasaki in 1945 during World War II, it took, on average, 2 years for the development of the disease. Why such a long time? From the time when a leukemia cell is formed, how much of a delay is there before the disease develops? There are animal models

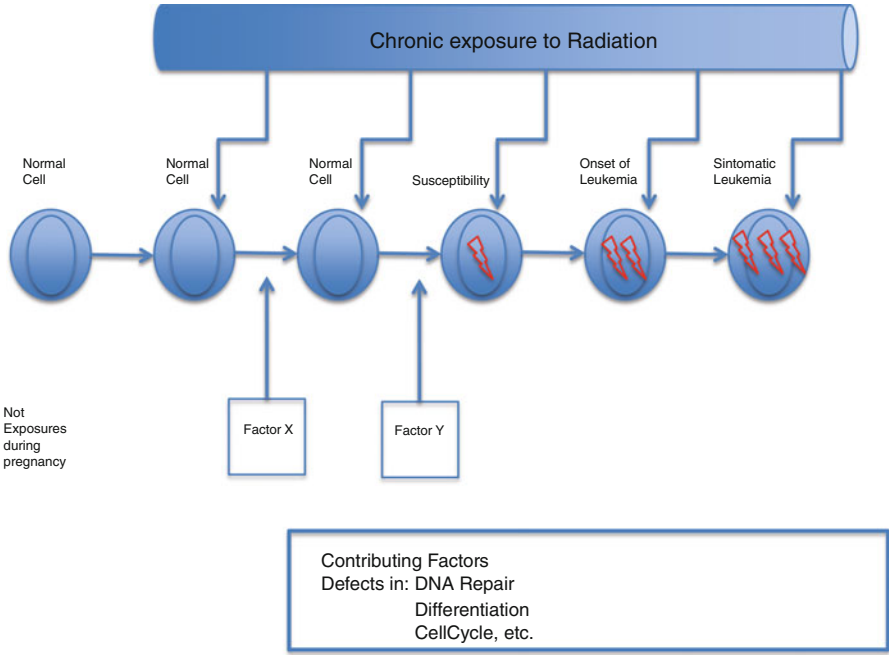

Fig. 6.6 Chronic exposure to a carcinogenic agent can very rapidly produce leukemia in a susceptible child. Chronic exposure would not have as much effect in a child who is not susceptible to leukemia. First, it is necessary that the child becomes susceptible; thereafter, the same exposure will cause the child to develop leukemia

showing that only 2 weeks is necessary from when the leukemia cell is formed to when the cell enters the peripheral circulation.

Exposure provides a standard that leads one to think that leukemia may be prevented by altering the duration or intensity of exposure, or both. It will be necessary to test various models of causality; the resulting information may make it possible to begin the search for measures to prevent this disease.

However, as mentioned earlier, environmental factors cannot be identified as being associated with leukemia if they are investigated without taking into account the susceptibility of the child to leukemia. For no matter how intense or how long the exposure may be, this will not produce leukemia in a child who is not susceptible to the disease (although, as mentioned before, if the cellular proliferation is intense, there could be disease).

As shown in Fig. 6.7, the presence of children in panel B demonstrates that, although the factor may be a true causal factor for leukemia, the child did not develop the disease for one of three reasons: (1) the child is not susceptible to the

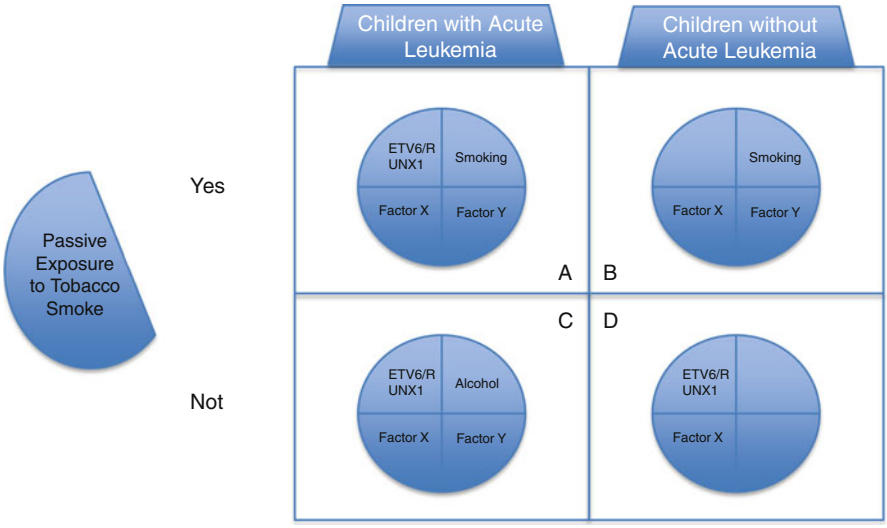

Fig. 6.7 Example of exposure to a risk factor. Passive exposure to tobacco smoke can cause a child to develop leukemia, when all the other components of the sufficient cause of leukemia are present together. Cell A shows that if the exposure is very intense and the cell is proliferating, as it may be in pre-B-cell ALL positive for common acute lymphoblastic leukemia antigen (CALLA$^+$) when the child is 2 years old, it is possible that this would be sufficient for the development of the disease. Cell B shows a group of children exposed to the factor, but because the other components of the sufficient cause are not present, they do not develop leukemia. Cell C shows the children who develop leukemia, but who were not exposed to the risk factor that is under study; that is, the component causes of the sufficient cause that provokes leukemia were not present in the exposure under study (here, passive exposure to tobacco smoke), but rather the sufficient causes have other component causes that are not included in the factor under study. Cell D shows the children who do not develop leukemia and who are not exposed to the factor under study

disease; (2) in these children, the other factors necessary to complete sufficient cause are missing; or (3) factors necessary to determine whether a leukemia cell can implant and then proliferate are missing.

When speaking of susceptibility to leukemia, at least five conditions are pertinent (Fig. 6.8). The first is the susceptibility that may well be the start of leukemia, as in the case of the child born with the *ETV6/RUNX1*, *MLL7/AF4*, or *AF9* rearrangement (Inaba et al. 2013); second, the susceptibility caused by the child's having a condition, such as Down syndrome, Fanconi anemia, or Bloom syndrome, which favors the development of leukemia (Seif 2011); third, the susceptibility

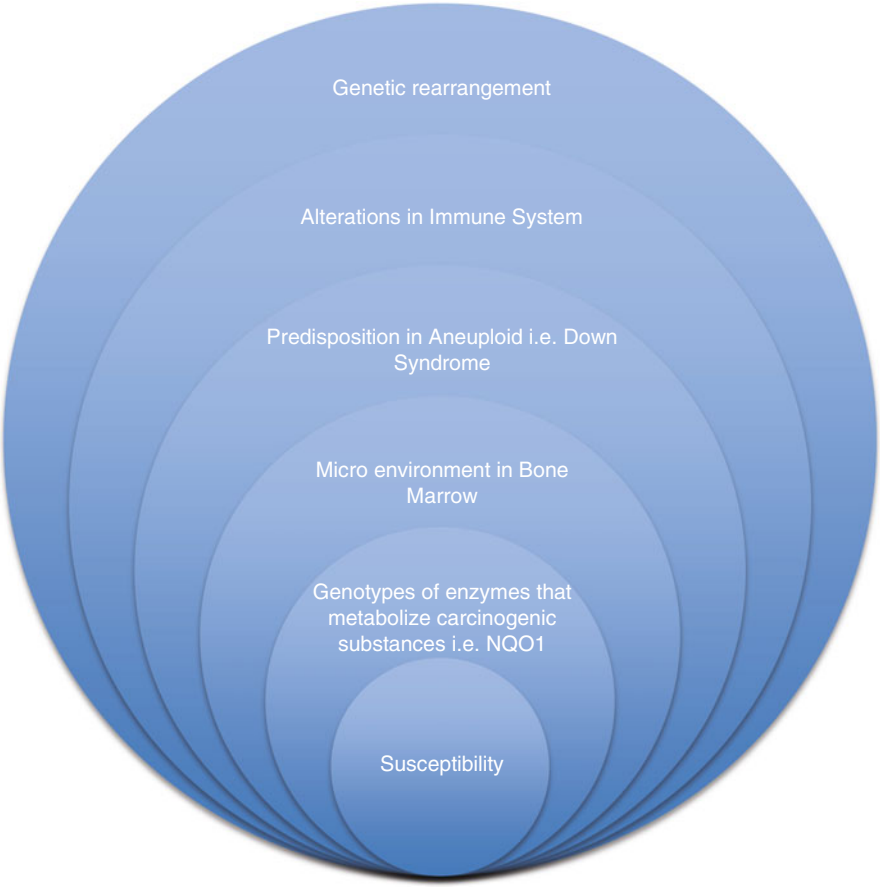

Fig. 6.8 It is not possible to speak of susceptibility, or predisposition to leukemia, as having only one aspect or as being a phenomenon that is a permanent state of the child. There are factors that appear at birth but that may be acquired at any moment of life. Examples are genetic rearrangements or changes in the niche of the bone marrow, which even may be modified by infectious processes (Prendergast and Essers 2014). Down syndrome is one of the aneuploidy syndromes associated with leukemia, but there are others such as the Li-Fraumeni syndrome (Seif 2011; Bhojmani et al. 2015)

generated by the immune system, the response of which very probably influences the proliferation of leukemia (Reither et al. 2015), as it is known that children with different immunological alterations have a greater disposition to leukemia; fourth, the susceptibility generated by the niche of bone marrow (Greim et al. 2014), where certain changes in the microenvironment increase susceptibility to the disease (see chapter by Rosana Pelayo et al., which focuses on this point); and fifth, the susceptibility generated by molecular mechanisms that do not permit adequate metabolism of carcinogenic agents, such as occurs with the NQOA1 family which influences the metabolism of hydrocarbons, similar to what happens with cytochrome P450, among others (Bhojwani et al. 2015).

If measurement of the role of exposure in causality of ALs is extraordinarily complex, the study of susceptibility is no simpler. Identification of the first genetic rearrangements in AL appears to have given us a guide for outlining causality of leukemias. Knowledge concerning rearrangements that occur at high frequency and the proteins involved may lead one to think that the basis for the development of AL in children is understood (Greaves 1999). However, as might be expected, there is no one specific rearrangement for leukemia. Understanding the causes of leukemia now appears to be more complex, in that the most frequent rearrangement, hyperdiploidy, is encountered only in approximately 25 % of cases; even with its high non-specificity, it appears to be the alteration most frequently associated with the development of ALs in children (Greaves 1999). Now we are also confronted by different molecular subtypes of AL, and each appears to have a different etiology. If it was difficult to know the causes of leukemia before, the complexity now is greater.

At the very least it is thought that these genetic rearrangements may be a necessary cause for the development of leukemia in which they are involved (Greaves and Wiemels 2003). If we decide to rename the leukemias based on their genetic rearrangement, evidently the rearrangement will be a necessary part for the development of said leukemia, just as occurs for infectious diseases. For example, it is not enough to be infected with the *Mycobacterium tuberculosis* for the individual to develop pulmonary tuberculosis; other factors are needed. However, without *M. tuberculosis* it would be impossible to have pulmonary tuberculosis. The infectious agent becomes part of the infirmity as well as being its cause. The same happens with genetic rearrangements: if we now decide to name the ALLs, for example, ETV6/RUNX1 ALL, then the rearrangement ETV6/RUNX1 becomes part of the diagnosis of the infirmity and evidently is a necessary cause for this particular disease to develop.

For infectious diseases, there are individuals who have an infectious agent, but who do not develop the corresponding disease; these are known as healthy carriers. The same occurs for AL. For example, it is known that approximately 1 in 12,000 newborns, as determined by blood samples, are born with the *ETV6/RUNX1* rearrangement, but only a small proportion of these individuals will develop ALL (5 % of the neonates with this genetic rearrangement have a twin who develops ALL). Thus, at least one other factor is needed for these children to develop ALL. Greaves and Wiemels (2003) proposed that a cell, previously mutated by

being presented with an intense demand to proliferate, could randomly undergo a second mutation that results in ALL being induced. For Greaves, this process is related to a later infection (Greaves 1999, Greaves and Wiemels 2003).

A rearrangement that appears very promising is the *MLL* rearrangement. This rearrangement is one of a very broad family associated with *MLL*; at present, more than 80 are known (Pendleton et al. 2014; Zhang et al. 2012). Henceforth in this chapter, MLL-r will be used to refer to rearrangements associated with MLL. These MLL-r appear to be very promising; in fact, they are considered as a sufficient cause of AL in infants (children <12 months of age), because 85 % of infants with AL have this genetic rearrangement. Evaluation of identical twins (monozygotic, monochorionic) has determined that 100 % of these twins, who at birth showed this rearrangement, developed AL (Greaves 1999). However, the optimism concerning the identification of the origin of AL in infants has faded, because not in all series of cases of infant leukemia is the frequency of MLL-r as high, and because articles have been published showing that children who were born with MLL-r can lose this rearrangement with the passage of time (Uckun et al. 1998; Uckun 1999). In addition, there are sensible questions concerning the role of MLL-r as a sufficient cause of ALs in infants (Greaves and Wiemels 2003): Why do carriers of this rearrangement not develop AL in the same period of time? What determines whether this rearrangement occurs? There is no doubt that children with this rearrangement have an elevated risk of presenting AL, but what is additionally required such that a carrier develops the disease? Is it possible that other factors are missing? Are these factors so frequent in a population that there is no way to avoid coming into contact with them at some moment and that, therefore, all children with this rearrangement will develop AL? However, if we can identify those factors, would it be possible to delay their presence in the child's environment, such that the onset of the infirmity would be delayed? Is it to be expected that children who have this rearrangement will all inevitably develop the disease and, consequently, that there is no way to prevent the infirmity?

If today we had a rapid, inexpensive, and easily scalable strategy that could be rolled out on a grand scale and which could scrutinize and identify those children with MLL-r, we would still have two major problems. On one hand, it cannot be determined which of the children will develop AL; therefore, to give treatment to all would generate an ethical problem, as treatments for cancer are very injurious—treatment could occasion serious damage. On the other hand, we could withhold treatment, as is done with the syndrome of transitory dysmyelopoiesis of the newborn, which manifests in children with DS and which is known to go into remission without the necessity of receiving specific treatment. It is known that 10–30 % of the children who present with this disorder will develop AL; it is because not all will develop AL that preventive treatment is not given (Mejía-Aranguré et al. 2011a, b; Maloney et al. 2015). Are we doing good by so doing? Would we be doing good if we were to leave neonates positive for MLL-r without treatment? Does the evidence that MLL-r behaves as a sufficient cause make it worth initiating treatment for all children from the time that a diagnosis is made via a screening test?

It is not easy to answer these last points. I venture to state that, if we decide not to treat all patients who are carriers of an MLL-r, it is because, at a basic level, it is assumed there are other factors determining that some will develop AL and others not. This, I believe, is where the value of prevention lies. If factors, other than MLL- wwwr, necessary for the child to develop AL are lacking, then we can prevent the development of the disease, because it would only be necessary to identify those other factors that the determine whether the disease develops: not, by any means, an easy task.

These susceptibility factors, such as ETV6/RUNX1 and MLL-r, could be taken as crucial points at which interaction with environmental factors (such as infections) could influence the development of AL (Greaves 2006a). Also, these susceptibility factors open the door to thinking that these same susceptibility factors result from the environmental factors. Especially with the *MLL* in adult secondary leukemias, it is known that this genetic rearrangement is strongly associated with the use of inhibitors of topoisomerase II (e.g., etoposide). For children the evidence is not so strong, but it is assumed that exposure to naturally occurring topoisomerase II inhibitors (flavonoids), through their consumption by the mother during pregnancy, is what causes the child to present with MLL-r and, consequently, to develop AL (Pendleton et al. 2014; Greaves and Wiemels 2003).

Thus, environmental exposure would not only be associated with the susceptibility to development of AL with which the child is born, but also such susceptibility would, in some cases, originate from the environmental exposure. The susceptibility we identify most often as being strongly associated with the development of AL is that related with genetic rearrangements; there is evidence that children can be born with this susceptibility. The fact that intense exposure to an environmental factor, such as an inhibitor of topoisomerase II, can provoke the post-uterine appearance of MLL-r (just as secondary leukemias after chemotherapy treatment of adults) suggests the possibility that this susceptibility not only is acquired during the intrauterine stage, but also may be acquired at any postnatal stage. It appears to me that the difference between a child developing AL at a very early age (the first 3 years of life) or at a later time, on one hand may be due to the child's having been born with the susceptibility to AL and the age at which the environmental exposure occurred. On the other hand, the age of onset could reflect the difference between the child who was born with the genetic susceptibility and the child who was born without susceptibility and became susceptible, and who, as a consequence, developed AL at a later time.

Susceptibility is related not only with genetic rearrangements associated directly with the development of AL, but also with the manner in which a cell responds to exposure to carcinogens. The cytochrome P450 family and those genes related to *NQO1* are directly involved with the pathway in which benzene and its derivatives are metabolized (Guha et al. 2008). Epidemiologists used to think that, solely because a factor is carcinogenic, it would therefore cause the disease. However, molecular epidemiology led us to think differently: that exposure alone is not enough and that, between exposure and the development of the disease, there is a

"black box," i.e., the manner in which one responds to carcinogens (Perera 2000; Vineis and Perera 2007). There are individuals who after low-dose exposure may develop the infirmity, whereas others may develop the disease only with high exposure to carcinogens; this difference, in great measure, has to do with the manner in which these compounds are metabolized.

This shows that susceptibility, evidenced by the presence of ETV6/RUNX1 or MLL-r, is not sufficient for a child to develop AL; nor is the appearance of these rearrangements sufficient to initiate the development of the disease. (This is analogous to what occurs with infectious diseases, whereby exposure to an infectious agent is not enough for an individual to develop the infection.) Exposure to an agent that leads to cancer is required; however, this agent may or may not be carcinogenic. It is possible that the presence of the ETV6/RUNX1 or MLL-r rearrangement does not require a carcinogen for the child to develop AL, but only needs something to trigger the mutated cells to proliferate and become dominant (Greaves and Wiemels 2003; Greaves 2006a). Environmental factors affect susceptibility, as in the case of immunity. It is known that persons with an immunodeficiency have a greater risk of developing AL. If a factor is capable of causing immunological vigilance to not function properly, then a degree of immunodepression may exist which will be sufficient to allow a cell with a genetic rearrangement linked to AL to proliferate and dominate its microenvironment. This would then result in the child's developing AL. In some studies, environmental factors, such as insecticides, have been shown to be associated with the development of AL. It is known that insecticides do not have a carcinogenic effect, but rather act as immunosuppressors (Chang et al. 2009). Based on the characteristics of such a factor, the evidence of its association with the development of AL will be inconsistent because, as mentioned before, this depends on the frequency of the exposure of the factor in the population. This factor would be more important in populations more exposed to insecticides, but would have less importance in populations where such exposure is almost nonexistent. For children with a genetic rearrangement linked to the development of AL, exposure to insecticides may permit the development of a propitious medium in which leukemic cells may proliferate and dominate their microenvironment. Thus, insecticides would participate in the development of AL in those children who have a genetic rearrangement linked to the development of AL; however, the insecticides do not have a carcinogenic effect, and much less are they capable, under laboratory conditions, of causing a pluripotent blood cell to be converted into a leukemic cell. Therefore, they do not have a carcinogenic effect, but are a cause in the development of AL in a determined population, which in this hypothetical case is a specific group of patients: children who have a genetic rearrangement linked to AL.

The basic sciences, too, have failed in the search for substances that produce AL, because such substances do not necessarily "produce" the disease. However, environmental factors that could have an important influence in the development of AL are those that generate a propitious microenvironment in which cells having a genetic rearrangement linked to AL can proliferate and dominate their microenvironment.

Some viruses could fall into the same category; with the exception of human T-cell lymphotropic virus (HTLV-1) (chapter by Arellano-Galindo et al., is specifically dedicated to this topic), no other viruses have been identified as a direct cause of AL (Greaves 2006a; Morales-Sánchez and Fuentes-Pananá 2013; Mackenzie et al. 2006). Although viruses may not be a direct mechanism for causing AL in children, it is known that they can affect the immune system, the repair system in DNA, etc. Such effects have been shown to be associated with the development of AL in two ways. First, there are viruses, such as adenovirus, that share sequences similar to some specific sequences of genes in the major histocompatibility complex. Thus, if these viruses infect a cell having a genetic rearrangement linked to AL, they can cause said cell to no longer be recognized by the immune system; as a consequence, the cell escapes the mechanisms of internal regulation, which inhibit the growth of cancerous cells (Dorak et al. 1999). In such a case, the child would have to be a carrier of a rearrangement linked to the development of AL, be infected with the virus (e.g., adenovirus), and in addition be a carrier of the specific HLA alleles (in the case of adenovirus, DR-53 is required). If, and only if, all of these conditions are met, would a propitious environment be generated for the leukemic cell to proliferate and dominate its microenvironment?

A second way in which viruses may participate is by affecting the DNA repair mechanisms of a cell (Zur Hausen and de Villiers 2005). Viruses do not necessarily want to kill the host; it is by means of the host that they obtain nutrients and reproduce. To subsist in the host, viruses can alter the DNA repair mechanisms, so that a cell with a genetic rearrangement linked to the development of AL may be exposed to factors that could provoke mutations which cause cells with such a rearrangement to proliferate and dominate their microenvironment.

We have worked with a natural model, children with DS (Mejía-Aranguré et al. 2011a, b). Children with DS have various characteristics that make them susceptible to developing AL: these children have increased chromosome fragility; their DNA repair mechanisms are affected; they are more vulnerable to the toxic effects of various mutagenic substances; and they become more vulnerable to the collateral effects of chemotherapy if they do develop AL (Hitzler 2010; Maloney et al. 2015). The child with DS has different molecular factors determining that he/she will develop AL (Chaps. 4 and 5 deal with this theme); yet, only 2 % of children with DS will have developed AL within the first 15 years of life. What determines that some children with DS develop AL and others do not? In my opinion, the child with DS is subject to the same mechanisms described above, although it is possible that the child with DS requires fewer mutations to develop AL in comparison with a healthy child (Valladares et al. 2005). As a consequence, it is not enough that the child with DS be exposed to an environmental factor; the exposure must occur during a window of vulnerability and be linked with the mechanisms of susceptibility, thus creating conditions propitious for a leukemic cell to proliferate and dominate its microenvironment.

The advantage of studying children with DS as a model to identify the environmental risk factors associated with the development of AL in other children is based on having so many pertinent mechanisms of susceptibility exhibited by only one group. With these children, it is not necessary to study many environmental

factors to identify which of these factors are more important in influencing the development of AL. In addition, as has been demonstrated by various studies concerning this topic, the sample size does not need to be large. In these studies, even without a large sample size, it was possible to determine that some environmental factors, such as smoking and alcohol consumption by the father, are strongly involved in the development of AL (Mejía-Aranguré et al. 2007). We have called this a paired case-control study for susceptibility (Mejía-Aranguré and Fajardo-Gutiérrez 2006). We have preferred to study children with DS rather than those with Fanconi anemia or ataxia telangiectasia, both of which are associated with a higher risk for developing AL than is DS, because of the frequency of these ailments and, therefore, the difficulty of identifying the risk factors associated with the development of AL in these children, as the sample size will be very small (Seif 2011). Just as with DS, not all children with these infirmities develop AL; therefore, the participation of other factors, distinct from those intrinsic to the disease, must be involved for some to develop AL, and others not.

How Should the Causality of AL Be Studied?

In recent years, important goals have been reached concerning the causal mechanisms of AL in children, such as the identification of the genetic rearrangements associated with AL and the understanding that not all factors are sufficient for the development of the disease. To further elucidate the causality of AL, time periods critical to the evolution of the disease must be studied. I make the following clarification. First, the factors to which the parents were exposed prior to the conception of the child or during pregnancy, but which could influence the development of AL in the child, must be identified and studied while not losing sight of the fact that such factors are not unique (see schematic in chapter by Pérez-Saldivar et al., which concerns these periods of study). The combination of factors necessary for a child to be born with a high susceptibility to AL should be studied. There are perhaps two great questions that should be answered concerning the period before the child with AL is born: first, which factors are conducive to a child being born with a high susceptibility to AL? And, second (perhaps the more important of the two), to how many factors must the mother and father, or both, be exposed prior to conception or during the pregnancy for a child to be born with a high susceptibility to AL?

After birth, the time between early-onset leukemia and the appearance of leukemias at later stages of extrauterine life can be divided into three stages:

- AL in the infant (during the first 12 months of life) is a special topic. Based on current knowledge, some consider this leukemia to be distinct to those that appear at later stages of the childhood (Yeoh et al. 2002)
- AL, especially early pre-B ALL, predominates during a peak age range (2–5 years old)
- ALs that appear after this peak age range

The ALs that appear during the first year of life are thought to have a genetic component; that is, there is a high susceptibility for the development of the disease, and very few factors and very little time are needed for the leukemia to develop (Greaves 1999; Greaves and Wiemels 2003; Wiemels 2012).

Among leukemias, ALL appears more frequently during the peak age range (2–5 years of age). This range coincides with the period of development and greatest proliferation of B cells. This finding is consistent with the concept that ALL must occur at a time biologically determined; the period when B cells are proliferating is found to be associated with the highest risk of developing ALL (Greaves 1999, 2006a; Greaves and Wiemels 2003; Wiemels 2012). As mentioned earlier, this may also occur with other cancers; the peak age at which the onset of a particular cancer is more frequent correlates with the period of higher proliferation of cells in the corresponding tissue (Mejía-Aranguré et al. 2005).

The ALs that appear after the peak age range in which ALLs predominate is another group worth studying. As has been shown in some studies (Nuñez-Enriquez et al. 2013; Flores-Lujano et al. 2009), the risk factors for AL are distinct, depending on the age of onset. It is very probable that the older the child, the greater will be the necessity of risk factors to which the child must be exposed for the disease to develop and the lesser will be the susceptibility to AL with which the child was born. For this reason, I venture to say that the age at onset of AL is a reflection of the degree of susceptibility to the disease and of the number of factors in exposure to carcinogens that are necessary for the development of the disease. All this also depends on the window of vulnerability in which the child finds himself or herself. This window of vulnerability is directly involved with the proliferation of the child's B or T cells, which will cause the interaction between the degree of susceptibility and the degree of exposure to carcinogens, thereby provoking the onset of AL.

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References

Anderson LM, et al. Critical windows of exposure for children's health: cancer in human epidemiological studies and neoplasms in experimental animal models. Environ Health Perspect. 2000;108 suppl 3:573–94.

Bassin EB, et al. Age-specific fluoride exposure in drinking water and osteosarcoma (United States). Cancer Causes Control. 2006;17:421–8.

Bhojwani D, et al. Biology of childhood acute lymphoblastic leukemia. Pediatr Clin N Am. 2015;62:47–60.

Bueno, et al. A human ESC model for MLL-AF4 leukemic fusion gene reveals an impaired early hematopoietic-endothelial specification. Cell Res. 2012;22:986–1002.

Chang JS. Parental smoking and childhood leukemia. In: Verma M, editor. Methods of molecular biology, cancer epidemiology, vol. 472. Totowa: Springer Science; 2009. p. 103–37.

Chang JS, et al. Allergies and childhood leukemia. Blood Cell Mol Dis. 2009;42:99–104.

Dorak MT, et al. Unravelling an HLA-DR association in childhood acute lymphoblastic leukemia. Blood. 1999;94:694–700.
Flores-Lujano J, et al. Breastfeeding and early infection in the aetiology of childhood leukaemia in Down syndrome. Br J Cancer. 2009;101:860–4.
Greaves M. Molecular genetics, natural history and the demise of childhood leukaemia. Eur J Cancer. 1999;35(14):1941–53.
Greaves M. Infection, immune responses and the aetiology of childhood leukaemia. Nat Rev Cancer. 2006a;6(3):193–203.
Greaves M. The causation of childhood leukemia: a paradox of progress? Discov Med. 2006b;6(31):24–8.
Greaves M. Is cancer an evolutionary inevitability? In: Cancer. The evolutionary legacy. Oxford University Press. New York: 2000a. p. 111–9.
Greaves M. How cáncer cells play the winning game. In: Cancer. The evolutionary legacy. Oxford University Press. New York: 2000b. p. 53–68.
Greaves MF, Wiemels J. Origins of chromosome translocations in childhood leukaemia. Nat Rev Cancer. 2003;3(9):639–49.
Greim H, et al. The bone marrow niche, stem cells, and leukemia: impact of drugs, chemicals, and the environment. Ann NY Acad Sci. 2014;1310:7–31.
Guha N, et al. NQO1 polymorphisms and de novo childhood leukemia: a HuGE review and meta-analysis. Am J Epidemiol. 2008;168:1221–32.
Henderson E. History of leukemia. In: Henderson ES, Lister TA, Greaves MF, editors. Leukemia. 7th ed. Philadelphia: Saunders; 2002. p. 1–7.
Hitzler JK. Cancer among persons with Down syndrome. Int Rev Res Ment Retard. 2010; 39:129–64.
Inaba, et al. Acute lymphoblastic leukaemia. Lancet. 2013;381:1943–55.
Kansara M, et al. Translational biology of osteosarcoma. Nat Rev Cancer. 2014;14:722–35.
Kheifets L, et al. Pooled analysis of recent studies on magnetic fields and childhood leukaemia. Br J Cancer. 2010;103:1128–35.
MacKenzie J, et al. The putative role of transforming viruses in childhood acute lymphoblastic leukemia. Haematologica. 2006;91:240–3.
Maloney KW, Taub JW, Ravindranath Y, Roberts I, Vyas P. Down syndrome preleukemia and leukemia. Pediatr Clin N Am. 2015;62:121–37.
Marshall GM, et al. The prenatal origins of cancer. Nat Rev Cancer. 2014;14:277–89.
McNally RJQ, Eden TOB. An infectious aetiology for childhood acute leukaemia: a review of the evidence. Br J Haematol. 2004;127:243–63.
Mejía-Aranguré JM. Model for identifying the etiology of acute lymphoblastic leukemia in children. In Mejia-Arangure JM, editor. Clinical epidemiology of acute lymphoblastic leukemia – from the molecules to the clinic. Croatia. InTech; 2013. p. 342.
Mejía-Aranguré JM, Fajardo-Gutierrez A. Selection by susceptibility as a design to identify environmental risk factors in children's acute leukemia. Epidemiology. 2006;17:S505–6.
Mejía-Aranguré JM, et al. Environmental factors contributing to the development of childhood leukemia in children with Down's syndrome. Leukemia. 2003;17:1905–7.
Mejía-Aranguré JM, et al. Age of onset of different malignant tumors in childhood. Rev Med Inst Mex Seguro Soc. 2005;43(1):25–37.
Mejía-Aranguré JM, et al. Magnetic fields and acute leukemia in children with Down syndrome. Epidemiology. 2007;18(1):158–61.
Mejía-Aranguré JM, et al. Childhood acute leukemias in Hispanic population: differences by age peak and Immunophenotype. In: Faderl S, editor. Novel aspects in acute lymphoblastic leukemia. Croatia: InTech; 2011a.
Mejía-Aranguré JM, et al. Infections and acute leukemia in children with Down syndrome. In: Dey S, editor. Prenatal diagnosis and screening for Down syndrome. Croatia: Intech; 2011b. p. 79–106. doi:10.5772/18533.

Metayer C, et al. The childhood leukemia international consortium. Cancer Epidemiol. 2013;37:336–47.
Morales-Sánchez A, Fuentes-Pananá E. In Mejía-Aranguré JM, editor. Clinical epidemiology of acute lymphoblastic leukemia – from the molecules to the clinic. Croatia. InTech; 2013. p. 19.
Muñoz P, et al. Specific marking of hESCs-derived hematopoietic lineage by WAS-promoter driven lentiviral vectors. PLoS ONE. 2012;7(6), e39091. doi:10.1371/journal.pone.0039091.
Nuñez-Enriquez JC, et al. Allergy and acute leukaemia in children with Down syndrome: a population study. Report from the Mexican inter-institutional group for the identification of the causes of childhood leukaemia. Br J Cancer. 2013;108:2334–8. doi:10.1038/bjc.2013.237.
Parkin DM, Darby SC. Cancers in 2010 attributable to ionising radiation exposure in the UK. Br J Cancer. 2011;105:S57–65.
Pendleton MJ, et al. Topoisomerase II and leukemia. Ann NY Acad Sci. 2014;1310:98–110.
Perera FP. Molecular epidemiology: on the path to prevention? J Natl Cancer Inst. 2000;92:602–12.
Perez-Saldivar ML, et al. Father's occupational exposure to carcinogenic agents and childhood acute leukemia: a new method to assess exposure (a case-control study). BMC Cancer. 2008;8:7.
Prendergast AM, Essers MAG. Hematopoietic stem cells, infection, and the niche. Ann NY Acad Sci. 2014;1310:51–7.
Pyatt D, Hays S. A review of the potential association between childhood leukemia and benzene. Chem Biol Interact. 2010;184:151–64.
Ramos-Mejía V, et al. iPSC lines that do not silence the expression of the ectopic reprogramming factors may display enhanced propensity to genomic instability. Cell Res. 2010;20:1092–5.
Ramos-Mejía V, et al. iPSCs from cancer cells: challenges and opportunities. Trends Mol Med. 2012;18(5):245–7.
Richardson RB. Promotional etiology for common childhood acute lymphoblastic leukemia: the infective lymphoid recovery hypothesis. Leuk Res. 2011;35(11):1425–31.
Riether C, et al. Regulation of hematopoietic and leukemic stem cells by the immune system. Cell Death Differ. 2015;22:187–98.
Rothman KJ. Causes. Am J Epidemiol. 1976;104(6):587–92.
Rudant J, et al. Selection bias in case–control studies on household exposure to pesticides and childhood acute leukemia. J Expo Sci Environ Epidemiol. 2010;20:299–309.
Schmiegelow K, Vestergaard T, Nielsen SM, Hjalgrim H. Etiology of common childhood acute lymphoblastic leukemia: the adrenal hypothesis. Leukemia. 2008;22(12):2137–41.
Schüz J, et al. Bias in studies of parental self-reported occupational exposure and childhood cancer. Am J Epidemiol. 2003;158:710–6.
Seif AE. Pediatric leukemia predisposition syndromes: clues to understanding leukemogenesis. Cancer Genet. 2011;204:227e244.
Taylor GM. Immunogenetics and the aetiology of childhood leukemia. Arch Dis Child. 1994;70(2):77–81.
Uckun FM. Re:MLL-AF4 fusion transcripts in normal and leukemic hematopoietic cells. Blood. 1999;93:1107–10.
Uckun FM, et al. Clinical significance of MLL-AF4 fusion transcript expression in the absence of a cytogenetically detectable t(4;11)(q21;q23) chromosomal translocation. Blood. 1998;92:810–21.
Valladares A, et al. Cytogenetic studies in children with Down syndrome and acute leukemia. Leuk Res. 2005;29:1241–6.
Vineis P, Perera F. Molecular epidemiology and biomarkers in etiologic cancer research: the new in light of the old. Cancer Epidemiol Biomarkers Prev. 2007;16:1954–65.
Wartenberg D, Groves FD, Adelman AS. Acute lymphoblastic leukemia: epidemiology and etiology. In: Estey EH, Faderl SH, Kantarjian HM, editors. Hematologic malignancies: acute leukemias. Germany: Springer; 2008. p. 77–93.

Wiemels J. Perspectives on the causes of childhood leukemia. Chem Biol Interact. 2012; 196:59–67.
Yeoh EJ, et al. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. Cancer Cell. 2002;1:133–43.
Zhang Y, et al. Disordered epigenetic regulation in MLL-related leukemia. Int J Hematol. 2012;96:428–37.
Zur Hausen H, de Villiers EM. Virus target cell conditioning model to explain some epidemiologic characteristics of childhood leukemias and lymphomas. Int J Cancer. 2005;115:1–5.

Chapter 7
Molecular Origin of Childhood Acute Lymphoblastic Leukemia

Esmé Waanders, Marjolijn C.J. Jongmans, and Charles G. Mullighan

Abstract Our understanding of the genetic etiology of pediatric acute lymphoblastic leukemia (ALL) has advanced greatly in the past few decades. Due to the advent of genome-wide profiling techniques for copy number alterations (CNAs) as well as sequence mutations, we have thoroughly characterized many different genetic subtypes of ALL. Each subtype harbors alterations activating leukemogenic pathways and differs in prevalence, prognosis, cell type, and treatment response. The interplay of founding leukemogenic aberrations, acquired mutations, and germline composition of the patient is important for the development and progression of the disease. Moreover, genomic profiling has identified genetic alterations that have been integrated into diagnostic testing algorithms and are being evaluated as targets for therapy. Despite these advances, the genetic basis of a minority of ALL cases remains unknown, and the frequency of these enigmatic cases rises with patient age. Much work remains in studying these last uncharacterized groups to fully understand leukemia development and improve outcomes.

Keywords Acute lymphoblastic leukemia • Genetic profiling • Mutations • Oncogenic pathways • Germline susceptibility

Introduction

Genomic profiling techniques have driven a revolution in cancer research. We are now able to quickly and accurately characterize inherited and somatic genetic alterations, tailor treatment strategies, and predict treatment outcome in leukemia. Historically, ALL is categorized based on cell lineage (B-progenitor

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or T cell), corresponding expression of cell surface markers, and structural and numerical chromosomal alterations. Leukemias of lymphoid origin can be subdivided into pro/pre/common B-lineage (BCP-ALL: CD19+, CD10+), T-lineage (T-ALL: cytoplasmic CD3+), and mixed lineage or biphenotypic ALL (MPAL: a single tumor population or multiple populations that express markers that fulfill classification for multiple lineages, most commonly myeloid and T-cell). Acute myeloid leukemia (AML) is diagnosed when the leukemia expresses myeloid markers.

Cytogenetic approaches, including karyotyping and fluorescence in situ hybridization (FISH), can identify chromosomal alterations including aneuploidy and translocations, which are hallmarks of many subtypes of acute leukemia. Recurrent chromosomal changes in B-cell precursor acute lymphoblastic leukemia (BCP-ALL) are the loss or gain of complete chromosomes (hypodiploidy with less than 44 chromosomes and high-hyperdiploidy with greater than 50 chromosomes, respectively) and translocations including *ETV6-RUNX1* (*TEL-AML1*), *BCR-ABL1* encoded by the Philadelphia (Ph) chromosome, *TCF3-PBX1* (*E2A-PBX1*), and *MLL* (Mullighan 2012) (Table 7.1, Fig. 7.1). T-lineage ALL is characterized by rearrangements of transcription factors *TLX1* (*HOX11*), *TLX3 (HOX11L2)*, *LYL1*, *TAL1*, *LMO1*, *LMO2*, and *MLL* (Aifantis et al. 2008) (Table 7.2, Fig. 7.1). The prevalence of the leukemia subtypes varies significantly with age (Fig. 7.2). *MLL*-rearranged leukemia is most common in very young children (<1 year of age), and high-hyperdiploid and *ETV6-RUNX1* are frequent in young children (aged 2–8), whereas the frequency of *BCR-ABL1* and *BCR-ABL1*-like ALL increases in older children, adolescents, and adults (Roberts et al. 2014).

Genomic copy number profiling, gene expression profiling, and next-generation sequencing including whole-exome, whole-genome, and transcriptome sequencing have now added another level of detail to the molecular classification of ALL. These approaches have identified recurrent genetic alterations not previously apparent on karyotyping and have demonstrated that each subtype is characterized by constellations of sequence and structural genetic alterations that perturb multiple cellular pathways (Mullighan 2013). Pediatric leukemia harbors relatively few sequence mutations and structural alterations compared to other malignancies. Chromosomal instability is uncommon, and CNAs are mostly focal deletions targeting one gene. The aberrations, though few in number, commonly target key pathways across multiple ALL subtypes, resulting in a block in lymphoid differentiation, perturbation of cell cycle regulation, and increased proliferation. The genes targeted, the type of alteration (e.g., chromosomal rearrangement, deletion/amplification, or sequence mutation), and the cell stage in which the lesions occurred (e.g., progenitor or lineage committed cell) and hence the molecular origin of the leukemia vary between subtypes. The founding chromosomal rearrangements or CNAs by which the diverse subtypes are characterized occur in a preleukemic (stem) cell which subsequently acquires additional driver mutations that cooperate with the initiating lesion, often a chromosomal translocation, to confer a growth or survival advantage for the leukemic clone (Fig. 7.3).

Table 7.1 Cytogenetic subtypes in BCP-ALL

Subtype	Cytogenetics	Frequency (%)	Prognosis	Genes/pathways targeted
B-lineage ALL				
Near-haploid	24–31 chromosomes	1–2	Poor	Ras, receptor tyrosine kinase signaling, *IKZF3*
Low-hypodiploid	32–39 chromosomes	1–2	Poor	*TP53*, *IKZF2*, *RB1*, Ras
Dicentric	Commonly from dic(7;9), dic(9;12), and dic(9;20)	~2	Unknown	*PAX5*
Hyperdiploid	>50 chromosomes	20–30	Excellent	Activated kinase and Ras pathways
ETV6-RUNX1 (*TEL-AML1*)	t(12;21)(p13;q22)	15–25	Excellent	Expression of myeloid antigens
TCF3-PBX1 (*E2A-PBX1*)	t(1;19)(q23;p13)	2–6	Excellent, association with CNS relapse	*PAX5*, *CDKN2A*/*CDKN2B*
Ph+ (*BCR-ABL1*)	t(9;22)(q34;q11.2)	2–4	Poor	*IKZF1*, *PAX5*, *EBF1*, *CDKN2A*/*CDKN2B*
Ph-like (*BCR-ABL1-like*)	Multiple rearrangements encoding chimeric proteins fusing 5′ partners with 3′ kinase domains (*ABL1*, *PDGFRB*, *JAK2*).	2–5	Poor	*IKZF1*, distinct gene expression profile
MLL-rearranged	*MLL-AF4* t(4;11)(q21;q23); t(11;v)	1–2[a]	Poor	Few cooperating lesions
ERG deletion		7	Good	Distinct gene expression profile
iAMP21		1	Poor	*P2RY8-CRLF2* fusion, *EBF1*, *ETV6*, *RB1*, *RUNX1*

[a]Common in infant ALL (especially <6 months of age)

In this chapter, we illustrate the various B-lineage and T-lineage pediatric ALL subtypes with a focus on the pattern of co-occurring lesions and oncogenic pathways involved in the development of these leukemias. Also, we describe common mechanisms of genetic alteration and the concept of tumor heterogeneity. It is now recognized that there is an important interplay of common and rare inherited variants and subsequent somatic genetic alterations that disrupt key pathways. Therefore, we end this chapter with a paragraph on germline variation and ALL risk.

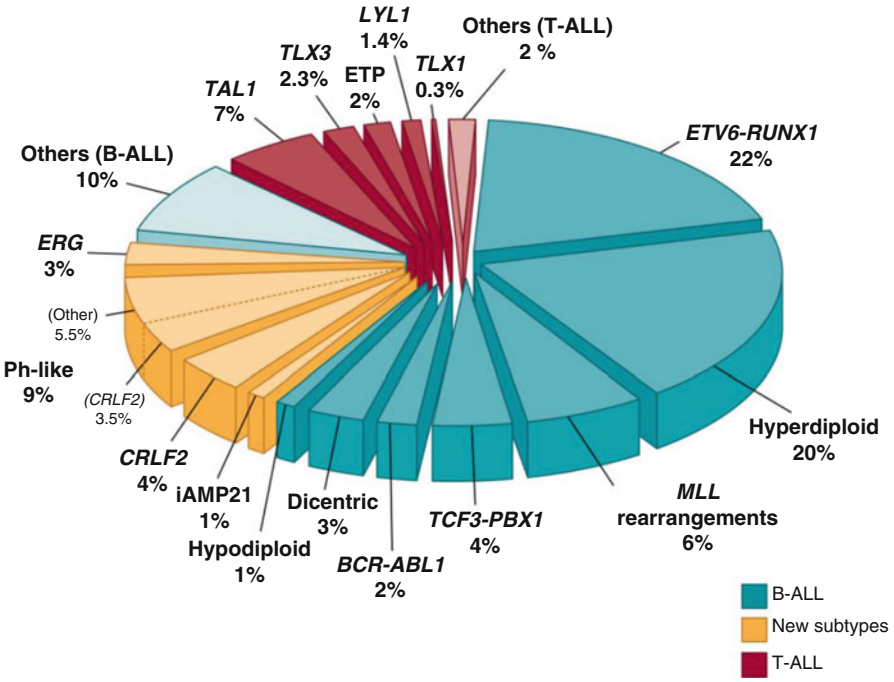


Fig. 7.1 Frequency of genetic subtypes of pediatric ALL. The pie chart includes all major B- and T-lineage subtypes of ALL, to illustrate the relative frequency of each (Data are derived from St Jude Children's Research Hospital Total Therapy studies. Reprinted from Mullighan 2013, with permission from Elsevier)

B-Lineage ALL

Hyperdiploid ALL

Hyperdiploid ALL comprises 25–30 % of BCP-ALL cases and is rare in T-cell acute lymphoblastic leukemia (T-ALL). The peak incidence is in children 2–4 years of age. Hyperdiploid ALL is characterized by recurring, nonrandom gains of at least five chromosomes, most commonly chromosomes 4, 6, 8, 10, 14, 17, 18, and X. Half of the cases include partial aneuploidies like a gain 1q, del 6q and isochromosomes 7q or 17q. The subtype rarely contains balanced translocations. Most genes on the gained chromosome show increased expression; however, some show absent or decreased expression, suggesting epigenetic regulation by methylation induced silencing (Andersson et al. 2005; Ross et al. 2003; Figueroa et al. 2013). Cooperating mutations are activated kinase and Ras pathways (mutually exclusive mutations of *FLT3* in 10–25 %, *KRAS*/*NRAS* in 15–30 %, and *PTPN11* in 10–15 %) (Paulsson et al. 2008).

Table 7.2 Cytogenetic subtypes in T-ALL

Subtype	Cytogenetics	Frequency (%)	Prognosis	Affected pathways
T-lineage ALL				
TAL1 deregulated	t(1;7)(p32;q35), t(1;14)(p32;q11) and interstitial 1p32 deletion	15–18	Good	Transcription regulation by enhancers
LMO1/*LMO2* deregulated	t(11;14) (p15;q11) and 5′ *LMO2* deletion	10	Good	Self-renewal
TLX1 (*HOX11*) deregulated	t(10;14) (q24;q11) and t(7;10)(q35;q24)	7	Good	Chromosomal missegregation and aneuploidy
TLX3 (*HOX11L2*) deregulated	t(5;14)(q35;q32)	20	Poor	del(5)(q35)
LEF1 inactivated		18		*NOTCH1*, *CDKN2A*/*CDKN2B*, *PTEN*, PI3K/AKT, *MYC*
PICALM-MLLT10 (*CALM-AF10*)	t(10;11) (p13;q14)	10	Poor	*MEIS1* and *HOX* upregulation
MLL-MLLT1 (*MLL-ENL*)	t(11;19) (q23;p13)	2–3	Superior prognosis to other *MLL*-rearranged leukemias	Distinct gene expression profile
Kinase rearrangements	*NUP214-ABL1*, *EML1-ABL1*, *ETV6-JAK2*, *ETV6-ABL1*	6		Activated kinase signaling
NOTCH1 rearranged	t(7;9)(q34;q34)	<1		
Early T-cell precursor (ETP ALL)	Heterogeneous translocations, deletions, mutations involving multiple cellular pathways	12	Poor, although improved in recent studies with risk-adapted therapy	Immature immunophenotype, expression of myeloid and/or stem cell markers, *MEF2C* dysregulation

Mechanisms leading to aneuploidies both in hypo- and hyperdiploid ALL are unknown. The specific patterns of gained chromosomes and copy-neutral loss of heterozygosity suggest an early catastrophic event with or without reduplication in contrast to sequential loss of single chromosomes which would lead to a more random pattern of losses and gains. One hypothesis presumes that all gains occur in one single aberrant cell division, but another suggests that the cells become tetraploid and subsequently lose certain chromosomes (Paulsson and Johansson 2009).

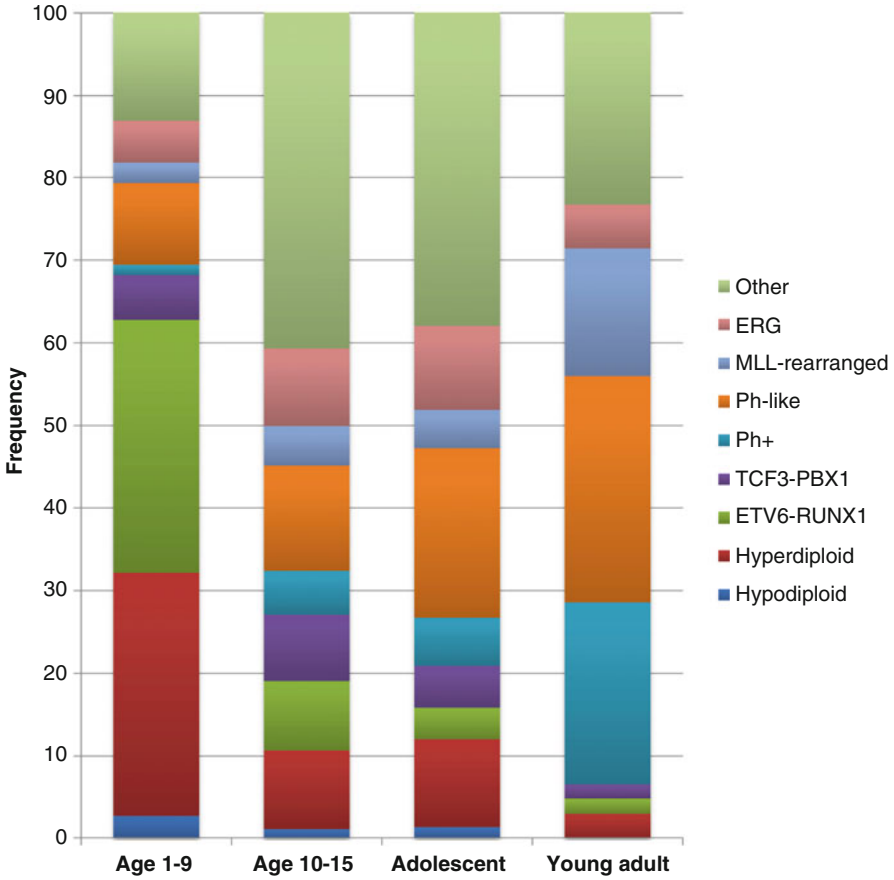


Fig. 7.2 Age distribution of B-ALL genetic subtypes. Ages 1–9: National Cancer Institute-classified standard-risk precursor B-cell ALL (age range of 1–9 years and peripheral blood leukocyte count at diagnosis <50,000/μL); ages 10–15: National Cancer Institute-classified high-risk precursor B-cell ALL (age range of 10–15 years or leukocyte count ≥50,000/μL); adolescent: age range of 16–20; young adult: age range of 21–39 (From Roberts et al. 2014. Copyright © 2014 Massachusetts Medical Society. Reprinted with permission)

Hypodiploid ALL

Hypodiploid ALL is found in up to 5 % of BCP-ALL cases and is characterized by the loss of two or more chromosomes. It can be subdivided into three subgroups, each with a characteristic mutational profile (Holmfeldt et al. 2013; Harrison et al. 2004; Heerema et al. 1999; Nachman et al. 2007). Near-haploid ALL cells have 24–31 chromosomes and harbor alterations targeting receptor tyrosine kinase signaling and activating Ras signaling (*NF1*, *NRAS*, *KRAS*, *PTPN11*) and a loss-of-function of the lymphoid transcription factor gene *IKZF3* (AIOLOS). Low-hypodiploid ALL contains 32–39 chromosomes and harbors loss-of-function

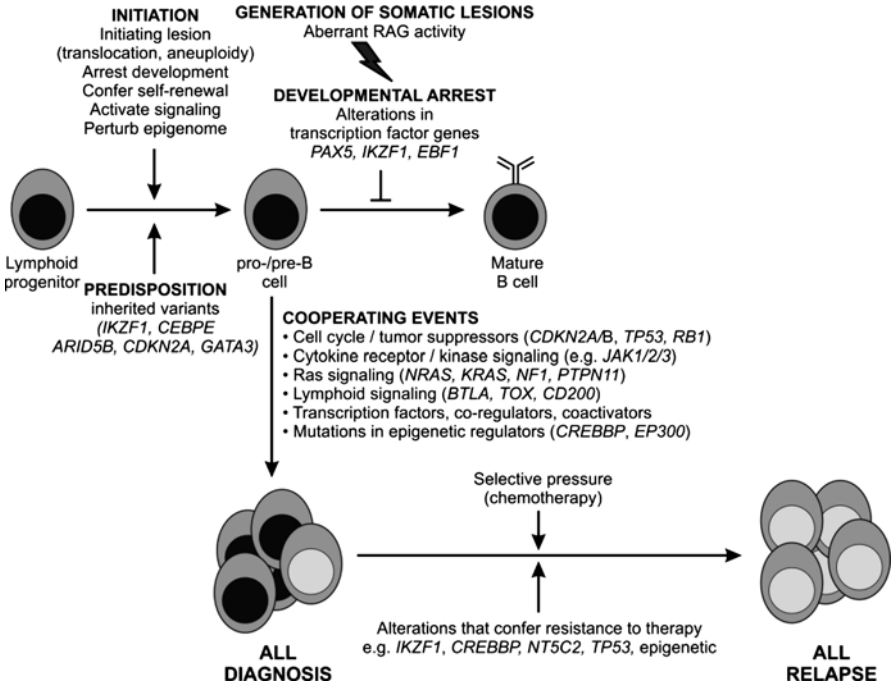


Fig. 7.3 Acquisition of genetic alterations in the pathogenesis of BCP-ALL. Chromosomal rearrangements and founding lesions initiate leukemogenesis by increasing self-renewal and deregulation of transcription and epigenetic signatures. Additional lesions subsequently disrupt lymphoid development and block differentiation, which confers susceptibility to additional genetic lesions targeting cellular pathways like cell cycle regulation, tumor suppression, cytokine receptor and kinase signaling, and chromatin modification. Diagnosis ALL samples are commonly clonally heterogeneous, and genetic alterations in minor clones may survive therapy and promote relapse. A similar diagram can be proposed for T-ALL, where lesions targeting lymphoid development, self-renewal, and kinase signaling are also observed (This figure was originally published in *Blood*; Mullighan 2014)

alterations in *TP53*, *IKZF2* (HELIOS), and *RB1*. The patterns of aneuploidy are stereotyped and most commonly involve chromosomes 1–7, 9, 11–13, 15–17, 19–20, and 22 in near-haploid ALL and chromosomes 2–4, 7, 9, 12–13, 15–17, and 20 in low-hypodiploid ALL (Holmfeldt et al. 2013). Notably, both copies of chromosome 21 are always retained. Both near-haploid and low-hypodiploid cells can undergo genome duplication leading to a hyperdiploid chromosome number ("masked" hypodiploid ALL), requiring careful examination of the patterns of aneuploidy and/or genomic analysis to distinguish from high-hyperdiploid ALL. Cytogenetic findings may be suggestive of masked hypodiploid ALL rather than high-hyperdiploid ALL. Masked hypodiploid cases typically exhibit disomy and tetrasomy, whereas high-hyperdiploid cases have trisomy of distinct chromosomes, most commonly 4, 10, 14, 17, 18, and 21. Deoxyribonucleic acid (DNA) index analysis may also reveal evidence of a nonmasked clone in hypodiploid cases.

Patients with low-hypodiploid ALL tend to be older (median age of 15 years) than patients with near-haploid ALL (median age of 7 years) (Holmfeldt et al. 2013).

The third subgroup is high-hypodiploid ALL in which cells contain 40–45 chromosomes which is less common, and it does not share the poor outcome of near-haploid and low-hypodiploid ALL. Common alterations are the loss of a sex chromosome and the presence of dicentric or isochromosomes involving chromosomes 7, 9, 12, and 20 (Harrison et al. 2004; Nachman et al. 2007).

The distinct roles of the loss-of-function alterations of Ikaros family members in low-hypodiploid and near-haploid ALL are unknown. In cell lines, loss of expression of *Ikzf2* and *Ikzf3* augments Ras signaling, suggesting a role other than only perturbation of B-lymphoid development. Overall, hypodiploid ALL is characterized by activated PI3K/mTOR and MEK/ERK signaling that represents potential avenues for therapeutic intervention in this high-risk form of leukemia.

ETV6-RUNX1 ALL

ETV6-RUNX1 ALL is characterized by the translocation t(12;21)(p13;q22) and occurs in 25–30 % of pediatric ALL cases; however, it is uncommon in adult ALL (1–4 %) (Aguiar et al. 1996; Al-Obaidi et al. 2002, Burmeister et al. 2010; Golub et al. 1995; Raynaud et al. 1996b; Romana et al. 1995b; Shurtleff et al. 1995). The t(12;21)(p13;q22) translocation, which is often cryptic on karyotyping, results in the fusion of the *N*-terminal helix-loop-helix domain of *ETV6* (encoding ETS variant 6, also known as *TEL*) to almost the entire *RUNX1* protein (runt-related transcription factor 1, also called *AML1*) (Golub et al. 1995; Romana et al. 1995a). The fusion protein recruits the nuclear corepressor complex (N-CoR), which confers histone deacetylase activity and contains the transcriptional repressor mSin3A (Fenrick et al. 1999; Guidez et al. 2000). A likely effect of the fusion protein is a transcriptional repression of *RUNX1* target genes. Both *ETV6* and *RUNX1* are master regulators in hematopoiesis, and alterations of these genes play a central role in leukemogenesis. *ETV6* is rearranged to over 20 translocation partners in a range of malignancies, but loss-of-function or expression by deletions or mutations occur in different types of leukemia (Bohlander 2005). *RUNX1* is part of the core binding factor transcription complex and contains a DNA binding domain. In addition to a role in *ETV6-RUNX1* ALL, *RUNX1* is rearranged in AML (*RUNX1-RUNX1T1* or *AML-ETO*), and amplification of *RUNX1* is found in iAMP21 intrachromosomal amplifications (Harewood et al. 2003; Robinson et al. 2003, 2005; Strefford et al. 2005a, b).

The *ETV6-RUNX1* rearrangement often arises in utero (Ford et al. 1998; Wiemels et al. 1999a, b). It has been reported to be present in cord blood samples in a 100-fold higher frequency than the risk of developing leukemia (Lausten-Thomsen et al. 2011; Mori et al. 2002), suggesting that some individuals acquire secondary genetic alterations required for the establishment of leukemia. Overexpression of *ETV6-RUNX1* in fetal hematopoietic cells inhibits B-cell differentiation and increases

self-renewal of B-cell progenitors (Andreasson et al. 2001; Morrow et al. 2004; Tsuzuki et al. 2004), but does not directly lead to leukemia development. Additional alterations, such as a deletion of the *CDKN2A*/*CDKN2B* genes (which encode the INK4/ARF tumor suppressors), are necessary (Bernardin et al. 2002; Wiemels et al. 1999b). Acquired somatic deletions are frequent in *ETV6-RUNX1* ALL. The most frequent co-occurring lesions identified in *ETV6-RUNX1* ALL are deletions of *PAX5*, *CDKN2A*/*CDKN2B*, *CD200*, *BTLA*, *BTG1*, *EBF1*, *FHIT*, *TBL1XR1*, *NR3C1*, and the other allele of *ETV6* (Al-Shehhi et al. 2013; Lilljebjorn et al. 2007, 2010; Mullighan et al. 2007a; Parker et al. 2008; Raynaud et al. 1996a; SennanaSendi et al. 1996; Waanders et al. 2012). A whole-genome sequencing study identified recombinase-activating gene (RAG)-mediated recombination as the main mechanism of deletion development (Papaemmanuil et al. 2014), which is discussed in more detail below.

TCF3-PBX1 *ALL*

TCF3-PBX1 ALL comprises 5 % of pediatric and 3–6 % of adult ALL, with a higher incidence in young adults and African Americans (Moorman et al. 2010; Privitera et al. 1992; Raimondi et al. 1990). This ALL subtype results most commonly from a t(1;19)(p13;q22) translocation which fuses the genes *TCF3* (*E2A*) and *PBX1*. *TCF3* is a basic helix-loop-helix (bHLH) transcription factor with two protein products, E12 and E47, both of which are required for B- and T-cell development. Knockout of *TCF3* in mice results in a differentiation block at the pro-B-cell stage (Bain et al. 1994). *PBX1* is required for hematopoiesis maintenance but is normally not expressed in lymphoid cells (DiMartino et al. 2001). *PBX1* contains a homeobox and binds *HOX* genes and *MEIS1* which in turn interacts with the *HOX* genes (Shanmugam et al. 1999). The fusion retains the transactivation domain of *TCF3* and pairs this to the homeobox domain of *PBX1*. The fusion protein can still bind HOX genes, but it can no longer bind *MEIS1* (Lu and Kamps 1997; Sykes and Kamps 2004), leading to deregulation of target genes. Co-occurring aberrations are mostly deletions of *PAX5* and *CDKN2A*/*CDKN2B*, deletion of chromosome 19p (including *TCF3*), and a gain of chromosome 1q (including *PBX1*).

BCR-ABL1 *ALL*

Between 3 % and 5 % of pediatric ALL cases harbor the t(9;22)(q34;q11.1) or variant translocations. The derivative chromosome 22 is known as the Ph chromosome, named after the city in which this translocation was first described in 1960 (Nowell and Hungerford 1960; Rowley 1973). The presence of *BCR-ABL1* is associated with a high incidence of central nervous system (CNS) involvement at diagnosis, a high peripheral blood leukocyte count, resistance to therapy, and poor outcome

(Arico et al. 2010). In addition, the *BCR-ABL1* translocation is associated with older age. The median age in pediatric cases is about 8 years, but *BCR-ABL1* leukemia frequency increases to 25 % in adults (Crist et al. 1990; Ribeiro et al. 1987; Seckerwalker et al. 1991). The translocation fuses the genes *BCR* and *ABL1*, resulting in expression of isoforms of variable size depending on the sites of translocation in *BCR* and *ABL1*, with the 190 kDa protein (p190) most commonly observed in ALL. A similar fusion, though with different breakpoints leading to the p210 and p230 isoforms, is found in chronic myeloid leukemia (CML) and AML (Melo 1996; Ben-Neriah et al. 1986; Grosveld et al. 1986; Heisterkamp et al. 1985; Mes-Masson et al. 1986; Shtivelman et al. 1985). The various fusions can be detected for diagnostics by cytogenetics and FISH but more accurately by polymerase chain reaction (PCR)(Radich et al. 1994; Vanrhee et al. 1995). *BCR* (breakpoint cluster region) is a gene with unknown function containing a serine/threonine kinase domain, and *ABL1* (ABL proto-oncogene 1) encodes a nonreceptor protein tyrosine kinase that is involved in cell division, adhesion, differentiation, and stress response. The fusions lead to overactivation of the *ABL1* kinase domain and deregulation of the *ABL1* targets.

Co-occurring lesions in *BCR-ABL1* leukemia include loss-of-function or dominant-negative alterations of *IKZF1* (encoding the lymphoid transcription factor IKAROS) in approximately 70 % of cases (Iacobucci et al. 2009; Mullighan et al. 2008a), gain of a second *BCR-ABL1* chromosome, hyperdiploid karyotype in a subset of cases, and monosomy 7 or 7q (Heerema et al. 2004). Additional genetic alterations in *BCR-ABL1*-positive ALL include deletions in lymphoid transcription factors *PAX5* and *EBF1* and deletions in *CDKN2A*/*CDKN2B* tumor suppressors in 50 % of samples. These lesions are also observed at the progression of CML to lymphoid blast crisis (acute leukemia). Accordingly, in mouse models of *BCR-ABL1*-positive leukemia, expression of BCR-ABL1 on its own induces CML, but together with loss of *Arf* (encoded by *Cdkn2a*) and/or *Ikzf1* induces B-ALL (Churchman et al. 2015; Daley et al. 1990; Williams et al. 2006, 2007). Affected pathways include the RAS/MAPK, STAT, PI3 kinase, JNK/SAPK, and NF-κB pathways (Sattler and Griffin 2003).

Ph-Like ALL

Gene expression profiling studies identified a subgroup of B-lineage ALL with a similar gene expression profile to *BCR-ABL1* ALL, but without the t(9;22) translocation or expression of *BCR-ABL1* (Den Boer et al. 2009; Mullighan et al. 2009b). The frequency of *BCR-ABL1*-like or Ph-like ALL increases from 10 % in children with ALL to 27 % in young adults (Roberts et al. 2012, 2014). Recently, a large sequencing effort identified a diverse range of genetic alterations, most commonly chromosomal rearrangements, that activate cytokine receptor and kinase signaling in 91 % of Ph-like ALL cases (Roberts et al. 2012, 2014). These rearrangements commonly lead to a fusion of an *N*-terminal gene (e.g., *ETV6*, *MYB*, *EBF1*) to part

of a cytokine receptor or tyrosine kinase gene (e.g., *ABL1*, *ABL2*, *CRLF2*, *PDGFRB*, or *JAK2*), resulting in overexpression and constitutive activation of kinase signaling in leukemic cells. Additional cases harbor rearrangements of the cytokine receptor genes *CRLF2* or *EPOR* into immunoglobulin loci (*IGH*, *IGK*) that deregulate expression of the receptor by juxtaposition to the immunoglobulin enhancer regions. Alternatively, Ph-like leukemia may harbor mutations or deletions that activate kinase signaling, including mutations in kinases *JAK1*, *JAK2*, *FLT3*, and *IL7R*, and focal deletions of *SH2B3* (LNK), which is an inhibitor of JAK signaling (Bersenev et al. 2010). Other accompanying lesions are frequent deletions or mutations in *IKZF1* (Den Boer et al. 2009; Mullighan et al. 2009b). The diverse range of alterations converge on a limited number of tyrosine kinase signaling pathways, particularly ABL1/2, PDFGRB, CSF1R, and JAK-STAT signaling, and therapeutic targeting of these pathways is being explored in prospective clinical trials.

MLL-*Rearranged ALL*

Rearrangements of the myeloid/lymphoid or mixed-lineage leukemia (*MLL*) gene are present in 1–3 % of pediatric ALL samples (Forestier et al. 2000a, b; Harrison 2001). In infants younger than 1 year of age, this subtype is most frequent; 70 % of infant ALL samples harbor an *MLL* rearrangement (Pui et al. 1994, 1995; Rubnitz et al. 1994). *MLL* rearrangements are also found in pediatric AML (15–20 %) (Balgobind et al. 2011; Forestier et al. 2003; Harrison et al. 2010) and in adult leukemia (4–9 %) (Group Francais de Cytogenetique Hematologique 1996; Mancini et al. 2005; Moorman et al. 2007a; Secker-Walker et al. 1997). Furthermore, *MLL* rearrangements are associated with secondary AML after treatment with topoisomerase inhibitors (Domer et al. 1995; Felix et al. 1995; Pui et al. 1991).

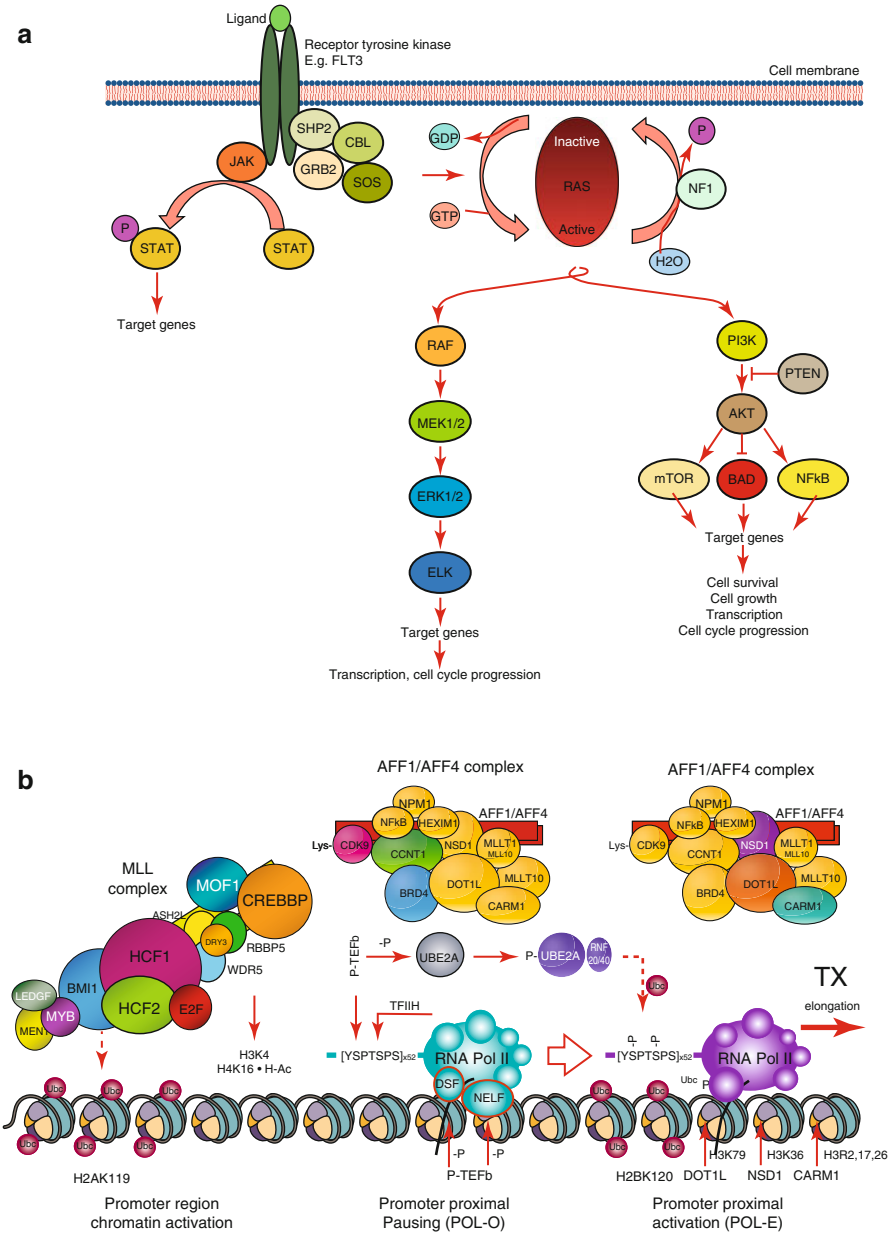
Translocations are the most frequent type of rearrangement (61 %), and over 120 fusion partners have been identified (Meyer et al. 2006, 2009). Eighty percent of the samples contain translocations t(1;11)(p32;q23) (*MLL-EPS15*), t(4;11)(q21;q23) (*MLL-AFF1/AF4*), t(6;11)(q27;q23) (*MLL-MLLT4/AF6*), t(9;11)(p22;q23) (*MLL-MLLT3/AF9*), t(10;11)(p12;q23) (*MLL-MLLT10/AF10*), or t(11;19)(q23;p13.3) (*MLL-MLLT1/ENL*). The translocations with partner genes *AF4* and *EPS15* are exclusively associated with ALL; the other partner genes are predominantly found in AML (Marschalek 2011). *MLL* is also affected by 11q23-qter deletions (6 %), 11q inversions (11 %), and focal duplications, amplifications, or partial tandem duplications (PTD) (Bernard et al. 1995; Caligiuri et al. 1994; Patel et al. 2012; Schichman et al. 1994; Meyer et al. 2006). Most rearrangements lead to expression of chimeric fusion proteins, but head-to-head orientation and out-of-frame fusions resulting in a loss-of-function have also been described (Meyer et al. 2009). The reciprocal fusion proteins may also play an important role in leukemogenesis. Cells expressing *AFF1-MLL* show increased cell cycling, as well as increased sensitivity to apoptosis. Co-expression of the reciprocal *MLL-AFF1* fusion results in a block of apoptosis (Gaussmann et al. 2007). In animal models, expression of *MLL* fusion

genes can induce leukemia, and different fusions can induce different phenotypes of leukemia. *MLL-MLLT3/AF9* induces AML in mice, but in human cord blood, *MLL-MLLT3/AF9* induces both AML and ALL, depending on the microenvironment (Corral et al. 1996; Barabe et al. 2007; Wei et al. 2008). In mice, *MLL-MLLT1/ENL* induces biphenotypic leukemia and AML, and *MLL-AFF1/AF4* induces mature B-lineage tumors (Forster et al. 2003; Metzler et al. 2006; Zeisig et al. 2003).

Perturbation of epigenetic regulation is a key mechanism of leukemogenesis in *MLL*-rearranged leukemia. *MLL* encodes two large proteins of 3969 and 4005 amino acids that contain multiple domains. The *N*-terminal MLL fragment harbors transcriptional activating functions, while the C-terminal region has repressor properties. The leukemic translocation breakpoints cluster in exons 8–14 (8.3 kb region) of the *MLL* gene which result in loss of the C-terminal SET domain and retention of the *N*-terminal activating domains. *MLL* is part of a large multi-protein complex which also includes known leukemia drivers CREBBP, E2F, and MYB (Fig. 7.4; reviewed in Marschalek 2011). The complex activates and maintains transcription by methylation of histone H3 lysine-4 (H3K4), acetylation of histone core particles, and mono-ubiquitination of histone H2A lysine-119 (H2AK119). At the activated promoter region, RNA polymerase II (RNA Pol II) assembles and associates with the AFF1/AFF4 protein complex containing p-TEFb, DOT1L, BRD4, and MLL translocation partners AFF1, MLLT1, and MLLT10. This AFF1/AFF4 complex facilitates chromatin remodeling to allow the transition of RNA Pol II from the promoter-proximal arrested state into the elongation state and thus efficient transcription (Marschalek 2011; Luo et al. 2012). P-TEFb phosphorylates the RNA Pol II complex and activates mono-ubiquitination of histone H2B by UBE2A. Next, histone methyltransferases DOT1L, NSD1, and CARM1 modify the chromatin at lysines K79 and K36 and at arginines R2, R17, R26, respectively, which enables additional elongation factors to associate with RNA Pol II.

Fig. 7.4 Signaling pathways involved in leukemia. (**a**) JAK-STAT, Ras, and PI3K/mTOR pathways. Binding of cytokines activates receptor tyrosine kinases and subsequent pathway-specific adapter molecules. Guanosine nucleotide exchange factors such as SOS1 convert RAS proteins into their active GTP-bound state. GTPase-activating proteins such as NF1 deactivate RAS proteins. Active RAS signals to several effector pathways: RAF-MEK-ERK and PI3K-mTOR-NFκB. JAK proteins autophosphorylate upon activation of the receptor tyrosine kinase and subsequently phosphorylate STAT proteins. Activated STATs dimerize and translocate to the nucleus where they promote transcription of STAT target genes. *GTP* guanosine triphosphate, *GDP* guanosine diphosphate. *JAK* indicates JAK1, JAK2, JAK3, or TYK2 proteins, *STAT* indicates STAT1, STAT2, STAT3, STAT4, or STAT5 proteins, *RAS* indicates NRAS, KRAS, or HRAS proteins, and *RAF* indicates ARAF, BRAF, or RAF-1 proteins (Adapted from Flotho et al. 2007; Knight and Irving 2014). (**b**) Mechanisms of the MLL and AFF1/AFF4 complexes. The MLL complex modifies the promoter regions of active genes by methylation, acetylation, and ubiquitination. P-TEFb associated with the AFF1/AFF4 complex activates UBE2A and phosphorylates the promoter-proximal-arrested RNA Pol II and associated factors. Subsequently, DOT1L, NSD1, and CARM1 modify the chromatin, which enables additional elongation factors to associate with RNA Pol II, converting it to the elongation form and inducing transcription (Adapted from Marschalek 2011)

Rearrangements of MLL deregulate the tightly controlled interaction of these complexes and promote atypical acetylation and active transcription of HOXA cluster genes by aberrantly targeting methyltransferase activity to their promoters (Benedikt et al. 2011; Lin et al. 2010; Luo et al. 2012; Marschalek 2011). In particular, *HOXA9*, *MEIS1*, and microRNAs miR-17-93 and miR-196b are upregulated (Armstrong et al. 2002; Faber et al. 2009; Li et al. 2012; Mi et al. 2010; Popovic et al. 2009). The microRNA miR-150 is downregulated which in turn upregulates



FLT3 (Armstrong et al. 2002). About half of t(4;11) cases do not show increased *HOXA* expression. These cases have a higher risk of relapse (Trentin et al. 2009; Stam et al. 2010). Therapeutic targeting of members of these deregulated complexes (e.g., DOT1L, BRD4, SET domain proteins, MENIN, p-TEFb) is currently a main focus of research.

Most MLL rearrangements occur in utero (Ford et al. 1993). MLL-rearranged leukemias have both lymphoid and myeloid features (reviewed by Greaves 2005), and they harbor very few CNAs or mutations. Ras mutations are the few mutations that do occur (*NRAS*/*KRAS*, *BRAF*, *NF1*) (Liang et al. 2006; Balgobind et al. 2008; Chandra et al. 2010; Bardini et al. 2010; Dobbins et al. 2013; Mullighan et al. 2007a; Andersson et al. 2015). Duplications and amplifications of *MLL* are associated with a complex karyotype and *TP53* mutations. The most frequent co-occurring lesion with *MLL* PTD is trisomy 11.

ERG-*ALL*

A new subgroup lacking any other known chromosomal rearrangements and with a distinct gene expression profile is *ERG*-altered ALL (Mullighan et al. 2007b). This group comprises 5–10 % of BCP-ALL cases. *ERG* is an ETS (erythroblast transformation-specific) transcription factor and plays a key role in embryonic development, hematopoiesis, angiogenesis, inflammation, as well as cell proliferation, differentiation, and apoptosis (Loughran et al. 2008; Iwamoto et al. 2001; McLaughlin et al. 2001). The gene has numerous isoforms resulting from splice variants and the use of alternative promoters and transcriptional start sites (Owczarek et al. 2004). A total of 75 % of ERG-ALL samples show a focal deletion of part of the ERG gene (Mullighan et al. 2007b). The deletions lead to the loss of an inhibitory domain and expression of an aberrant C-terminal ERG fragment (from alternative start site) that retains the ETS and transactivation domains. The oncogenetic mechanism of ERG rearrangements in leukemia is unknown. Interestingly, ERG translocations are also found in prostate cancer and Ewing's sarcoma, and ERG is overexpressed in AML and adult T-ALL (Tomlins et al. 2005; Delattre et al. 1994; Sorensen et al. 1994; Ichikawa et al. 1994; Baldus et al. 2004, 2006). *IKZF1* alterations co-occur in this subtype, but in contrast to other leukemia subtypes, these do not confer a poor prognosis (Clappier et al. 2014; Harvey et al. 2010b; Zaliova et al. 2014).

ALL with Intrachromosomal Amplification of Chromosome 21

Leukemia of the intrachromosomal amplification of chromosome 21 (iAMP21) subtype is characterized by complex rearrangements of chromosome 21 involving regions of gain, amplification, inversion, and deletion, all resulting in a gain of at

least three extra copies of an approximately 5.1-Mb region containing the genes *RUNX1*, *DYRK1A*, and *ETS2* (Li et al. 2014; Moorman et al. 2007b; Robinson et al. 2007; Strefford et al. 2006). ALL with iAMP21 is generally considered a distinct subtype, but iAMP21 is also occasionally observed in subtypes with recurring aneuploidy or founding translocations (hyperdiploidy, *ETV6-RUNX1*, *BCR-ABL1*, or Ph-like ALL) (Harrison et al. 2014; Haltrich et al. 2013; Ma et al. 2001). The subtype is found in 2 % of pediatric BCP-ALL and is associated with an older age at diagnosis in pediatric cases (median age of 9 years), but has not been described in adults (Harrison et al. 2014).

The iAMP21 rearrangement arises through a bridge-fusion-bridge mechanism creating a dicentric chromosome 21 (Robinson et al. 2007; Li et al. 2014). The two centromeres in these dicentric chromosomes are pulled to opposite poles during mitosis and form anaphase bridges (Crasta et al. 2012; Hatch et al. 2013; Kuchinskaya et al. 2007). It is hypothesized that these chromosomes are processed separately in the micronuclei, where they are pulverized or undergo chromothripsis. After the chromothripsis event, the derivate chromosome 21 is reassembled and duplicated as a full chromosome or by isochromosome or ring chromosome formation. In this scenario, the chromothripsis event in contrast to other tumors is nonrandom, but occurs in a coordinated sequence of events. Co-occurring lesions include gains of chromosomes X (21 %), 10 (4 %), or 14 (4 %), monosomy 7 (5 %), or deletion of chromosome arms like 1q (11 %), 6q (4 %), 7q (11 %), 9p (10 %), 11q (including *ATM* and *MLL*; 12 %), 12p (11 %), 13q (6 %), and 16q (6 %). Focal aberrations in this leukemia subtype include the *P2RY8-CRLF2* fusion (18 %) and deletions in the genes *EBF1* (8 %), *ETV6* (37 %), and *RB1* (41 %) (Harrison et al. 2014; Rand et al. 2011; Schwab et al. 2013).

T-Lineage ALL

T-ALL develops from T-lineage progenitor cells and is characterized by older age of onset and a male gender predominance as compared to BCP-ALL (Aifantis et al. 2008). Up to 70 % of T-ALLs contain chromosomal rearrangements, most of which involve the T-cell receptor loci *TRA* and *TRC* at chromosome 14q11, *TRB* (7q34), and *TRG* (7p14). The translocations involve and deregulate expression of transcription factor genes like the bHLH family (*MYC*, *TAL1*, *TAL2*, *LYL1*, and *BHLHB1*), genes encoding the LIM-domain-only proteins (*LMO1* and *LMO2*), and homeodomain genes (*HOX11* and *HOX11L2*) (Bernard et al. 2001; Boehm et al. 1991; Cauwelier et al. 2006; Chen et al. 1990; Finger et al. 1986; Hatano et al. 1991; Kennedy et al. 1991; McGuire et al. 1989; McKeithan et al. 1986; Mellentin et al. 1989; Royer-Pokora et al. 1991; Shima et al. 1986; Wang et al. 2000; Xia et al. 1991). The translocations are mutually exclusive, are associated with generally distinct gene expression profiles, and are thus considered to define different distinct T-ALL subtypes (Ferrando et al. 2002; Soulier et al. 2005; Van Vlierberghe et al. 2008b; Homminga et al. 2011).

Co-occurring lesions in T-ALL frequently involve sequence mutations of *NOTCH1* (>50 %), amplification of *MYB* (8–15 %), and deletions or mutations in *CDKN2A*/*CDKN2B* (>70 %), *PTEN* (35 %), *FBXW7* (9–16 %), *WT1* (13 %), and *BCL11B* (9 %) (Clappier et al. 2007; Gutierrez et al. 2011, 2009; Lahortiga et al. 2007; O'Neil et al. 2007; Thompson et al. 2007; Tosello et al. 2009; Weng et al. 2004; Zuurbier et al. 2012). Ribosomal proteins *RPL5* and *RPL10* are mutated in 10 % of pediatric T-ALL but not in adult T-ALL (De Keersmaecker et al. 2013). In contrast, *CNOT3* encoding part of a transcriptional regulatory complex is mutated in 8 % of adult T-ALL, but less commonly in pediatric T-ALL (De Keersmaecker et al. 2013). Loss-of-function of *PHF6* through mutations or deletions is found in 16 % of pediatric ALL cases and 38 % of adult T-ALL and is associated with *TLX1*, *TLX3*, and *TAL1* ALL (Van Vlierberghe et al. 2010). Finally, mutations and chimeric fusions including kinases like *ABL1, PTK2B* (*FAK*) and *JAK2* occur in T-ALL (Atak et al. 2013; Graux et al. 2004).

TAL1 *ALL*

TAL1 and family members *TAL2* and *LYL1* are deregulated most often in pediatric T-ALL. *TAL1*, *TAL2*, and *LYL2* belong to the bHLH family of proteins. This gene family also encompasses E47 and E12 encoded by the *E2A* gene involved in *TCF3-PBX1* ALL. HLH proteins form heterodimers and bind the E-box motif of transcriptional enhancers, thereby regulating transcription. A cryptic interstitial deletion at chromosome 1p32 leads to a fusion of the genes *SIL* (*STIL*) and *TAL1* and is present in 15–25 % of cases (Xia et al. 1991; Brown et al. 1990; Jonsson et al. 1991). The t(1;14)(p32;q11) translocation (3 % of cases) juxtaposes *TAL1* to the *TRA/TRD* locus. The translocation t(7;9)(q34;q32) juxtaposes *TAL2* to the *TRB* locus and the t(7;19)(q34;p13) juxtaposes *LYL1* to the TRB locus (Cleary et al. 1988). Recently, another mechanism resulting in overexpression of *TAL1* was identified. Insertions and deletions of 2–18 base pairs introducing *MYB* binding motifs in a noncoding region 7.5 kb upstream of *TAL1* occur in about 6 % of T-ALL cases (Mansour et al. 2014). These extra *MYB* binding sites create a region of dense histone 3 lysine 27 (H3K27) acetylation commonly referred to as a superenhancer, which recruits transcription factors *CREBBP*, *RUNX1*, *GATA3*, and *TAL1*, resulting a positive feedback loop and overexpression of *TAL1*. Other mutations creating superenhancers might be involved in samples with unexplained overexpression of oncogenic driver genes (Groschel et al. 2014; Herranz et al. 2014).

LMO1/LMO2 *ALL*

A second subtype of T-ALL has a similar gene expression profile to *TAL1* ALL and is characterized by rearrangements of the LIM-domain-only genes *LMO1* and *LMO2* by t(11;14)(p15;q11) and t(11;14)(p13;q11) or t(7;11)(q35;p13), placing

these genes under control of the *TRA* and *TRD* loci, respectively (McGuire et al. 1989; Boehm et al. 1991; Royer-Pokora et al. 1991; Homminga et al. 2011). An additional mechanism is a focal deletion del(11)(p12p13) of a regulatory region upstream of *LMO2* that results in increased expression of *LMO2* (Mullighan et al. 2007a; Van Vlierberghe et al. 2006). This deletion was also found in the germline of a patient who developed two primary T-ALL occurrences, indicating that this lesion may confer susceptibility to leukemia development (Szczepanski et al. 2011).

LMO1 and *LMO2* encode transcription factors that contain a cysteine-rich domain. They function as scaffolds in protein-protein interactions and form cell type-specific transcriptional complexes with *LDB1*, *ETO2*, *GATA1*, *GATA2*, *GATA3*, *TAL1*, *LYL1*, *RUNX*, and ETS proteins, which regulate expression of thousands of target genes and are essential for hematopoiesis (Wilson et al. 2010; Palii et al. 2011; Sanda et al. 2012; Soler et al. 2010; Tripic et al. 2009). Overexpression of *LMO1* or *LMO2* induces leukemia and lymphoma with long latency in mice, though co-occurrence with lesions in *TAL1*, *NOTCH1*, and *CDKN2A*/*CDKN2B* (Arf) vastly accelerates the process (Aplan et al. 1997; Chervinsky et al. 1999; Larson et al. 1996; McGuire et al. 1992; Neale et al. 1995; Wadman et al. 1994). Aberrant expression of *LMO1* or *LMO2* in mouse thymocytes induces self-renewal and stem cell characteristics, which sensitizes these cells for additional mutation occurrence (McCormack et al. 2010; Treanor et al. 2011; Gerby et al. 2014).

HOX Gene-Deregulated ALL

Two HOX genes are involved in T-ALL development and comprise a subgroup of T-ALL: *HOX11* (*TLX1*) and *HOX11L2* (*TLX3*). The HOX genes are essential in anterior/posterior patterning, differentiation, and regulation of hematopoiesis and leukemogenesis (Argiropoulos and Humphries 2007). Overexpression of *TLX1* occurs in 7 % of cases and arises from t(10;14)(q24;q11) or t(7;10)(q35;q24) translocations juxtaposing *TLX1* to the *TRA* or *TRB* loci, respectively (Dube et al. 1991; Hatano et al. 1991; Kennedy et al. 1991; Lu et al. 1991). Expression of *TLX1* in animal models immortalizes hematopoietic progenitors but induces T-ALL only after prolonged latency, indicating that additional lesions are needed (Hawley et al. 1997, 2008; Keller et al. 1998). *TLX1* downregulates mitotic checkpoint genes like *CHEK1*, which results in chromosomal missegregation and aneuploidy (De Keersmaecker et al. 2010). Overexpression of *TLX3* occurs in approximately 20 % of pediatric T-ALL and results from a cryptic t(5;14)(q35;q32) fusing *TLX3* with *BCL11B* (Ballerini et al. 2002; Berger et al. 2003; Bernard et al. 2001; Cave et al. 2004). *BCL11B* is expressed during T-cell development and is somatically affected by deletions and sequence mutations in T-ALL development (De Keersmaecker et al. 2010). Other translocations involving *BCL11B-NKX2-5* and *CDK6-TLX3* result in a similar type of leukemia (Nagel et al. 2003, 2007; Su et al. 2004). A frequent co-occurring lesion specific for *TLX3* overexpressing T-ALL is a cryptic deletion on chromosome 5, del(5)(q35), containing 30 genes just downstream of the translocation breakpoint (Van Vlierberghe et al. 2008a).

LEF1-*Inactivated ALL*

The transcription factor lymphoid enhancer-binding factor 1 (*LEF1*) is inactivated by monoallelic deletions, biallelic deletions, or truncating mutations in approximately 18 % of pediatric T-ALL cases (Gutierrez et al. 2010). *LEF1* is essential for hematopoietic stem cell and progenitor maintenance and function (Edmaier et al. 2014). It binds the T-cell receptor alpha enhancer and interacts with Wnt/β-catenin signaling, which controls self-renewal, proliferation, and differentiation of many types of stem cells, and transforming growth factor beta (TGF-β)/*SMAD4* signaling, which is involved in cell growth, differentiation, apoptosis, and cellular homeostasis (Nishita et al. 2000). Deregulated *LEF1* expression (either up or down) has been associated with B-ALL, AML, chronic lymphocytic leukemia, and myelodysplastic syndromes (Edmaier et al. 2014; Erdfelder et al. 2010; Kuhnl et al. 2011; Metzeler et al. 2012; Pellagatti et al. 2009; Gutierrez et al. 2010; Petropoulos et al. 2008). *LEF1*-inactivated ALL shows a differentiation arrest at an early cortical stage with expression of cell surface markers CD1b, CD1e, and CD8, but absence of CD34. The subtype distinguishes from the other T-ALL subtypes in that it shows no overexpression of *TAL1*, *HOX11*, *HOX11L2*, or *HOXA*/*MEIS1* (Gutierrez et al. 2010). Co-occurring lesions are activating *NOTCH1* mutations, biallelic *CDKN2A*/*CDKN2B* deletions, and *PTEN* loss-of-function, activating mutations in the PI3K/AKT pathway, and overexpression of *MYC* and its target genes (Gutierrez et al. 2010). Patients present generally at a younger age (Gutierrez et al. 2010).

MLL-*Rearranged T-ALL*

MLL-rearranged T-ALL most often involves t(11;19)(q23;p13.3) (*MLL*-*MLLT1*/*ENL*; 4–8 % of cases), but other fusions also occur (Hayette et al. 2002). This subtype is mostly found in adolescents (Rubnitz et al. 1999). The transcriptional profile of *MLL*-rearranged T-ALL differs significantly from *MLL*-rearranged BCP-ALL (Ferrando et al. 2002, 2003). In 8 % of pediatric and 10 % of adult T-ALL cases (Asnafi et al. 2003; Dreyling et al. 1996; Atak et al. 2013) and occasionally in AML (Bohlander et al. 2000; Dreyling et al. 1998), the translocation t(10;11)(p13;q14) is found which fuses *PICALM* and *MLL10* (*CALM-AF10*). This translocation does not involve *MLL* itself, but interestingly, both *PICALM* and *MLL10* are described as fusion partners for *MLL*, and *PICALM-MLL10* results in the characteristic upregulation of *MEIS1* and *HOX* genes.

Early T-Cell Precursor ALL

Early T-cell precursor (ETP) ALL is characterized by an immature immunophenotype with expression of the T-lineage marker cytoplasmic CD3; a lack of expression of other T-cell markers such as CD1a, CD8, and CD5; and an aberrant expression of

myeloid or stem cell markers (Coustan-Smith et al. 2009). ETP ALL likely represents one of a spectrum of primitive neoplasms of progenitor cells that retain their multi-lineage potential that may also include biphenotypic and bilineal ALL.

ETP ALL cells harbor recurring alterations of multiple pathways in the majority of cases. These include loss-of-function aberrations – mutations, deletions, or translocations – in hematopoietic development genes (*RUNX1*, *IKZF1*, *ETV6*, and *GATA3*); activating mutations in Ras or cytokine signaling (*NRAS*, *KRAS*, *NF1*, *PTPN11*, *FLT3*, *JAK1*, *JAK3*, *IL7R*, and *SH2B3*); and mutations in chromatin-modifying genes, particularly PRC2 complex genes (*EZH2*, *SUZ12*, and *EED*) which confer H3K27 trimethylation, *SETD2* and *EP300* (Della Gatta et al. 2012; Ntziachristos et al. 2012; Zhang et al. 2012; Shochat et al. 2011; Zenatti et al. 2011). Mutations in IL7R, the alpha chain of interleukin-7 receptor (IL7R), involve in-frame insertions that introduce a cysteine in the transmembrane domain of IL7R, which dimerizes the receptor and results in constitutive activation, which in turn activates JAK-STAT signaling in the absence of ligand. Activated JAK-STAT signaling measured by phosphoflow cytometry or gene profiling studies is present in the majority of ETP ALL (Zhang et al. 2012) even without IL7R mutations. In $Arf^{-/-}$ mice, IL7R mutations were shown to be potent driver mutations and initiators of ETP ALL (Treanor et al. 2014). *EZH2* encodes the catalytic component of PRC2 and contains *MLL*-like SET domain that mediates histone methylation. The PRC2 complex interacts with *DNMT3A*, which is mutated in adult AML and adult ETP ALL, but not pediatric ETP ALL (Ley et al. 2010). In ETP ALL, *EZH2* is targeted by loss-of-function mutations in the SET domain and elsewhere. In mice, loss-of-function *EZH2* mutations result in T-ALL development (Simon et al. 2012; Neumann et al. 2013). In contrast, gain-of-function mutation p.Tyr641 in the SET domain which enhances di- and trimethylation is not found in ETP ALL, but is characteristic of lymphoma (Morin et al. 2010; Sneeringer et al. 2010; Yap et al. 2011). ETP ALL is also characterized by overexpression of *MEF2C*, a member of MADS-box transcription factor family, which causes overexpression of *MEF2C* target genes *LYL1*, *LMO2*, and *HHEX* (Homminga et al. 2011; Smith et al. 2014).

Tumor Heterogeneity, Disease Progression, and Relapse

Leukemic tumors are not composed of a single clone of cells all containing the same aberrations, but are commonly multiclonal. Clonal architecture and composition are dynamic and evolve during leukemogenesis and therapy. This evolution does not proceed in a sequential linear fashion, but it develops in a complex branching pattern. Mutations and CNAs continue to occur independently and repeatedly through external or intrinsic factors in some but not all cells without a preferential order (Anderson et al. 2011; Notta et al. 2011). A new clone will grow out when the cells overcome diverse evolutionary bottlenecks by advantages in competitive regenerative capacity, treatment resistance, proliferation in particular stroma or environments, or the capability to undergo senescence. This dynamic is most elaborately

shown when comparing matched diagnosis and relapse samples. Founding chromosomal translocations are almost always conserved from diagnosis to relapse, along with a proportion of CNAs and point mutations, but most cases exhibit substantial genomic changes during disease progression, with acquisition of new deletions and mutations and loss of diagnosis-specific lesions (Mullighan et al. 2008b; Yang et al. 2008; Kawamata et al. 2009). Many relapse-acquired lesions, especially those influencing drug resistance, appear to be present at low levels at time of diagnosis and may be detected with current highly sensitive sequencing techniques (Ma et al. 2015). This is important for the molecular monitoring of minimal residual disease and early detection of relapse development in leukemia patients (Faham et al. 2012). One such example are mutations in *TP53* which are uncommon in major clones at diagnosis but frequent in relapsed ALL and are associated with treatment failure (Blau et al. 1997; Diccianni et al. 1994; Gump et al. 2001; Hof et al. 2011; Hsiao et al. 1994). In addition, Ras pathway mutations and mutations in *CREBBP* are enriched in relapsed ALL. *CREBBP* is part of the MLL complex and mediates transcriptional response to glucocorticoids (Inthal et al. 2012; Kino et al. 1999; Lambert and Nordeen 2003; Mullighan et al. 2011). Finally, relapse-associated genes *NT5C2* and *PRPS1* play a role in purine analogue resistance (Meyer et al. 2013; Tzoneva et al. 2013; Ma et al. 2015; Li et al. 2015).

Deregulation of Multiple Pathways in ALL

At least four pathways are frequently mutated in the majority of cases of ALL: hematopoietic and lymphoid maturation; cell cycle regulation; cytokine receptor, kinase, and Ras signaling; and epigenetic modification. The block in differentiation arises from focal deletions, translocations, and loss-of-function or dominant negative mutations in hematopoietic and lymphoid transcription factors such as *PAX5*, *IKZF1*, and *EBF1*. Similarly, cell cycle regulation and tumor suppression genes *CDKN2A*/*CDKN2B*, *PTEN*, *RB1*, and *TP53* are targeted by both mutations and deletions as well as promoter methylation. In contrast, gain-of-function mutations are found in various pathways inducing proliferation like sequence mutations in Ras pathway genes (*NRAS*, *KRAS*, and *NF1*) and mutations and translocations resulting in increased expression or activity of cytokine receptor *IL7R*, cytokine-like receptor *CRLF2*, and JAK kinases (*JAK1*, *JAK2*, *JAK3*, and *TYK2*).

Illegitimate VDJ Recombination

Focal deletions in ALL frequently result from illegitimate VDJ recombination. In early stages of lymphocyte development, tightly regulated VDJ recombination mediated by recombination-activating genes 1 and 2 (*RAG1* and *RAG2*) creates the diversity in the antigen receptor repertoire. The developmental arrest of a (pre-)

leukemic cell in a stage where the *RAG1* and *RAG2* genes are highly expressed may increase the risk of off-target recombinational events in transcriptionally active and thus accessible genes. As these genes are mostly involved in cell differentiation and proliferation at these stages, the recombinational events can easily cause the cell to spiral out of control and evolve into a full-blown leukemia. Illegitimate, off-target activity of the RAG recombinases causes aberrations with one or more of the following characteristics: (1) breakpoints located in active promoter and enhancer regions, (2) recombination signal sequences (RSS) or RSS-like motif directly adjacent to the breakpoints, (3) tightly clustered breakpoints, and (4) nontemplated nucleotides between the breakpoints resulting from terminal deoxynucleotidyl transferase (TdT) activity. Aberrations with this fingerprint can be recognized in multiple ALL subtypes like *ETV6-RUNX1*-positive ALL (B-cell differentiation genes), Ph+ and Ph-like ALL (intragenic *IKZF1* deletions), and T-ALL (*TAL1* translocations, *LMO2* deletions, and *CDKN2A* and *CDKN2B* deletions) (Mullighan et al. 2008a, b; Iacobucci et al. 2009; Marculescu et al. 2002; Papaemmanuil et al. 2014; Waanders et al. 2012; Holmfeldt et al. 2013). RAG-mediated recombination is an important mutational process in ALL, and targeted single-cell sequencing indicated that it occurs continuously throughout leukemia evolution (Papaemmanuil et al. 2014).

Ras Pathway and Receptor Tyrosine Kinase Mutations

The Ras pathway plays a role in differentiation, apoptosis, and proliferation (reviewed in Pylayeva-Gupta et al. 2011). The signaling cascade includes Ras, Raf, MEK (MAPKK), and ERK (MAPK), which transfers and integrates the extracellular signal to various nuclear and cytosolic targets (Fig. 7.4). The pathway is activated by ligand binding to the cell surface receptor tyrosine kinase (*FLT3*), which autophosphorylates its intracellular SH2 domain and recruits Grb2 and guanine nucleotide exchange factors (GEFs). The GEFs activate membrane-associated GTPases *NRAS*, *KRAS*, and *HRAS* by converting the inactive GDP-bound state to the active GTP-bound state. Active Ras phosphorylates Raf at specific serine residues resulting in homo- or heterodimers of Raf isoforms. Raf then activates MEK1/2 which in turn activates ERK1/2. Activated ERK translocates to the nucleus where it phosphorylates transcription factors such as ELK1, which in turn regulate gene transcription.

The Ras pathway genes most commonly affected in leukemia include *NRAS*, *KRAS*, *NF1*, *PTPN11*, and *FLT3*. The most common alterations in leukemia are activating sequence mutations at codons 12–13 and 59–63 of NRAS and KRAS, which inhibit the effect of GTPase-activating proteins (GAPs) such as NF1. NF1 hydrolyzes GTP and converts the active GTP-bound NRAS/KRAS to the inactive GDP-bound state. In leukemia, *NF1* is mostly targeted by focal deletions, which inactivate the protein (Balgobind et al. 2008; Mullighan et al. 2007a; Holmfeldt et al. 2013). All these aberrations result in a constitutively active GTP-bound

NRAS/KRAS and thus activation of the signaling pathway. The receptor tyrosine kinase *FLT3* is mutated in 2–9 % of ALL cases and in 30 % of AML cases (Case et al. 2008; Paulsson et al. 2008; Small 2006). Activating mutations in the tyrosine kinase domain or mutations abolishing the autoinhibitory function of the juxtamembrane region cause ligand-independent constitutive activation and hypersignaling of the Ras pathway. In *MLL*-rearranged ALL, *FLT3* is overexpressed rather than mutated (Armstrong et al. 2003; Stam et al. 2005). Recently, another receptor tyrosine kinase, *MERTK*, was found to be overexpressed in B-ALL and *TCF3-PBX1* ALL in particular (Linger et al. 2013). *PTPN11* encodes the protein Shp2, which is a phosphatase and regulator of the Ras and JAK-STAT pathways. *PTPN11* is thought to dephosphorylate the GAP binding sites on the receptor tyrosine kinases, thereby switching off the signaling. Mutations in this gene (2–10 % of ALL cases) eliminate the negative regulation and thus activate the various pathways (Case et al. 2008; Molteni et al. 2010; Tartaglia et al. 2004; Yamamoto et al. 2006; Paulsson et al. 2008).

Activation of the Ras pathway is a hallmark of many tumor types (Pylayeva-Gupta et al. 2011) but is present only in certain ALL subtypes. At diagnosis, Ras mutations are found in hyperdiploid, hypodiploid, and *MLL*-rearranged ALL, *ERG* ALL, Ph+ ALL, Ph-like ALL, and ETP ALL (Paulsson et al. 2008; Holmfeldt et al. 2013; Andersson et al. 2015; Roberts et al. 2014; Zhang et al. 2011, 2012), though at relapse Ras activation is found to be acquired in many subtypes. The activation of the Ras pathway signifies many new and important therapeutic targets (Knight and Irving 2014). Importantly, it has been shown that the Ras pathway cross talks with the PI3K/Akt/mTOR and the RalGEF/RAL pathways (Castellano and Downward 2011; Cooper et al. 2013; Mendoza et al. 2011), and leukemic cells with Ras pathway alterations commonly exhibit activation of PI3K signaling (Holmfeldt et al. 2013).

CRLF2 *and* IL7R *Alterations*

CRLF2 (cytokine receptor-like factor 2) is located at the pseudoautosomal region 1 at Xq21.3/Yp11.2. With the alpha chain of IL7R, it forms a heterodimeric type I cytokine receptor for thymic stromal lymphopoietin (TSLP). Ligand engagement activates JAK-STAT signaling, and physiologic signaling through the receptor is important for T-cell and dendritic cell development. Several genomic aberrations result in lineage-inappropriate and autonomous JAK-STAT, PI3K/mTOR, and BCL-2 signaling in B-ALL. *CRLF2* expression can be deregulated through translocation with the immunoglobulin heavy chain (IGH) gene locus at chromosome 14q32 (IGH-CRLF2, t(X;14)(p22;q32), or t(Y;14)(p11;q32)) or by a deletion (del(X)(p22.33p22.33) or del(Y)(p11.32p11.32)) which juxtaposes *CRLF2* to the active promoter of P2Y purinergic receptor 8 gene (*P2RY8*) (Mullighan et al. 2009a; Russell et al. 2009). The p.Phe232Cys also found in ALL results in receptor dimerization and thus constitutive active *CRLF2* (Chapiro et al. 2010). *CRLF2*

rearrangements are common in Ph-like ALL (50 % of cases) and Down syndrome-associated ALL (55–60 % of cases), but they are also found in other leukemia subtypes including iAMP21 ALL (Russell et al. 2009). Mutations in IL7R are found both in B- (Ph-like) and T-lineage ALL (Zenatti et al. 2011; Zhang et al. 2012; Shochat et al. 2011; Roberts et al. 2014). In-frame insertions that introduce a cysteine in the transmembrane domain of IL7R result in dimerization and constitutive activation of the receptor. This in turn activates JAK-STAT signaling in the absence of ligand. *JAK1* or *JAK2* are co-mutated in 50 % of *CRLF2*-affected cases.

JAK-STAT Signaling Alterations in ALL

Activation of JAK-STAT signaling may also arise from gain-of-function mutations or translocations of the Janus kinase family members. The Janus kinase family consists of *JAK1*, *JAK2*, *JAK3*, and *TYK2*. Mutations can occur in the kinase domain, but more frequently they affect the pseudokinase domain. The pseudokinase domain inhibits the kinase domain function, and mutations are thought to remodel the complex which leads to activation of the kinase (Bandaranayake et al. 2012; Lupardus et al. 2014; Toms et al. 2013). Mutations in the JAK family differ between leukemia subtypes. *JAK2* p.Val617Phe is very frequent in myeloproliferative diseases but is rare in ALL (Levine and Gilliland 2008). In BCP-ALL, *JAK2* is most frequently affected by p.Arg683Gly/Ser especially in *CRLF2*-rearranged Ph-like ALL (Harvey et al. 2010a; Mullighan et al. 2009c; Zhang et al. 2011). In T-ALL, *JAK1* and *JAK3* are more commonly mutated (Bellanger et al. 2014; Zhang et al. 2012). A subset of Ph-like ALL cases have rearrangements leading to fusion and constitutive activation of JAK2 and rearrangement of the erythropoietin receptor gene to immunoglobulin loci, which also activates JAK-STAT signaling (Roberts et al. 2014).

NOTCH Signaling

The main pathway affected in T-ALL is the NOTCH pathway represented by mutations in *NOTCH1* (>50 % of cases), *FBXW7* (9–16 %), and *PTEN* (35 %) (Gutierrez et al. 2009; O'Neil et al. 2007; Thompson et al. 2007; Weng et al. 2004). *NOTCH1* is a member of the transmembrane receptor family consisting of another three members (*NOTCH2*, *NOTCH3*, and *NOTCH4*) (reviewed in Suresh and Irvine 2015). NOTCH proteins mediate cell-cell interaction and transduce extracellular signals resulting in the regulation of self-renewal, differentiation, proliferation, and apoptosis. Epidermal growth factor-like repeats in the extracellular domain bind ligands Delta-like 1, Delta-like 3, Delta-like 4, Jagged 1, and Jagged 2 on neighboring cells. Each NOTCH protein has its own ligand specificity, which is also dependent on the cell type it expresses. Binding of ligand results in cleavage by metalloproteases and γ-secretases and internalization and localization to the nucleus of the intracellular

domain of NOTCH (ICN). In the nucleus, ICN forms a transcription complex with transcription factors which binds to promoter regions of target genes and recruits the chromatin remodeling proteins histone acetyltransferases (HATs) to initiate transcription. *NOTCH1* has many target genes including *HES1*, *HEY*, c-*MYC*, *GATA*-3, *CCND1* (Cyclin D1), *CDKN1A* (p21), *IL7R*, and the homeobox genes *HOXA5*, *HOXA9*, and *HOXA10* (Cohen et al. 2010; Guo et al. 2009; Hozumi et al. 2008; Monastirioti et al. 2010; Wang et al. 2014; Weerkamp et al. 2006; Weng et al. 2006). The intracellular domain of NOTCH1 consists of an RBP-J κ-associated domain (RAM), seven Ankyrin repeats, two nuclear localization signals, a transactivation domain, and a proline-glutamate-serine-threonine (PEST)-rich domain. The RAM and ankyrin domains infer signal transduction, and the RAM domain binds transcription factors. The PEST domain contains phosphorylation sites to regulate the stability and ubiquitination of the intracellular domain. Mutations in *NOTCH1* are mostly localized in the heterodimerization domain, the transactivation domain, and the PEST domain, all of which result in constitutional activation or an increased stability of ICN (Weng et al. 2004; Ferrando 2009). *FBXW7* is an E3 ubiquitin protein ligase, and a loss-of-function mutation deregulates *NOTCH1* degradation (Thompson et al. 2007; O'Neil et al. 2007). *PTEN* negatively regulates the PI3K-AKT pathway and is inhibited by the NOTCH pathway. An overactivated NOTCH thus strongly inhibits PI3K-AKT. Loss-of-function mutations in *PTEN* uncouple NOTCH1 signaling from the PI3K-AKT pathway and activate the AKT pathway, giving the cells an additional stimulus for growth and survival (Palomero et al. 2007, 2008).

Chromatin Remodeling

Mutations in genes that mediate chromatin remodeling and histone modification are common in many ALL subtypes and in relapsed ALL. In *MLL*-rearranged ALL, epigenetic remodeling is the main pathway affected, and very few co-occurring lesions are present. Translocations involving *MLL* and genes from the AFF1/AFF4 complex deregulate the control of methylation, acetylation, and ubiquitination of promoter regions by the MLL complex and the activation of RNA Pol II by the AFF1/AFF4 complex, resulting in aberrant expression of *HOXA9* and *MEIS1* (Luo et al. 2012; Marschalek 2011). Further, ETP ALL harbors mutations in polycomb repressor complex 2 (PRC2) components *EZH2*, *SUZ12*, and *EED*, which influences repressive H3K27 trimethylation (Zhang et al. 2012). *ETV6-RUNX1* ALL harbors mutations in methyltransferase *WHSC1* (*NSD2*) (Jaffe et al. 2013), and hypodiploid and relapsed ALL often show mutations in the H3K18 and H3K27 acetylase *CREBBP* (CREB-binding protein) (Mullighan et al. 2011; Holmfeldt et al. 2013). Relapsed ALL further contains mutations in *SETD2* (H3K36 trimethylase), *KDM6A* (lysine-specific demethylase of histone H3), and *MLL2*/*KMT2D* (H3K4 methyltransferase) (Mar et al. 2014; Ma et al. 2015).

Germline Genetic Variation and ALL Risk

Like in many cancers, the onset of a proportion of childhood leukemia is significantly influenced by genetic predisposing factors. The Swedish family cancer database revealed evidence for inherited predisposition to childhood ALL, independent of the concordance in monozygotic twins (Kharazmi et al. 2012). The excess risk in monozygotic twins may be due more to intraplacental transmission rather than highly penetrant risk alleles (Greaves et al. 2003; Kharazmi et al. 2012). Inherited predisposition may be composed of common inherited polymorphisms with modest effect sizes and rare germline variants that induce a high risk of leukemia. The detection of multiple variants in both categories in the last decade can be attributed to impressive developments in the field of whole-genome analyses.

Common Genetic Variants Predisposing to Childhood ALL

Initially, many association studies of ALL have been based on the candidate gene approach and have evaluated a restricted number of polymorphisms, primarily in genes implicated in the metabolism of carcinogens, folate metabolism, DNA repair, and cell cycle regulation (Vijayakrishnan and Houlston 2010). Reports from most candidate gene studies have been disappointing, with many positive associations initially being reported which subsequent studies failed to replicate. Hence, few if any definitive susceptibility alleles for ALL have been identified through candidate gene association studies (Vijayakrishnan and Houlston 2010).

Genome-wide association studies (GWAS) have identified multiple reproducible associations between common inherited variants and the risk of ALL. Notably, these studies have also identified associations with specific ALL subtypes, ALL in specific ethnic populations, and outcomes. GWAS studies compare the DNA of two groups of participants: people with the disease (cases) and people without the disease (controls). From each individual, millions of genetic variants (single nucleotide polymorphisms (SNPs)) are genotyped using microarrays. If one type of the variant is statistically significantly more frequent in people with the disease as compared to healthy controls, the SNP is said to be "associated" with the disease. Variants associated with ALL include SNPs in *IKZF1* (7p12.2), *CDKN2A*/*CDKN2B* (9p21), *ARID5B* (10q21.2), *CEBPE* (14q11.2), *PIP4K2A* (10p12.2), and *GATA3* (10p14) (Migliorini et al. 2013; Papaemmanuil et al. 2009; Perez-Andreu et al. 2013, 2015; Sherborne et al. 2010; Trevino et al. 2009). The risk of ALL associated with each of the variants individually is modest, but they still make a significant contribution to disease burden because of their high frequencies in the population. Some of these variants are typically associated with a specific ALL subtype. Examples are the relationship between *ARID5B* and *PIP4K2A* genotype and hyperdiploid ALL, whereas the risk allele in *GATA3* has been associated with Ph-like ALL (Migliorini et al. 2013; Perez-Andreu et al. 2013). It is unknown how these variants infer their risk. For some, it was shown that the variants influence gene expression.

Inherited genetic factors may play a role in determining the natural course of the disease and its response to therapies. *GATA3* variants are associated with Ph-like ALL and poor ALL outcome (Migliorini et al. 2013; Perez-Andreu et al. 2013). Different outcomes of treatment regimens between ethnic groups may also in part be explained by genetic variation. Hispanic children have a greater incidence of ALL (Yamamoto and Goodman 2008) and increased relapse relative to Europeans (Yang et al. 2011). It was shown that *GATA3* risk alleles contribute to this increased ALL incidence and may underlie their poorer outcomes (Walsh et al. 2013). Racial disparities in the incidence and treatment outcome of childhood ALL have also been linked to *ARID5B* genetic polymorphisms (Xu et al. 2012).

The detection of risk alleles that contribute significantly to the development of childhood ALL is meaningful in understanding the etiology of this disease. The application of GWAS on large sample sizes of more narrowly defined subtypes of childhood ALL and the implementation of complete genome sequencing as an alternative to genotyping array-based GWAS studies contribute in achieving this goal.

Rare Genetic Variants Predisposing to Childhood ALL

Families with multiple relatives affected by ALL are of value to identify rare genetic variants that confer a much higher risk of developing leukemia compared to variants identified by GWAS studies. A recent example is the identification of a novel *PAX5* sequence mutation, p.Gly183Ser, in three unrelated kindreds with autosomal dominant ALL (Shah et al. 2013; Auer et al. 2014). Somatic *PAX5* sequence mutations are common in BCP-ALL and typically involve the DNA binding paired domain or the C-terminal transactivating domain. The Ser183 mutation results in partial loss of transcriptional activation and may act by impeding interaction between PAX5 and cofactors that enhance PAX5 activity. All patients with this mutation exhibited loss of the *PAX5* wild-type gene by deletion of chromosome 9p, indicating that transmission of this mutation is tolerable in the heterozygous state, but severe attenuation of *PAX5* activity is required for leukemogenesis. This mutation was not detected in over 30 additional ALL kindreds; thus, additional mutations are likely to contribute to leukemogenesis in familial ALL. Germline mutations in *ETV6* were identified in association with familial thrombocytopenia and hematologic malignancies (Zhang et al. 2015; Noetzli et al. 2015). The mutations identified correspond to hotspots for recurrent somatic mutation in malignancies and affect DNA binding efficiency and altered the intracellular localization of the protein. Moreover, they had a dominant negative effect on the transcriptional repressor function of wild-type *ETV6*. This novel cancer-predisposing syndrome is characterized by diverse hematologic malignancies, including MDS, pre-B-cell ALL, and multiple myeloma, and affects both children and adults. No deletion or mutation of the remaining wild-type *ETV6* allele was observed in any of the neoplasms.

It is likely that not all patients who have developed ALL as a result of carrying a high-risk germline mutation will be recognized by their family history. For example, *de novo* mutations and mutations that follow incomplete penetrance may be found in

sporadic patients. Recently, it was shown that the *TP53* alterations observed in 91.2 % of childhood cases of low-hypodiploid ALL are also present in nontumor cells in 43.3 % of the mutation-carrying cases (Holmfeldt et al. 2013; Powell et al. 2013). Hence, low-hypodiploid ALL represents a manifestation of Li-Fraumeni syndrome (LFS), a hereditary cancer predisposition syndrome that affects children, adolescents, and adults and predisposes them to a wide spectrum of malignancies. This syndrome has a high de novo mutation rate (estimated between 7 % and 20 %) (Gonzalez et al. 2009), and therefore several of these patients with low-hypodiploid ALL will have represented the first presentation of a cancer syndrome in the family.

The association between germline *TP53* mutations and low-hypodiploid ALL was identified by a detailed description of the mutational landscape of one particular subtype of ALL (Holmfeldt et al. 2013). In this study, germline variants were also identified in *NRAS* and *PTPN11* in near-haploid ALL, suggesting association with other susceptibility syndromes. Likewise, it was shown in a thorough study of iAMP21 ALL that individuals born with the rare constitutional Robertsonian translocation rob(15;21)(q10;q10)c have an approximately 2700-fold increased risk of developing iAMP21 ALL compared to the general population (Li et al. 2014). The translocation results in a dicentric chromosome which is susceptible to chromothripsis and iAMP21 formation. Additional novel cancer-predisposing mechanisms may emerge from ongoing studies describing the mutation spectrum of subtypes of ALL.

A direct approach to detect high-risk childhood ALL predisposing mutations in sporadic patients is by studying patients who develop two primary events of ALL. Szczepanski and colleagues studied a cohort of patients with a late relapse (at least 2.5 years from diagnosis) of T-ALL (Szczepanski et al. 2011). In 36 % ($n=8$) of the patients, *NOTCH1* mutation patterns and T cell receptor gene rearrangement sequences had completely changed between diagnosis and relapse, and gene copy number analysis showed markedly different patterns of genomic aberrations, suggesting a second T-ALL rather than a resurgence of the original clone. In one patient, SNP analysis revealed a germline del(11)(p12;p13), a known recurrent aberration in T-ALL. Further studies will likely reveal additional germline-predisposing mutations in these patients.

Rare Genetic Variants Predisposing to Childhood ALL: "Syndromic" ALL

A more easily recognizable group of patients with ALL predisposition are the patients with a germline mutation that results in a syndrome characterized by congenital anomalies, intellectual disability, dysmorphisms, or a combination of these. Probably the most well-known example is a markedly elevated risk of both ALL and AML in Down syndrome (Hasle et al. 2000). The actual risk of developing ALL varies among these "multisystem syndromes" from very low in patients with Noonan syndrome caused by activating mutations in genes involved in the RAS-MAPK pathway (Jongmans et al. 2011) to high in several DNA repair disorders like

ataxia telangiectasia (Olsen et al. 2001). The fact that many of these syndromes are extremely rare can hamper the judgment of whether a condition indeed is an ALL-predisposing syndrome, since proper epidemiological studies are impossible due to small sample sizes. To secure an increase of knowledge regarding associations between rare syndromes and cancer predisposition, publications of case reports are important.

Children with ALL have a 5-year survival rate of more than 90 % (Bienemann et al. 2011). It may well be that a proportion of the 10 % of children who do not survive are enriched for children with cancer-predisposing conditions that make them prone for comorbidity, second primary malignancies, and more severe toxic side effects of treatment. In patients with ataxia telangiectasia, for instance, the treatment of malignancies is hampered by therapy-associated toxicity and infectious complications, and these patients benefit from significantly reduced-intensity chemotherapy (Hunger et al. 2012). In order to achieve a cure rate significantly above 90 % for childhood ALL, studies of treatment outcome and side effects experienced in patients with rare syndromes are extremely valuable for the adjustment of treatment protocols in future patients.

References

Aguiar RC, Sohal J, Van Rhee F, Carapeti M, Franklin IM, Goldstone AH, Goldman JM, Cross NC. TEL-AML1 fusion in acute lymphoblastic leukaemia of adults. M.R.C. Adult Leukaemia Working Party. Br J Haematol. 1996;95:673–7.

Aifantis I, Raetz E, Buonamici S. Molecular pathogenesis of T-cell leukaemia and lymphoma. Nat Rev Immunol. 2008;8:380–90.

Al-Obaidi MSJ, Martineau M, Bennett CF, Franklin IM, Goldstone AH, Harewood L, Jalali GR, Prentice HG, Richards SM, Roberts K, Harrison CJ, Council MR. ETV6/AML1 fusion by FISH in adult acute lymphoblastic leukemia. Leukemia. 2002;16:669–74.

Al-Shehhi H, Konn ZJ, Schwab CJ, Erhorn A, Barber KE, Wright SL, Gabriel AS, Harrison CJ, Moorman AV. Abnormalities of the der(12)t(12;21) in ETV6-RUNX1 acute lymphoblastic leukemia. Genes Chromosomes Cancer. 2013;52:202–13.

Anderson K, Lutz C, Van Delft FW, Bateman CM, Guo Y, Colman SM, Kempski H, Moorman AV, Titley I, Swansbury J, Kearney L, Enver T, Greaves M. Genetic variegation of clonal architecture and propagating cells in leukaemia. Nature. 2011;469:356–61.

Andersson A, Olofsson T, Lindgren D, Nilsson B, Ritz C, Eden P, Lassen C, Rade J, Fontes M, Morse H, Heldrup J, Behrendtz M, Mitelman F, Hoglund M, Johansson B, Fioretos T. Molecular signatures in childhood acute leukemia and their correlations to expression patterns in normal hematopoietic subpopulations. Proc Natl Acad Sci U S A. 2005;102:19069–74.

Andersson AK, Ma J, Wang J, Chen X, Gedman AL, Dang J, Nakitandwe J, Holmfeldt L, Parker M, Easton J, Huether R, Kriwacki R, Rusch M, Wu G, Li Y, Mulder H, Raimondi S, Pounds S, Kang G, Shi L, Becksfort J, Gupta P, Payne-Turner D, Vadodaria B, Boggs K, Yergeau D, Manne J, Song G, Edmonson M, Nagahawatte P, Wei L, Cheng C, Pei D, Sutton R, Venn NC, Chetcuti A, Rush A, Catchpoole D, Heldrup J, Fioretos T, Lu C, Ding L, Pui CH, Shurtleff S, Mullighan CG, Mardis ER, Wilson RK, Gruber TA, Zhang J, Downing JR, St. Jude Children's Research Hospital-Washington University Pediatric Cancer Genome Project. The landscape of somatic mutations in infant MLL-rearranged acute lymphoblastic leukemias. Nat Genet. 2015;47(4):330–7.

Andreasson P, Schwaller J, Anastasiadou E, Aster J, Gilliland DG. The expression of ETV6/CBFA2 (TEL/AML1) is not sufficient for the transformation of hematopoietic cell lines in vitro or the induction of hematologic disease in vivo. Cancer Genet Cytogenet. 2001;130:93–104.

Aplan PD, Jones CA, Chervinsky DS, Zhao X, Ellsworth M, Wu C, McGuire EA, Gross KW. An scl gene product lacking the transactivation domain induces bony abnormalities and cooperates with LMO1 to generate T-cell malignancies in transgenic mice. EMBO J. 1997;16:2408–19.

Argiropoulos B, Humphries RK. Hox genes in hematopoiesis and leukemogenesis. Oncogene. 2007;26:6766–76.

Arico M, Schrappe M, Hunger SP, Carroll WL, Conter V, Galimberti S, Manabe A, Saha V, Baruchel A, Vettenranta K, Horibe K, Benoit Y, Pieters R, Escherich G, Silverman LB, Pui CH, Valsecchi MG. Clinical outcome of children with newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia treated between 1995 and 2005. J Clin Oncol. 2010;28:4755–61.

Armstrong SA, Staunton JE, Silverman LB, Pieters R, den Boer ML, Minden MD, Sallan SE, Lander ES, Golub TR, Korsmeyer SJ. MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. Nat Genet. 2002;30:41–7.

Armstrong SA, Kung AL, Mabon ME, Silverman LB, Stam RW, den Boer ML, Pieters R, Kersey JH, Sallan SE, Fletcher JA, Golub TR, Griffin JD, Korsmeyer SJ. Inhibition of FLT3 in MLL. Validation of a therapeutic target identified by gene expression based classification. Cancer Cell. 2003;3:173–83.

Asnafi V, Radford-Weiss I, Dastugue N, Bayle C, Leboeuf D, Charrin C, Garand R, Lafage-Pochitaloff M, Delabesse E, Buzyn A, Troussard X, Macintyre E. CALM-AF10 is a common fusion transcript in T-ALL and is specific to the TCRgammadelta lineage. Blood. 2003;102:1000–6.

Atak ZK, Gianfelici V, Hulselmans G, de Keersmaecker K, Devasia AG, Geerdens E, Mentens N, Chiaretti S, Durinck K, Uyttebroeck A, Vandenberghe P, Wlodarska I, Cloos J, Foa R, Speleman F, Cools J, Aerts S. Comprehensive analysis of transcriptome variation uncovers known and novel driver events in T-cell acute lymphoblastic leukemia. PLoS Genet. 2013;9:e1003997.

Auer F, Ruschendorf F, Gombert M, Husemann P, Ginzel S, Izraeli S, Harit M, Weintraub M, Weinstein OY, Lerer I, Stepensky P, Borkhardt A, Hauer J. Inherited susceptibility to pre B-ALL caused by germline transmission of PAX5 c.547G>A. Leukemia. 2014;28:1136–8.

Bain G, Maandag EC, Izon DJ, Amsen D, Kruisbeek AM, Weintraub BC, Krop I, Schlissel MS, Feeney AJ, Van Roon M, et al. E2A proteins are required for proper B cell development and initiation of immunoglobulin gene rearrangements. Cell. 1994;79:885–92.

Baldus CD, Liyanarachchi S, Mrozek K, Auer H, Tanner SM, Guimond M, Ruppert AS, Mohamed N, Davuluri RV, Caligiuri MA, Bloomfield CD, de la Chapelle A. Acute myeloid leukemia with complex karyotypes and abnormal chromosome 21: amplification discloses overexpression of APP, ETS2, and ERG genes. Proc Natl Acad Sci U S A. 2004;101:3915–20.

Baldus CD, Burmeister T, Martus P, Schwartz S, Gokbuget N, Bloomfield CD, Hoelzer D, Thiel E, Hofmann WK. High expression of the ETS transcription factor ERG predicts adverse outcome in acute T-lymphoblastic leukemia in adults. J Clin Oncol. 2006;24:4714–20.

Balgobind BV, Van Vlierberghe P, van den Ouweland AM, Beverloo HB, Terlouw-Kromosoeto JN, Van Wering ER, Reinhardt D, Horstmann M, Kaspers GJ, Pieters R, Zwaan CM, Van Den Heuvel-Eibrink MM, Meijerink JP. Leukemia-associated NF1 inactivation in patients with pediatric T-ALL and AML lacking evidence for neurofibromatosis. Blood. 2008;111:4322–8.

Balgobind BV, Zwaan CM, Pieters R, Van Den Heuvel-Eibrink MM. The heterogeneity of pediatric MLL-rearranged acute myeloid leukemia. Leukemia. 2011;25:1239–48.

Ballerini P, Blaise A, Busson-Le Coniat M, Su XY, Zucman-Rossi J, Adam M, Van Den Akker J, Perot C, Pellegrino B, Landman-Parker J, Douay L, Berger R, Bernard OA. HOX11L2 expression defines a clinical subtype of pediatric T-ALL associated with poor prognosis. Blood. 2002;100:991–7.

Bandaranayake RM, Ungureanu D, Shan Y, Shaw DE, Silvennoinen O, Hubbard SR. Crystal structures of the JAK2 pseudokinase domain and the pathogenic mutant V617F. Nat Struct Mol Biol. 2012;19:754–9.

Barabe F, Kennedy JA, Hope KJ, Dick JE. Modeling the initiation and progression of human acute leukemia in mice. Science. 2007;316:600–4.

Bardini M, Spinelli R, Bungaro S, Mangano E, Corral L, Cifola I, Fazio G, Giordan M, Basso G, de Rossi G, Biondi A, Battaglia C, Cazzaniga G. DNA copy-number abnormalities do not occur in infant ALL with t(4;11)/MLL-AF4. Leukemia. 2010;24:169–76.

Bellanger D, Jacquemin V, Chopin M, Pierron G, Bernard OA, Ghysdael J, Stern MH. Recurrent JAK1 and JAK3 somatic mutations in T-cell prolymphocytic leukemia. Leukemia. 2014;28:417–9.

Benedikt A, Baltruschat S, Scholz B, Bursen A, Arrey TN, Meyer B, Varagnolo L, Muller AM, Karas M, Dingermann T, Marschalek R. The leukemogenic AF4-MLL fusion protein causes P-TEFb kinase activation and altered epigenetic signatures. Leukemia. 2011;25:135–44.

Ben-Neriah Y, Daley GQ, Mes-Masson AM, Witte ON, Baltimore D. The chronic myelogenous leukemia-specific P210 protein is the product of the bcr/abl hybrid gene. Science. 1986;233:212–4.

Berger R, Dastugue N, Busson M, Van Den Akker J, Perot C, Ballerini P, Hagemeijer A, Michaux L, Charrin C, Pages MP, Mugneret F, Andrieux J, Talmant P, Helias C, Mauvieux L, Lafage-Pochitaloff M, Mozziconacci MJ, Cornillet-Lefebvre P, Radford I, Asnafi V, Bilhou-Nabera C, Nguyen Khac F, Leonard C, Speleman F, Poppe B, Bastard C, Taviaux S, Quilichini B, Herens C, Gregoire MJ, Cave H, Bernard OA, Groupe Francais De Cytogenetique Hematologique (GFCH). t(5;14)/HOX11L2-positive T-cell acute lymphoblastic leukemia. A collaborative study of the Groupe Francais de Cytogenetique Hematologique (GFCH). Leukemia. 2003;17:1851–7.

Bernard OA, Romana SP, Schichman SA, Mauchauffe M, Jonveaux P, Berger R. Partial duplication of HRX in acute leukemia with trisomy 11. Leukemia. 1995;9:1487–90.

Bernard OA, Busson-Leconiat M, Ballerini P, Mauchauffe M, Della Valle V, Monni R, Nguyen Khac F, Mercher T, Penard-Lacronique V, Pasturaud P, Gressin L, Heilig R, Daniel MT, Lessard M, Berger R. A new recurrent and specific cryptic translocation, t(5;14)(q35;q32), is associated with expression of the Hox11L2 gene in T acute lymphoblastic leukemia. Leukemia. 2001;15:1495–504.

Bernardin F, Yang Y, Cleaves R, Zahurak M, Cheng L, Civin CI, Friedman AD. TEL-AML1, expressed from t(12;21) in human acute lymphocytic leukemia, induces acute leukemia in mice. Cancer Res. 2002;62:3904–8.

Bersenev A, Wu C, Balcerek J, Jing J, Kundu M, Blobel GA, Chikwava KR, Tong W. Lnk constrains myeloproliferative diseases in mice. J Clin Invest. 2010;120:2058–69.

Bienemann K, Burkhardt B, Modlich S, Meyer U, Moricke A, Bienemann K, Mauz-Korholz C, Escherich G, Zimmermann M, Korholz D, Janka-Schaub G, Schrappe M, Reiter A, Borkhardt A. Promising therapy results for lymphoid malignancies in children with chromosomal breakage syndromes (Ataxia teleangiectasia or Nijmegen-breakage syndrome): a retrospective survey. Br J Haematol. 2011;155:468–76.

Blau O, Avigad S, Stark B, Kodman Y, Luria D, Cohen IJ, Zaizov R. Exon 5 mutations in the p53 gene in relapsed childhood acute lymphoblastic leukemia. Leuk Res. 1997;21:721–9.

Boehm T, Foroni L, Kaneko Y, Perutz MF, Rabbitts TH. The rhombotin family of cysteine-rich LIM-domain oncogenes: distinct members are involved in T-cell translocations to human chromosomes 11p15 and 11p13. Proc Natl Acad Sci U S A. 1991;88:4367–71.

Bohlander SK. ETV6: a versatile player in leukemogenesis. Semin Cancer Biol. 2005;15:162–74.

Bohlander SK, Muschinsky V, Schrader K, Siebert R, Schlegelberger B, Harder L, Schemmel V, Fonatsch C, Ludwig WD, Hiddemann W, Dreyling MH. Molecular analysis of the CALM/AF10 fusion: identical rearrangements in acute myeloid leukemia, acute lymphoblastic leukemia and malignant lymphoma patients. Leukemia. 2000;14:93–9.

Brown L, Cheng JT, Chen Q, Siciliano MJ, Crist W, Buchanan G, Baer R. Site-specific recombination of the tal-1 gene is a common occurrence in human T cell leukemia. EMBO J. 1990;9:3343–51.

Burmeister T, Gokbuget N, Schwartz S, Fischer L, Hubert D, Sindram A, Hoelzer D, Thiel E. Clinical features and prognostic implications of TCF3-PBX1 and ETV6-RUNX1 in adult acute lymphoblastic leukemia. Haematologica. 2010;95:241–6.

Caligiuri MA, Schichman SA, Strout MP, Mrozek K, Baer MR, Frankel SR, Barcos M, Herzig GP, Croce CM, Bloomfield CD. Molecular rearrangement of the ALL-1 gene in acute myeloid leukemia without cytogenetic evidence of 11q23 chromosomal translocations. Cancer Res. 1994;54:370–3.

Case M, Matheson E, Minto L, Hassan R, Harrison CJ, Bown N, Bailey S, Vormoor J, Hall AG, Irving JA. Mutation of genes affecting the RAS pathway is common in childhood acute lymphoblastic leukemia. Cancer Res. 2008;68:6803–9.

Castellano E, Downward J. RAS interaction with PI3K: more than just another effector pathway. Genes Cancer. 2011;2:261–74.

Cauwelier B, Dastugue N, Cools J, Poppe B, Herens C, de Paepe A, Hagemeijer A, Speleman F. Molecular cytogenetic study of 126 unselected T-ALL cases reveals high incidence of TCRbeta locus rearrangements and putative new T-cell oncogenes. Leukemia. 2006;20:1238–44.

Cave H, Suciu S, Preudhomme C, Poppe B, Robert A, Uyttebroeck A, Malet M, Boutard P, Benoit Y, Mauvieux L, Lutz P, Mechinaud F, Grardel N, Mazingue F, Dupont M, Margueritte G, Pages MP, Bertrand Y, Plouvier E, Brunie G, Bastard C, Plantaz D, Vande Velde I, Hagemeijer A, Speleman F, Lessard M, Otten J, Vilmer E, Dastugue N, Eortc CLG. Clinical significance of HOX11L2 expression linked to t(5;14)(q35;q32), of HOX11 expression, and of SIL-TAL fusion in childhood T-cell malignancies: results of EORTC studies 58881 and 58951. Blood. 2004;103:442–50.

Chandra P, Luthra R, Zuo Z, Yao H, Ravandi F, Reddy N, Garcia-Manero G, Kantarjian H, Jones D. Acute myeloid leukemia with t(9;11)(p21-22;q23): common properties of dysregulated ras pathway signaling and genomic progression characterize de novo and therapy-related cases. Am J Clin Pathol. 2010;133:686–93.

Chapiro E, Russell L, Lainey E, Kaltenbach S, Ragu C, Della-Valle V, Hanssens K, Macintyre EA, Radford-Weiss I, Delabesse E, Cave H, Mercher T, Harrison CJ, Nguyen-Khac F, Dubreuil P, Bernard OA. Activating mutation in the TSLPR gene in B-cell precursor lymphoblastic leukemia. Leukemia. 2010;24:642–5.

Chen Q, Cheng JT, Tasi LH, Schneider N, Buchanan G, Carroll A, Crist W, Ozanne B, Siciliano MJ, Baer R. The tal gene undergoes chromosome translocation in T cell leukemia and potentially encodes a helix-loop-helix protein. EMBO J. 1990;9:415–24.

Chervinsky DS, Zhao XF, Lam DH, Ellsworth M, Gross KW, Aplan PD. Disordered T-cell development and T-cell malignancies in SCL LMO1 double-transgenic mice: parallels with E2A-deficient mice. Mol Cell Biol. 1999;19:5025–35.

Churchman ML, Low J, Qu C, et al. Efficacy of Retinoids in IKZF1-Mutated BCR-ABL1 Acute Lymphoblastic Leukemia. Cancer cell 2015;28:343–56.

Clappier E, Cuccuini W, Kalota A, Crinquette A, Cayuela JM, Dik WA, Langerak AW, Montpellier B, Nadel B, Walrafen P, Delattre O, Aurias A, Leblanc T, Dombret H, Gewirtz AM, Baruchel A, Sigaux F, Soulier J. The C-MYB locus is involved in chromosomal translocation and genomic duplications in human T-cell acute leukemia (T-ALL), the translocation defining a new T-ALL subtype in very young children. Blood. 2007;110:1251–61.

Clappier E, Auclerc MF, Rapion J, Bakkus M, Caye A, Khemiri A, Giroux C, Hernandez L, Kabongo E, Savola S, Leblanc T, Yakouben K, Plat G, Costa V, Ferster A, Girard S, Fenneteau O, Cayuela JM, Sigaux F, Dastugue N, Suciu S, Benoit Y, Bertrand Y, Soulier J, Cave H. An intragenic ERG deletion is a marker of an oncogenic subtype of B-cell precursor acute lymphoblastic leukemia with a favorable outcome despite frequent IKZF1 deletions. Leukemia. 2014;28:70–7.

Cleary ML, Mellentin JD, Spies J, Smith SD. Chromosomal translocation involving the beta T cell receptor gene in acute leukemia. J Exp Med. 1988;167:682–7.

Cohen B, Shimizu M, Izrailit J, Ng NF, Buchman Y, Pan JG, Dering J, Reedijk M. Cyclin D1 is a direct target of JAG1-mediated Notch signaling in breast cancer. Breast Cancer Res Treat. 2010;123:113–24.

Cooper JM, Bodemann BO, White MA. The RalGEF/Ral pathway: evaluating an intervention opportunity for Ras cancers. Enzymes. 2013;34(Pt. B):137–56.

Corral J, Lavenir I, Impey H, Warren AJ, Forster A, Larson TA, Bell S, McKenzie AN, King G, Rabbitts TH. An Mll-AF9 fusion gene made by homologous recombination causes acute leukemia in chimeric mice: a method to create fusion oncogenes. Cell. 1996;85:853–61.

Coustan-Smith E, Mullighan CG, Onciu M, Behm FG, Raimondi SC, Pei D, Cheng C, Su X, Rubnitz JE, Basso G, Biondi A, Pui CH, Downing JR, Campana D. Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. Lancet Oncol. 2009;10:147–56.

Crasta K, Ganem NJ, Dagher R, Lantermann AB, Ivanova EV, Pan Y, Nezi L, Protopopov A, Chowdhury D, Pellman D. DNA breaks and chromosome pulverization from errors in mitosis. Nature. 2012;482:53–8.

Crist W, Carroll A, Shuster J, Jackson J, Head D, Borowitz M, Behm F, Link M, Steuber P, Ragab A, et al. Philadelphia chromosome positive childhood acute lymphoblastic leukemia: clinical and cytogenetic characteristics and treatment outcome. A Pediatric Oncology Group study. Blood. 1990;76:489–94.

Daley GQ, Van Etten RA, Baltimore D. Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. Science. 1990;247:824–30.

De Keersmaecker K, Real PJ, Gatta GD, Palomero T, Sulis ML, Tosello V, Van Vlierberghe P, Barnes K, Castillo M, Sole X, Hadler M, Lenz J, Aplan PD, Kelliher M, Kee BL, Pandolfi PP, Kappes D, Gounari F, Petrie H, Van Der Meulen J, Speleman F, Paietta E, Racevskis J, Wiernik PH, Rowe JM, Soulier J, Avran D, Cave H, Dastugue N, Raimondi S, Meijerink JP, Cordon-Cardo C, Califano A, Ferrando AA. The TLX1 oncogene drives aneuploidy in T cell transformation. Nat Med. 2010;16:1321–7.

De Keersmaecker K, Atak ZK, Li N, Vicente C, Patchett S, Girardi T, Gianfelici V, Geerdens E, Clappier E, Porcu M, Lahortiga I, Luca R, Yan J, Hulselmans G, Vranckx H, Vandepoel R, Sweron B, Jacobs K, Mentens N, Wlodarska I, Cauwelier B, Cloos J, Soulier J, Uyttebroeck A, Bagni C, Hassan BA, Vandenberghe P, Johnson AW, Aerts S, Cools J. Exome sequencing identifies mutation in CNOT3 and ribosomal genes RPL5 and RPL10 in T-cell acute lymphoblastic leukemia. Nat Genet. 2013;45:186–90.

Delattre O, Zucman J, Melot T, Garau XS, Zucker JM, Lenoir GM, Ambros PF, Sheer D, Turc-Carel C, Triche TJ, et al. The Ewing family of tumors – a subgroup of small-round-cell tumors defined by specific chimeric transcripts. N Engl J Med. 1994;331:294–9.

Della Gatta G, Palomero T, Perez-Garcia A, Ambesi-Impiombato A, Bansal M, Carpenter ZW, De Keersmaecker K, Sole X, Xu L, Paietta E, Racevskis J, Wiernik PH, Rowe JM, Meijerink JP, Califano A, Ferrando AA. Reverse engineering of TLX oncogenic transcriptional networks identifies RUNX1 as tumor suppressor in T-ALL. Nat Med. 2012;18:436–40.

Den Boer ML, Van Slegtenhorst M, de Menezes RX, Cheok MH, Buijs-Gladdines JG, Peters ST, Van Zutven LJ, Beverloo HB, Van Der Spek PJ, Escherich G, Horstmann MA, Janka-Schaub GE, Kamps WA, Evans WE, Pieters R. A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. Lancet Oncol. 2009;10:125–34.

Diccianni MB, Yu J, Hsiao M, Mukherjee S, Shao LE, Yu AL. Clinical significance of p53 mutations in relapsed T-cell acute lymphoblastic leukemia. Blood. 1994;84:3105–12.

Dimartino JF, Selleri L, Traver D, Firpo MT, Rhee J, Warnke R, O'Gorman S, Weissman IL, Cleary ML. The Hox cofactor and proto-oncogene Pbx1 is required for maintenance of definitive hematopoiesis in the fetal liver. Blood. 2001;98:618–26.

Dobbins SE, Sherborne AL, Ma YP, Bardini M, Biondi A, Cazzaniga G, Lloyd A, Chubb D, Greaves MF, Houlston RS. The silent mutational landscape of infant MLL-AF4 pro-B acute lymphoblastic leukemia. Genes Chromosomes Cancer. 2013;52:954–60.

Domer PH, Head DR, Renganathan N, Raimondi SC, Yang E, Atlas M. Molecular analysis of 13 cases of MLL/11q23 secondary acute leukemia and identification of topoisomerase II consensus-binding sequences near the chromosomal breakpoint of a secondary leukemia with the t(4;11). Leukemia. 1995;9:1305–12.

Dreyling MH, Martinez-Climent JA, Zheng M, Mao J, Rowley JD, Bohlander SK. The t(10;11)(p13;q14) in the U937 cell line results in the fusion of the AF10 gene and CALM, encoding a new member of the AP-3 clathrin assembly protein family. Proc Natl Acad Sci U S A. 1996;93:4804–9.

Dreyling MH, Schrader K, Fonatsch C, Schlegelberger B, Haase D, Schoch C, Ludwig W, Loffler H, Buchner T, Wormann B, Hiddemann W, Bohlander SK. MLL and CALM are fused to

AF10 in morphologically distinct subsets of acute leukemia with translocation t(10;11): both rearrangements are associated with a poor prognosis. Blood. 1998;91:4662–7.
Dube ID, Kamel-Reid S, Yuan CC, Lu M, Wu X, Corpus G, Raimondi SC, Crist WM, Carroll AJ, Minowada J, et al. A novel human homeobox gene lies at the chromosome 10 breakpoint in lymphoid neoplasias with chromosomal translocation t(10;14). Blood. 1991;78:2996–3003.
Edmaier KE, Stahnke K, Vegi N, Mulaw M, Ihme S, Scheffold A, Rudolph KL, Buske C. Expression of the lymphoid enhancer factor 1 is required for normal hematopoietic stem and progenitor cell function. Leukemia. 2014;28:227–30.
Erdfelder F, Hertweck M, Filipovich A, Uhrmacher S, Kreuzer KA. High lymphoid enhancer-binding factor-1 expression is associated with disease progression and poor prognosis in chronic lymphocytic leukemia. Hematol Rep. 2010;2:e3.
Faber J, Krivtsov AV, Stubbs MC, Wright R, Davis TN, Van Den Heuvel-Eibrink M, Zwaan CM, Kung AL, Armstrong SA. HOXA9 is required for survival in human MLL-rearranged acute leukemias. Blood. 2009;113:2375–85.
Faham M, Zheng J, Moorhead M, Carlton VE, Stow P, Coustan-Smith E, Pui CH, Campana D. Deep-sequencing approach for minimal residual disease detection in acute lymphoblastic leukemia. Blood. 2012;120:5173–80.
Felix CA, Hosler MR, Winick NJ, Masterson M, Wilson AE, Lange BJ. ALL-1 gene rearrangements in DNA topoisomerase II inhibitor-related leukemia in children. Blood. 1995;85:3250–6.
Fenrick R, Amann JM, Lutterbach B, Wang LL, Westendorf JJ, Downing JR, Hiebert SW. Both TEL and AML-1 contribute repression domains to the t(12;21) fusion protein. Mol Cell Biol. 1999;19:6566–74.
Ferrando AA. The role of NOTCH1 signaling in T-ALL. Hematol Am Soc Hematol Educ Program. 2009:353–61.
Ferrando AA, Neuberg DS, Staunton J, Loh ML, Huard C, Raimondi SC, Behm FG, Pui CH, Downing JR, Gilliland DG, Lander ES, Golub TR, Look AT. Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia. Cancer Cell. 2002;1:75–87.
Ferrando AA, Armstrong SA, Neuberg DS, Sallan SE, Silverman LB, Korsmeyer SJ, Look AT. Gene expression signatures in MLL-rearranged T-lineage and B-precursor acute leukemias: dominance of HOX dysregulation. Blood. 2003;102:262–8.
Figueroa ME, Chen SC, Andersson AK, Phillips LA, Li Y, Sotzen J, Kundu M, Downing JR, Melnick A, Mullighan CG. Integrated genetic and epigenetic analysis of childhood acute lymphoblastic leukemia. J Clin Invest. 2013;123:3099–111.
Finger LR, Harvey RC, Moore RC, Showe LC, Croce CM. A common mechanism of chromosomal translocation in T- and B-cell neoplasia. Science. 1986;234:982–5.
Flotho C, Kratz CP, Niemeyer CM. How a rare pediatric neoplasia can give important insights into biological concepts: a perspective on juvenile myelomonocytic leukemia. Haematologica. 2007;92:1441–6.
Ford AM, Ridge SA, Cabrera ME, Mahmoud H, Steel CM, Chan LC, Greaves M. In utero rearrangements in the trithorax-related oncogene in infant leukaemias. Nature. 1993;363:358–60.
Ford AM, Bennett CA, Price CM, Bruin MC, Van Wering ER, Greaves M. Fetal origins of the TEL-AML1 fusion gene in identical twins with leukemia. Proc Natl Acad Sci U S A. 1998;95:4584–8.
Forestier E, Johansson B, Borgstrom G, Kerndrup G, Johansson J, Heim S. Cytogenetic findings in a population-based series of 787 childhood acute lymphoblastic leukemias from the Nordic countries. The NOPHO Leukemia Cytogenetic Study Group. Eur J Haematol. 2000a;64:194–200.
Forestier E, Johansson B, Gustafsson G, Borgstrom G, Kerndrup G, Johannsson J, Heim S. Prognostic impact of karyotypic findings in childhood acute lymphoblastic leukaemia: a Nordic series comparing two treatment periods. For the Nordic Society of Paediatric Haematology and Oncology (NOPHO) Leukaemia Cytogenetic Study Group. Br J Haematol. 2000b;110:147–53.
Forestier E, Heim S, Blennow E, Borgstrom G, Holmgren G, Heinonen K, Johannsson J, Kerndrup G, Andersen MK, Lundin C, Nordgren A, Rosenquist R, Swolin B, Johansson B, Nordic Society Of Paediatric Hematology and Oncology, Swedish Cytogenetic Leukaemia Study

Group, NOPHO Leukaemia Cytogenetic Study Group. Cytogenetic abnormalities in childhood acute myeloid leukaemia: a Nordic series comprising all children enrolled in the NOPHO-93-AML trial between 1993 and 2001. Br J Haematol. 2003;121:566–77.

Forster A, Pannell R, Drynan LF, McCormack M, Collins EC, Daser A, Rabbitts TH. Engineering de novo reciprocal chromosomal translocations associated with Mll to replicate primary events of human cancer. Cancer Cell. 2003;3:449–58.

Gaussmann A, Wenger T, Eberle I, Bursen A, Bracharz S, Herr I, Dingermann T, Marschalek R. Combined effects of the two reciprocal t(4;11) fusion proteins MLL.AF4 and AF4.MLL confer resistance to apoptosis, cell cycling capacity and growth transformation. Oncogene. 2007;26:3352–63.

Gerby B, Tremblay CS, Tremblay M, Rojas-Sutterlin S, Herblot S, Hebert J, Sauvageau G, Lemieux S, Lecuyer E, Veiga DF, Hoang T. SCL, LMO1 and Notch1 reprogram thymocytes into self-renewing cells. PLoS Genet. 2014;10:e1004768.

Golub T, Mclean T, Stegmaier K, Ritz J, Sallan S, Neuberg D, Gilliland DG. TEL-AML1: the most common gene rearrangement in childhood ALL. Blood. 1995;86:2377.

Gonzalez KD, Buzin CH, Noltner KA, Gu D, Li W, Malkin D, Sommer SS. High frequency of de novo mutations in Li-Fraumeni syndrome. J Med Genet. 2009;46:689–93.

Graux C, Cools J, Melotte C, Quentmeier H, Ferrando A, Levine R, Vermeesch JR, Stul M, Dutta B, Boeckx N, Bosly A, Heimann P, Uyttebroeck A, Mentens N, Somers R, Macleod RA, Drexler HG, Look AT, Gilliland DG, Michaux L, Vandenberghe P, Wlodarska I, Marynen P, Hagemeijer A. Fusion of NUP214 to ABL1 on amplified episomes in T-cell acute lymphoblastic leukemia. Nat Genet. 2004;36:1084–9.

Greaves M. In utero origins of childhood leukaemia. Early Hum Dev. 2005;81:123–9.

Greaves MF, Maia AT, Wiemels JL, Ford AM. Leukemia in twins: lessons in natural history. Blood. 2003;102:2321–33.

Groschel S, Sanders MA, Hoogenboezem R, de Wit E, Bouwman BA, Erpelinck C, Van Der Velden VH, Havermans M, Avellino R, Van Lom K, Rombouts EJ, Van Duin M, Dohner K, Beverloo HB, Bradner JE, Dohner H, Lowenberg B, Valk PJ, Bindels EM, de Laat W, Delwel R. A single oncogenic enhancer rearrangement causes concomitant EVI1 and GATA2 deregulation in leukemia. Cell. 2014;157:369–81.

Grosveld G, Verwoerd T, Van Agthoven T, de Klein A, Ramachandran KL, Heisterkamp N, Stam K, Groffen J. The chronic myelocytic cell line K562 contains a breakpoint in bcr and produces a chimeric bcr/c-abl transcript. Mol Cell Biol. 1986;6:607–16.

Guidez F, Petrie K, Ford AM, Lu H, Bennett CA, Macgregor A, Hannemann J, Ito Y, Ghysdael J, Greaves M, Wiedemann LM, Zelent A. Recruitment of the nuclear receptor corepressor N-CoR by the TEL moiety of the childhood leukemia-associated TEL-AML1 oncoprotein. Blood. 2000;96:2557–61.

Gump J, Mcgavran L, Wei Q, Hunger SP. Analysis of TP53 mutations in relapsed childhood acute lymphoblastic leukemia. J Pediatr Hematol Oncol. 2001;23:416–9.

Guo D, Ye J, Dai J, Li L, Chen F, Ma D, Ji C. Notch-1 regulates Akt signaling pathway and the expression of cell cycle regulatory proteins cyclin D1, CDK2 and p21 in T-ALL cell lines. Leuk Res. 2009;33:678–85.

Gutierrez A, Sanda T, Grebliunaite R, Carracedo A, Salmena L, Ahn Y, Dahlberg S, Neuberg D, Moreau LA, Winter SS, Larson R, Zhang J, Protopopov A, Chin L, Pandolfi PP, Silverman LB, Hunger SP, Sallan SE, Look AT. High frequency of PTEN, PI3K, and AKT abnormalities in T-cell acute lymphoblastic leukemia. Blood. 2009;114:647–50.

Gutierrez A, Sanda T, Ma W, Zhang J, Grebliunaite R, Dahlberg S, Neuberg D, Protopopov A, Winter SS, Larson RS, Borowitz MJ, Silverman LB, Chin L, Hunger SP, Jamieson C, Sallan SE, Look AT. Inactivation of LEF1 in T-cell acute lymphoblastic leukemia. Blood. 2010;115:2845–51.

Gutierrez A, Kentsis A, Sanda T, Holmfeldt L, Chen SC, Zhang J, Protopopov A, Chin L, Dahlberg SE, Neuberg DS, Silverman LB, Winter SS, Hunger SP, Sallan SE, Zha S, Alt FW, Downing JR, Mullighan CG, Look AT. The BCL11B tumor suppressor is mutated across the major molecular subtypes of T-cell acute lymphoblastic leukemia. Blood. 2011;118:4169–73.

Haltrich I, Csoka M, Kovacs G, Torok D, Alpar D, Ottoffy G, Fekete G. Six cases of rare gene amplifications and multiple copy of fusion gene in childhood acute lymphoblastic leukemia. Pathol Oncol Res. 2013;19:123–8.

Harewood L, Robinson H, Harris R, Al-Obaidi MJ, Jalali GR, Martineau M, Moorman AV, Sumption N, Richards S, Mitchell C, Harrison CJ. Amplification of AML1 on a duplicated chromosome 21 in acute lymphoblastic leukemia: a study of 20 cases. Leukemia. 2003;17:547–53.

Harrison CJ. The detection and significance of chromosomal abnormalities in childhood acute lymphoblastic leukaemia. Blood Rev. 2001;15:49–59.

Harrison CJ, Moorman AV, Broadfield ZJ, Cheung KL, Harris RL, Reza Jalali G, Robinson HM, Barber KE, Richards SM, Mitchell CD, Eden TO, Hann IM, Hill FG, Kinsey SE, Gibson BE, Lilleyman J, Vora A, Goldstone AH, Franklin IM, Durrant J, Martineau M, Childhood & Adult Leukaemia Working Parties. Three distinct subgroups of hypodiploidy in acute lymphoblastic leukaemia. Br J Haematol. 2004;125:552–9.

Harrison CJ, Hills RK, Moorman AV, Grimwade DJ, Hann I, Webb DK, Wheatley K, de Graaf SS, Van Den Berg E, Burnett AK, Gibson BE. Cytogenetics of childhood acute myeloid leukemia: United Kingdom Medical Research Council Treatment trials AML 10 and 12. J Clin Oncol. 2010;28:2674–81.

Harrison CJ, Moorman AV, Schwab C, Carroll AJ, Raetz EA, Devidas M, Strehl S, Nebral K, Harbott J, Teigler-Schlegel A, Zimmerman M, Dastuge N, Baruchel A, Soulier J, Auclerc MF, Attarbaschi A, Mann G, Stark B, Cazzaniga G, Chilton L, Vandenberghe P, Forestier E, Haltrich I, Raimondi SC, Parihar M, Bourquin JP, Tchinda J, Haferlach C, Vora A, Hunger SP, Heerema NA, Haas OA, Ponte Di Legno International Workshop In Childhood Acute Lymphoblastic, Leukemia. An international study of intrachromosomal amplification of chromosome 21 (iAMP21): cytogenetic characterization and outcome. Leukemia. 2014;28:1015–21.

Harvey RC, Mullighan CG, Chen IM, Wharton W, Mikhail FM, Carroll AJ, Kang H, Liu W, Dobbin KK, Smith MA, Carroll WL, Devidas M, Bowman WP, Camitta BM, Reaman GH, Hunger SP, Downing JR, Willman CL. Rearrangement of CRLF2 is associated with mutation of JAK kinases, alteration of IKZF1, Hispanic/Latino ethnicity, and a poor outcome in pediatric B-progenitor acute lymphoblastic leukemia. Blood. 2010a;115:5312–21.

Harvey RC, Mullighan CG, Wang X, Dobbin KK, Davidson GS, Bedrick EJ, Chen IM, Atlas SR, Kang H, Ar K, Wilson CS, Wharton W, Murphy M, Devidas M, Carroll AJ, Borowitz MJ, Bowman WP, Downing JR, Relling M, Yang J, Bhojwani D, Carroll WL, Camitta B, Reaman GH, Smith M, Hunger SP, Willman CL. Identification of novel cluster groups in pediatric high-risk B-precursor acute lymphoblastic leukemia with gene expression profiling: correlation with genome-wide DNA copy number alterations, clinical characteristics, and outcome. Blood. 2010b;116:4874–84.

Hasle H, Clemmensen IH, Mikkelsen M. Risks of leukaemia and solid tumours in individuals with Down's syndrome. Lancet. 2000;355:165–9.

Hatano M, Roberts CW, Minden M, Crist WM, Korsmeyer SJ. Deregulation of a homeobox gene, HOX11, by the t(10;14) in T cell leukemia. Science. 1991;253:79–82.

Hatch EM, Fischer AH, Deerinck TJ, Hetzer MW. Catastrophic nuclear envelope collapse in cancer cell micronuclei. Cell. 2013;154:47–60.

Hawley RG, Fong AZ, Reis MD, Zhang N, Lu M, Hawley TS. Transforming function of the HOX11/TCL3 homeobox gene. Cancer Res. 1997;57:337–45.

Hawley RG, Hawley TS, Cantor AB. TLX1 (HOX11) immortalization of embryonic stem cell-derived and primary murine hematopoietic progenitors. Curr Protoc Stem Cell Biol. 2008;Chapter 1:Unit 1F 7.

Hayette S, Tigaud I, Maguer-Satta V, Bartholin L, Thomas X, Charrin C, Gadoux M, Magaud JP, Rimokh R. Recurrent involvement of the MLL gene in adult T-lineage acute lymphoblastic leukemia. Blood. 2002;99:4647–9.

Heerema NA, Nachman JB, Sather HN, Sensel MG, Lee MK, Hutchinson R, Lange BJ, Steinherz PG, Bostrom B, Gaynon PS, Uckun F. Hypodiploidy with less than 45 chromosomes confers adverse risk in childhood acute lymphoblastic leukemia: a report from the Children's Cancer Group. Blood. 1999;94:4036–45.

Heerema NA, Harbott J, Galimberti S, Camitta BM, Gaynon PS, Janka-Schaub G, Kamps W, Basso G, Pui CH, Schrappe M, Auclerc MF, Carroll AJ, Conter V, Harrison CJ, Pullen J, Raimondi SC, Richards S, Riehm H, Sather HN, Shuster JJ, Silverman LB, Valsecchi MG, Arico M, Acute Lymphoblastic Leukemia Study Groups, ALL, B. F. M, COALL, AIEOP, DCLSG, FRALLE, CCG, DFCI, POG, ST, J., UKALL. Secondary cytogenetic aberrations in childhood Philadelphia chromosome positive acute lymphoblastic leukemia are nonrandom and may be associated with outcome. Leukemia. 2004;18:693–702.

Heisterkamp N, Stam K, Groffen J, de Klein A, Grosveld G. Structural organization of the bcr gene and its role in the Ph' translocation. Nature. 1985;315:758–61.

Group Francais de Cytogenetique Hematologique. Cytogenetic abnormalities in adult acute lymphoblastic leukemia: correlations with hematologic findings outcome. A Collaborative Study of the Group Francais de Cytogenetique Hematologique. Blood. 1996;87:3135–42.

Herranz D, Ambesi-Impiombato A, Palomero T, Schnell SA, Belver L, Wendorff AA, Xu L, Castillo-Martin M, Llobet-Navas D, Cordon-Cardo C, Clappier E, Soulier J, Ferrando AA. A NOTCH1-driven MYC enhancer promotes T cell development, transformation and acute lymphoblastic leukemia. Nat Med. 2014;20:1130–7.

Hof J, Krentz S, Van Schewick C, Korner G, Shalapour S, Rhein P, Karawajew L, Ludwig WD, Seeger K, Henze G, Von Stackelberg A, Hagemeier C, Eckert C, Kirschner-Schwabe R. Mutations and deletions of the TP53 gene predict nonresponse to treatment and poor outcome in first relapse of childhood acute lymphoblastic leukemia. J Clin Oncol. 2011;29:3185–93.

Holmfeldt L, Wei L, Diaz-Flores E, Walsh M, Zhang J, Ding L, Payne-Turner D, Churchman M, Andersson A, Chen SC, Mccastlain K, Becksfort J, Ma J, Wu G, Patel SN, Heatley SL, Phillips LA, Song G, Easton J, Parker M, Chen X, Rusch M, Boggs K, Vadodaria B, Hedlund E, Drenberg C, Baker S, Pei D, Cheng C, Huether R, Lu C, Fulton RS, Fulton LL, Tabib Y, Dooling DJ, Ochoa K, Minden M, Lewis ID, To LB, Marlton P, Roberts AW, Raca G, Stock W, Neale G, Drexler HG, Dickins RA, Ellison DW, Shurtleff SA, Pui CH, Ribeiro RC, Devidas M, Carroll AJ, Heerema NA, Wood B, Borowitz MJ, Gastier-Foster JM, Raimondi SC, Mardis ER, Wilson RK, Downing JR, Hunger SP, Loh ML, Mullighan CG. The genomic landscape of hypodiploid acute lymphoblastic leukemia. Nat Genet. 2013;45:242–52.

Homminga I, Pieters R, Langerak AW, de Rooi JJ, Stubbs A, Verstegen M, Vuerhard M, Buijs-Gladdines J, Kooi C, Klous P, Van Vlierberghe P, Ferrando AA, Cayuela JM, Verhaaf B, Beverloo HB, Horstmann M, de Haas V, Wiekmeijer AS, Pike-Overzet K, Staal FJ, de Laat W, Soulier J, Sigaux F, Meijerink JP. Integrated transcript and genome analyses reveal NKX2-1 and MEF2C as potential oncogenes in T cell acute lymphoblastic leukemia. Cancer Cell. 2011;19:484–97.

Hozumi K, Negishi N, Tsuchiya I, Abe N, Hirano K, Suzuki D, Yamamoto M, Engel JD, Habu S. Notch signaling is necessary for GATA3 function in the initiation of T cell development. Eur J Immunol. 2008;38:977–85.

Hsiao MH, Yu AL, Yeargin J, Ku D, Haas M. Nonhereditary p53 mutations in T-cell acute lymphoblastic leukemia are associated with the relapse phase. Blood. 1994;83:2922–30.

Hunger SP, Lu X, Devidas M, Camitta BM, Gaynon PS, Winick NJ, Reaman GH, Carroll WL. Improved survival for children and adolescents with acute lymphoblastic leukemia between 1990 and 2005: a report from the Children's Oncology Group. J Clin Oncol. 2012;30:1663–9.

Iacobucci I, Storlazzi CT, Cilloni D, Lonetti A, Ottaviani E, Soverini S, Astolfi A, Chiaretti S, Vitale A, Messa F, Impera L, Baldazzi C, D'Addabbo P, Papayannidis C, Lonoce A, Colarossi S, Vignetti M, Piccaluga PP, Paolini S, Russo D, Pane F, Saglio G, Baccarani M, Foa R, Martinelli G. Identification and molecular characterization of recurrent genomic deletions on 7p12 in the IKZF1 gene in a large cohort of BCR-ABL1-positive acute lymphoblastic leukemia patients: on behalf of Gruppo Italiano Malattie Ematologiche dell'Adulto Acute Leukemia Working Party (GIMEMA AL WP). Blood. 2009;114:2159–67.

Ichikawa H, Shimizu K, Hayashi Y, Ohki M. An RNA-binding protein gene, TLS/FUS, is fused to ERG in human myeloid leukemia with t(16;21) chromosomal translocation. Cancer Res. 1994;54:2865–8.

Inthal A, Zeitlhofer P, Zeginigg M, Morak M, Grausenburger R, Fronkova E, Fahrner B, Mann G, Haas OA, Panzer-Grumayer R. CREBBP HAT domain mutations prevail in relapse cases of high hyperdiploid childhood acute lymphoblastic leukemia. Leukemia. 2012;26:1797–803.

Iwamoto M, Higuchi Y, Enomoto-Iwamoto M, Kurisu K, Koyama E, Yeh H, Rosenbloom J, Pacifici M. The role of ERG (ets related gene) in cartilage development. Osteoarthritis Cartilage. 2001;9(Suppl A):S41–7.

Jaffe JD, Wang Y, Chan HM, Zhang J, Huether R, Kryukov GV, Bhang HE, Taylor JE, Hu M, Englund NP, Yan F, Wang Z, Robert McDonald 3rd E, Wei L, Ma J, Easton J, Yu Z, Debeaumount R, Gibaja V, Venkatesan K, Schlegel R, Sellers WR, Keen N, Liu J, Caponigro G, Barretina J, Cooke VG, Mullighan C, Carr SA, Downing JR, Garraway LA, Stegmeier F. Global chromatin profiling reveals NSD2 mutations in pediatric acute lymphoblastic leukemia. Nat Genet. 2013;45:1386–91.

Jongmans MC, Van Der Burgt I, Hoogerbrugge PM, Noordam K, Yntema HG, Nillesen WM, Kuiper RP, Ligtenberg MJ, Van Kessel AG, Van Krieken JH, Kiemeney LA, Hoogerbrugge N. Cancer risk in patients with Noonan syndrome carrying a PTPN11 mutation. Eur J Hum Genet. 2011;19:870–4.

Jonsson OG, Kitchens RL, Baer RJ, Buchanan GR, Smith RG. Rearrangements of the tal-1 locus as clonal markers for T cell acute lymphoblastic leukemia. J Clin Invest. 1991;87:2029–35.

Kawamata N, Ogawa S, Seeger K, Kirschner-Schwabe R, Huynh T, Chen J, Megrabian N, Harbott J, Zimmermann M, Henze G, Schrappe M, Bartram CR, Koeffler HP. Molecular allelokaryotyping of relapsed pediatric acute lymphoblastic leukemia. Int J Oncol. 2009;34:1603–12.

Keller G, Wall C, Fong AZ, Hawley TS, Hawley RG. Overexpression of HOX11 leads to the immortalization of embryonic precursors with both primitive and definitive hematopoietic potential. Blood. 1998;92:877–87.

Kennedy MA, Gonzalez-Sarmiento R, Kees UR, Lampert F, Dear N, Boehm T, Rabbitts TH. HOX11, a homeobox-containing T-cell oncogene on human chromosome 10q24. Proc Natl Acad Sci U S A. 1991;88:8900–4.

Kharazmi E, Da Silva Filho MI, Pukkala E, Sundquist K, Thomsen H, Hemminki K. Familial risks for childhood acute lymphocytic leukaemia in Sweden and Finland: far exceeding the effects of known germline variants. Br J Haematol. 2012;159:585–8.

Kino T, Nordeen SK, Chrousos GP. Conditional modulation of glucocorticoid receptor activities by CREB-binding protein (CBP) and p300. J Steroid Biochem Mol Biol. 1999;70:15–25.

Knight T, Irving JA. Ras/Raf/MEK/ERK pathway activation in childhood acute lymphoblastic leukemia and its therapeutic targeting. Front Oncol. 2014;4:160.

Kuchinskaya E, Nordgren A, Heyman M, Schoumans J, Corcoran M, Staaf J, Borg A, Soderhall S, Grander D, Nordenskjold M, Blennow E. Tiling-resolution array-CGH reveals the pattern of DNA copy number alterations in acute lymphoblastic leukemia with 21q amplification: the result of telomere dysfunction and breakage/fusion/breakage cycles? Leukemia. 2007;21:1327–30.

Kuhnl A, Gokbuget N, Kaiser M, Schlee C, Stroux A, Burmeister T, Mochmann LH, Hoelzer D, Hofmann WK, Thiel E, Baldus CD. Overexpression of LEF1 predicts unfavorable outcome in adult patients with B-precursor acute lymphoblastic leukemia. Blood. 2011;118:6362–7.

Lahortiga I, de Keersmaecker K, Van Vlierberghe P, Graux C, Cauwelier B, Lambert F, Mentens N, Beverloo HB, Pieters R, Speleman F, Odero MD, Bauters M, Froyen G, Marynen P, Vandenberghe P, Wlodarska I, Meijerink JP, Cools J. Duplication of the MYB oncogene in T cell acute lymphoblastic leukemia. Nat Genet. 2007;39:593–5.

Lambert JR, Nordeen SK. CBP recruitment and histone acetylation in differential gene induction by glucocorticoids and progestins. Mol Endocrinol. 2003;17:1085–94.

Larson RC, Lavenir I, Larson TA, Baer R, Warren AJ, Wadman I, Nottage K, Rabbitts TH. Protein dimerization between Lmo2 (Rbtn2) and Tal1 alters thymocyte development and potentiates T cell tumorigenesis in transgenic mice. EMBO J. 1996;15:1021–7.

Lausten-Thomsen U, Madsen HO, Vestergaard TR, Hjalgrim H, Nersting J, Schmiegelow K. Prevalence of t(12;21)[ETV6-RUNX1]-positive cells in healthy neonates. Blood. 2011;117:186–9.

Levine RL, Gilliland DG. Myeloproliferative disorders. Blood. 2008;112:2190–8.

Ley TJ, Ding L, Walter MJ, Mclellan MD, Lamprecht T, Larson DE, Kandoth C, Payton JE, Baty J, Welch J, Harris CC, Lichti CF, Townsend RR, Fulton RS, Dooling DJ, Koboldt DC, Schmidt H, Zhang Q, Osborne JR, Lin L, O'Laughlin M, Mcmichael JF, Delehaunty KD, Mcgrath SD, Fulton LA, Magrini VJ, Vickery TL, Hundal J, Cook LL, Conyers JJ, Swift GW, Reed JP, Alldredge PA, Wylie T, Walker J, Kalicki J, Watson MA, Heath S, Shannon WD, Varghese N, Nagarajan R, Westervelt P, Tomasson MH, Link DC, Graubert TA, Dipersio JF, Mardis ER, Wilson RK. DNMT3A mutations in acute myeloid leukemia. N Engl J Med. 2010;363:2424–33.

Li Z, Huang H, Chen P, He M, Li Y, Arnovitz S, Jiang X, He C, Hyjek E, Zhang J, Zhang Z, Elkahloun A, Cao D, Shen C, Wunderlich M, Wang Y, Neilly MB, Jin J, Wei M, Lu J, Valk PJ, Delwel R, Lowenberg B, Le Beau MM, Vardiman J, Mulloy JC, Zeleznik-Le NJ, Liu PP, Zhang J, Chen J. miR-196b directly targets both HOXA9/MEIS1 oncogenes and FAS tumour suppressor in MLL-rearranged leukaemia. Nat Commun. 2012;3:688.

Li Y, Schwab C, Ryan SL, Papaemmanuil E, Robinson HM, Jacobs P, Moorman AV, Dyer S, Borrow J, Griffiths M, Heerema NA, Carroll AJ, Talley P, Bown N, Telford N, Ross FM, Gaunt L, Mcnally RJ, Young BD, Sinclair P, Rand V, Teixeira MR, Joseph O, Robinson B, Maddison M, Dastugue N, Vandenberghe P, Haferlach C, Stephens PJ, Cheng J, Van Loo P, Stratton MR, Campbell PJ, Harrison CJ. Constitutional and somatic rearrangement of chromosome 21 in acute lymphoblastic leukaemia. Nature. 2014;508:98–102.

Li B, Li H, Bai Y, Kirschner-Schwabe R, Yang JJ, Chen Y, et al. Negative feedback-defective PRPS1 mutants drive thiopurine resistance in relapsed childhood ALL. Nature medicine 2015; 21(6):563–71.

Liang DC, Shih LY, Fu JF, Li HY, Wang HI, Hung IJ, Yang CP, Jaing TH, Chen SH, Liu HC. K-Ras mutations and N-Ras mutations in childhood acute leukemias with or without mixed-lineage leukemia gene rearrangements. Cancer. 2006;106:950–6.

Lilljebjorn H, Heidenblad M, Nilsson B, Lassen C, Horvat A, Heldrup J, Behrendtz M, Johansson B, Andersson A, Fioretos T. Combined high-resolution array-based comparative genomic hybridization and expression profiling of ETV6/RUNX1-positive acute lymphoblastic leukemias reveal a high incidence of cryptic Xq duplications and identify several putative target genes within the commonly gained region. Leukemia. 2007;21:2137–44.

Lilljebjorn H, Soneson C, Andersson A, Heldrup J, Behrendtz M, Kawamata N, Ogawa S, Koeffler HP, Mitelman F, Johansson B, Fontes M, Fioretos T. The correlation pattern of acquired copy number changes in 164 ETV6/RUNX1-positive childhood acute lymphoblastic leukemias. Hum Mol Genet. 2010;19:3150–8.

Lin C, Smith ER, Takahashi H, Lai KC, Martin-Brown S, Florens L, Washburn MP, Conaway JW, Conaway RC, Shilatifard A. AFF4, a component of the ELL/P-TEFb elongation complex and a shared subunit of MLL chimeras, can link transcription elongation to leukemia. Mol Cell. 2010;37:429–37.

Linger RM, Lee-Sherick AB, Deryckere D, Cohen RA, Jacobsen KM, Mcgranahan A, Brandao LN, Winges A, Sawczyn KK, Liang X, Keating AK, Tan AC, Earp HS, Graham DK. Mer receptor tyrosine kinase is a therapeutic target in pre-B-cell acute lymphoblastic leukemia. Blood. 2013;122:1599–609.

Loughran SJ, Kruse EA, Hacking DF, de Graaf CA, Hyland CD, Willson TA, Henley KJ, Ellis S, Voss AK, Metcalf D, Hilton DJ, Alexander WS, Kile BT. The transcription factor Erg is essential for definitive hematopoiesis and the function of adult hematopoietic stem cells. Nat Immunol. 2008;9:810–9.

Lu Q, Kamps MP. Heterodimerization of Hox proteins with Pbx1 and oncoprotein E2a-Pbx1 generates unique DNA-binding specificities at nucleotides predicted to contact the N-terminal arm of the Hox homeodomain – demonstration of Hox-dependent targeting of E2a-Pbx1 in vivo. Oncogene. 1997;14:75–83.

Lu M, Gong ZY, Shen WF, Ho AD. The tcl-3 proto-oncogene altered by chromosomal translocation in T-cell leukemia codes for a homeobox protein. EMBO J. 1991;10:2905–10.

Luo Z, Lin C, Shilatifard A. The super elongation complex (SEC) family in transcriptional control. Nat Rev Mol Cell Biol. 2012;13:543–7.

Lupardus PJ, Ultsch M, Wallweber H, Bir Kohli P, Johnson AR, Eigenbrot C. Structure of the pseudokinase-kinase domains from protein kinase TYK2 reveals a mechanism for Janus kinase (JAK) autoinhibition. Proc Natl Acad Sci U S A. 2014;111:8025–30.

Ma SK, Wan TS, Cheuk AT, Fung LF, Chan GC, Chan SY, Ha SY, Chan LC. Characterization of additional genetic events in childhood acute lymphoblastic leukemia with TEL/AML1 gene fusion: a molecular cytogenetics study. Leukemia. 2001;15:1442–7.

Ma X, Edmonson M, Yergeau D, Muzny DM, Hampton OA, Rusch M, Song G, Easton J, Harvey RC, Wheeler DA, Ma J, Doddapaneni H, Vadodaria B, Wu G, Nagahawatte P, Carroll WL, Chen IM, Gastier-Foster JM, Relling MV, Smith MA, Devidas M, Guidry Auvil JM, Downing JR, Loh ML, Willman CL, Gerhard DS, Mullighan CG, Hunger SP, Zhang J. Rise and fall of subclones from diagnosis to relapse in pediatric B-acute lymphoblastic leukaemia. Nat Commun. 2015;6:6604.

Mancini M, Scappaticci D, Cimino G, Nanni M, Derme V, Elia L, Tafuri A, Vignetti M, Vitale A, Cuneo A, Castoldi G, Saglio G, Pane F, Mecucci C, Camera A, Specchia G, Tedeschi A, di Raimondo F, Fioritoni G, Fabbiano F, Marmont F, Ferrara F, Cascavilla N, Todeschini G, Nobile F, Kropp MG, Leoni P, Tabilio A, Luppi M, Annino L, Mandelli F, Foa R. A comprehensive genetic classification of adult acute lymphoblastic leukemia (ALL): analysis of the GIMEMA 0496 protocol. Blood. 2005;105:3434–41.

Mansour MR, Abraham BJ, Anders L, Berezovskaya A, Gutierrez A, Durbin AD, Etchin J, Lawton L, Sallan SE, Silverman LB, Loh ML, Hunger SP, Sanda T, Young RA, Look AT. Oncogene regulation. An oncogenic super-enhancer formed through somatic mutation of a noncoding intergenic element. Science. 2014;346:1373–7.

Mar BG, Bullinger LB, Mclean KM, Grauman PV, Harris MH, Stevenson K, Neuberg DS, Sinha AU, Sallan SE, Silverman LB, Kung AL, Lo Nigro L, Ebert BL, Armstrong SA. Mutations in epigenetic regulators including SETD2 are gained during relapse in paediatric acute lymphoblastic leukaemia. Nat Commun. 2014;5:3469.

Marculescu R, Le T, Simon P, Jaeger U, Nadel B. V(D)J-mediated translocations in lymphoid neoplasms: a functional assessment of genomic instability by cryptic sites. J Exp Med. 2002;195:85–98.

Marschalek R. Mechanisms of leukemogenesis by MLL fusion proteins. Br J Haematol. 2011;152:141–54.

McCormack MP, Young LF, Vasudevan S, de Graaf CA, Codrington R, Rabbitts TH, Jane SM, Curtis DJ. The Lmo2 oncogene initiates leukemia in mice by inducing thymocyte self-renewal. Science. 2010;327:879–83.

McGuire EA, Hockett RD, Pollock KM, Bartholdi MF, O'Brien SJ, Korsmeyer SJ. The t(11;14)(p15;q11) in a T-cell acute lymphoblastic leukemia cell line activates multiple transcripts, including Ttg-1, a gene encoding a potential zinc finger protein. Mol Cell Biol. 1989;9:2124–32.

McGuire EA, Rintoul CE, Sclar GM, Korsmeyer SJ. Thymic overexpression of Ttg-1 in transgenic mice results in T-cell acute lymphoblastic leukemia/lymphoma. Mol Cell Biol. 1992;12:4186–96.

Mckeithan TW, Shima EA, le Beau MM, Minowada J, Rowley JD, Diaz MO. Molecular cloning of the breakpoint junction of a human chromosomal 8;14 translocation involving the T-cell receptor alpha-chain gene and sequences on the 3′ side of MYC. Proc Natl Acad Sci U S A. 1986;83:6636–40.

McLaughlin F, Ludbrook VJ, Cox J, Von Carlowitz I, Brown S, Randi AM. Combined genomic and antisense analysis reveals that the transcription factor Erg is implicated in endothelial cell differentiation. Blood. 2001;98:3332–9.

Mellentin JD, Smith SD, Cleary ML. lyl-1, a novel gene altered by chromosomal translocation in T cell leukemia, codes for a protein with a helix-loop-helix DNA binding motif. Cell. 1989;58:77–83.

Melo JV. The diversity of BCR-ABL fusion proteins and their relationship to leukemia phenotype. Blood. 1996;88:2375–84.

Mendoza MC, Er EE, Blenis J. The Ras-ERK and PI3K-mTOR pathways: cross-talk and compensation. Trends Biochem Sci. 2011;36:320–8.

Mes-Masson AM, McLaughlin J, Daley GQ, Paskind M, Witte ON. Overlapping cDNA clones define the complete coding region for the P210c-abl gene product associated with chronic myelogenous leukemia cells containing the Philadelphia chromosome. Proc Natl Acad Sci U S A. 1986;83:9768–72.

Metzeler KH, Heilmeier B, Edmaier KE, Rawat VP, Dufour A, Dohner K, Feuring-Buske M, Braess J, Spiekermann K, Buchner T, Sauerland MC, Dohner H, Hiddemann W, Bohlander SK, Schlenk RF, Bullinger L, Buske C. High expression of lymphoid enhancer-binding factor-1 (LEF1) is a novel favorable prognostic factor in cytogenetically normal acute myeloid leukemia. Blood. 2012;120:2118–26.

Metzler M, Forster A, Pannell R, Arends MJ, Daser A, Lobato MN, Rabbitts TH. A conditional model of MLL-AF4 B-cell tumourigenesis using invertor technology. Oncogene. 2006;25:3093–103.

Meyer C, Schneider B, Jakob S, Strehl S, Attarbaschi A, Schnittger S, Schoch C, Jansen MW, Van Dongen JJ, Den Boer ML, Pieters R, Ennas MG, Angelucci E, Koehl U, Greil J, Griesinger F, Zur Stadt U, Eckert C, Szczepanski T, Niggli FK, Schafer BW, Kempski H, Brady HJ, Zuna J, Trka J, Nigro LL, Biondi A, Delabesse E, Macintyre E, Stanulla M, Schrappe M, Haas OA, Burmeister T, Dingermann T, Klingebiel T, Marschalek R. The MLL recombinome of acute leukemias. Leukemia. 2006;20:777–84.

Meyer C, Kowarz E, Hofmann J, Renneville A, Zuna J, Trka J, Ben Abdelali R, Macintyre E, De Braekeleer E, De Braekeleer M, Delabesse E, De Oliveira MP, Cave H, Clappier E, Van Dongen JJ, Balgobind BV, Van Den Heuvel-Eibrink MM, Beverloo HB, Panzer-Grumayer R, Teigler-Schlegel A, Harbott J, Kjeldsen E, Schnittger S, Koehl U, Gruhn B, Heidenreich O, Chan LC, Yip SF, Krzywinski M, Eckert C, Moricke A, Schrappe M, Alonso CN, Schafer BW, Krauter J, Lee DA, Zur Stadt U, Te Kronnie G, Sutton R, Izraeli S, Trakhtenbrot L, Lo Nigro L, Tsaur G, Fechina L, Szczepanski T, Strehl S, Ilencikova D, Molkentin M, Burmeister T, Dingermann T, Klingebiel T, Marschalek R. New insights to the MLL recombinome of acute leukemias. Leukemia. 2009;23:1490–9.

Meyer JA, Wang J, Hogan LE, Yang JJ, Dandekar S, Patel JP, Tang Z, Zumbo P, Li S, Zavadil J, Levine RL, Cardozo T, Hunger SP, Raetz EA, Evans WE, Morrison DJ, Mason CE, Carroll WL. Relapse-specific mutations in NT5C2 in childhood acute lymphoblastic leukemia. Nat Genet. 2013;45:290–4.

Mi S, Li Z, Chen P, He C, Cao D, Elkahloun A, Lu J, Pelloso LA, Wunderlich M, Huang H, Luo RT, Sun M, He M, Neilly MB, Zeleznik-Le NJ, Thirman MJ, Mulloy JC, Liu PP, Rowley JD, Chen J. Aberrant overexpression and function of the miR-17-92 cluster in MLL-rearranged acute leukemia. Proc Natl Acad Sci U S A. 2010;107:3710–5.

Migliorini G, Fiege B, Hosking FJ, Ma Y, Kumar R, Sherborne AL, Da Silva Filho MI, Vijayakrishnan J, Koehler R, Thomsen H, Irving JA, Allan JM, Lightfoot T, Roman E, Kinsey SE, Sheridan E, Thompson P, Hoffmann P, Nothen MM, Muhleisen TW, Eisele L, Zimmermann M, Bartram CR, Schrappe M, Greaves M, Stanulla M, Hemminki K, Houlston RS. Variation at 10p12.2 and 10p14 influences risk of childhood B-cell acute lymphoblastic leukemia and phenotype. Blood. 2013;122:3298–307.

Molteni CG, te Kronnie G, Bicciato S, Villa T, Tartaglia M, Basso G, Biondi A, Cazzaniga G. PTPN11 mutations in childhood acute lymphoblastic leukemia occur as a secondary event associated with high hyperdiploidy. Leukemia. 2010;24:232–5.

Monastirioti M, Giagtzoglou N, Koumbanakis KA, Zacharioudaki E, Deligiannaki M, Wech I, Almeida M, Preiss A, Bray S, Delidakis C. Drosophila Hey is a target of Notch in asymmetric divisions during embryonic and larval neurogenesis. Development. 2010;137:191–201.

Moorman AV, Harrison CJ, Buck GA, Richards SM, Secker-Walker LM, Martineau M, Vance GH, Cherry AM, Higgins RR, Fielding AK, Foroni L, Paietta E, Tallman MS, Litzow MR, Wiernik PH, Rowe JM, Goldstone AH, Dewald GW, Adult Leukaemia Working Party, M. R. C. N. C. R. I. Karyotype is an independent prognostic factor in adult acute lymphoblastic leukemia (ALL): analysis of cytogenetic data from patients treated on the Medical Research Council (MRC) UKALLXII/Eastern Cooperative Oncology Group (ECOG) 2993 trial. Blood. 2007a;109:3189–97.

Moorman AV, Richards SM, Robinson HM, Strefford JC, Gibson BE, Kinsey SE, Eden TO, Vora AJ, Mitchell CD, Harrison CJ, U. K. M. R. C. N. C. R. I. C. L. W. Party. Prognosis of children with acute lymphoblastic leukemia (ALL) and intrachromosomal amplification of chromosome 21 (iAMP21). Blood. 2007b;109:2327–30.

Moorman AV, Chilton L, Wilkinson J, Ensor HM, Bown N, Proctor SJ. A population-based cytogenetic study of adults with acute lymphoblastic leukemia. Blood. 2010;115:206–14.

Mori H, Colman SM, Xiao Z, Ford AM, Healy LE, Donaldson C, Hows JM, Navarrete C, Greaves M. Chromosome translocations and covert leukemic clones are generated during normal fetal development. Proc Natl Acad Sci U S A. 2002;99:8242–7.

Morin RD, Johnson NA, Severson TM, Mungall AJ, An J, Goya R, Paul JE, Boyle M, Woolcock BW, Kuchenbauer F, Yap D, Humphries RK, Griffith OL, Shah S, Zhu H, Kimbara M, Shashkin P, Charlot JF, Tcherpakov M, Corbett R, Tam A, Varhol R, Smailus D, Moksa M, Zhao Y, Delaney A, Qian H, Birol I, Schein J, Moore R, Holt R, Horsman DE, Connors JM, Jones S, Aparicio S, Hirst M, Gascoyne RD, Marra MA. Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. Nat Genet. 2010;42:181–5.

Morrow M, Horton S, Kioussis D, Brady HJ, Williams O. TEL-AML1 promotes development of specific hematopoietic lineages consistent with preleukemic activity. Blood. 2004;103:3890–6.

Mullighan CG. Molecular genetics of B-precursor acute lymphoblastic leukemia. J Clin Invest. 2012;122:3407–15.

Mullighan CG. Genome sequencing of lymphoid malignancies. Blood. 2013a;122:3899–907.

Mullighan CG. Genomic characterization of childhood acute lymphoblastic leukemia. Semin Hematol. 2013b;50(4):312–24.

Mullighan CG. The genomic landscape of acute lymphoblastic leukemia in children and young adults. Hematol Am Soc Hematol Educ Program. 2014;2014:174–80.

Mullighan CG, Goorha S, Radtke I, Miller CB, Coustan-Smith E, Dalton JD, Girtman K, Mathew S, Ma J, Pounds SB, Su X, Pui CH, Relling MV, Evans WE, Shurtleff SA, Downing JR. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. Nature. 2007a;446:758–64.

Mullighan CG, Miller CB, Su XP, Radtke I, Dalton J, Song GC, Zhou XD, Pui CH, Shurtleff SA, Downing JR. ERG deletions define a novel subtype of B-progenitor acute lymphoblastic leukemia. Blood. 2007b;110:212a–3.

Mullighan CG, Miller CB, Radtke I, Phillips LA, Dalton J, Ma J, White D, Hughes TP, Le Beau MM, Pui CH, Relling MV, Shurtleff SA, Downing JR. BCR-ABL1 lymphoblastic leukaemia is characterized by the deletion of Ikaros. Nature. 2008a;453:110–4.

Mullighan CG, Phillips LA, Su X, Ma J, Miller CB, Shurtleff SA, Downing JR. Genomic analysis of the clonal origins of relapsed acute lymphoblastic leukemia. Science. 2008b;322:1377–80.

Mullighan CG, Collins-Underwood JR, Phillips LA, Loudin MG, Liu W, Zhang J, Ma J, Coustan-Smith E, Harvey RC, Willman CL, Mikhail FM, Meyer J, Carroll AJ, Williams RT, Cheng J, Heerema NA, Basso G, Pession A, Pui CH, Raimondi SC, Hunger SP, Downing JR, Carroll WL, Rabin KR. Rearrangement of CRLF2 in B-progenitor- and Down syndrome-associated acute lymphoblastic leukemia. Nat Genet. 2009a;41:1243–6.

Mullighan CG, Su X, Zhang J, Radtke I, Phillips LA, Miller CB, Ma J, Liu W, Cheng C, Schulman BA, Harvey RC, Chen IM, Clifford RJ, Carroll WL, Reaman G, Bowman WP, Devidas M, Gerhard DS, Yang W, Relling MV, Shurtleff SA, Campana D, Borowitz MJ, Pui CH, Smith M, Hunger SP, Willman CL, Downing JR, Children's Oncology Group. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. N Engl J Med. 2009b;360:470–80.

Mullighan CG, Zhang J, Harvey RC, Collins-Underwood JR, Schulman BA, Phillips LA, Tasian SK, Loh ML, Su X, Liu W, Devidas M, Atlas SR, Chen IM, Clifford RJ, Gerhard DS, Carroll WL, Reaman GH, Smith M, Downing JR, Hunger SP, Willman CL. JAK mutations in high-risk childhood acute lymphoblastic leukemia. Proc Natl Acad Sci U S A. 2009c;106:9414–8.

Mullighan CG, Zhang J, Kasper LH, Lerach S, Payne-Turner D, Phillips LA, Heatley SL, Holmfeldt L, Collins-Underwood JR, Ma J, Buetow KH, Pui CH, Baker SD, Brindle PK, Downing JR. CREBBP mutations in relapsed acute lymphoblastic leukaemia. Nature. 2011;471:235–9.

Nachman JB, Heerema NA, Sather H, Camitta B, Forestier E, Harrison CJ, Dastugue N, Schrappe M, Pui CH, Basso G, Silverman LB, Janka-Schaub GE. Outcome of treatment in children with hypodiploid acute lymphoblastic leukemia. Blood. 2007;110:1112–5.

Nagel S, Kaufmann M, Drexler HG, Macleod RA. The cardiac homeobox gene NKX2-5 is deregulated by juxtaposition with BCL11B in pediatric T-ALL cell lines via a novel t(5;14)(q35.1;q32.2). Cancer Res. 2003;63:5329–34.

Nagel S, Scherr M, Kel A, Hornischer K, Crawford GE, Kaufmann M, Meyer C, Drexler HG, Macleod RA. Activation of TLX3 and NKX2-5 in t(5;14)(q35;q32) T-cell acute lymphoblastic leukemia by remote 3′-BCL11B enhancers and coregulation by PU.1 and HMGA1. Cancer Res. 2007;67:1461–71.

Neale GA, Rehg JE, Goorha RM. Ectopic expression of rhombotin-2 causes selective expansion of CD4-CD8- lymphocytes in the thymus and T-cell tumors in transgenic mice. Blood. 1995;86:3060–71.

Neumann M, Heesch S, Schlee C, Schwartz S, Gokbuget N, Hoelzer D, Konstandin NP, Ksienzyk B, Vosberg S, Graf A, Krebs S, Blum H, Raff T, Bruggemann M, Hofmann WK, Hecht J, Bohlander SK, Greif PA, Baldus CD. Whole-exome sequencing in adult ETP-ALL reveals a high rate of DNMT3A mutations. Blood. 2013;121:4749–52.

Nishita M, Hashimoto MK, Ogata S, Laurent MN, Ueno N, Shibuya H, Cho KW. Interaction between Wnt and TGF-beta signalling pathways during formation of Spemann's organizer. Nature. 2000;403:781–5.

Noetzli L, Lo RW, Lee-Sherick AB, Callaghan M, Noris P, Savoia A, Rajpurkar M, Jones K, Gowan K, Balduini CL, Pecci A, Gnan C, De Rocco D, Doubek M, Li L, Lu L, Leung R, Landolt-Marticorena C, Hunger S, Heller P, Gutierrez-Hartmann A, Xiayuan L, Pluthero FG, Rowley JW, Weyrich AS, Kahr WH, Porter CC, Di Paola J. Germline mutations in ETV6 are associated with thrombocytopenia, red cell macrocytosis and predisposition to lymphoblastic leukemia. Nat Genet. 2015;47:535–8.

Notta F, Mullighan CG, Wang JC, Poeppl A, Doulatov S, Phillips LA, Ma J, Minden MD, Downing JR, Dick JE. Evolution of human BCR-ABL1 lymphoblastic leukaemia-initiating cells. Nature. 2011;469:362–7.

Nowell PC, Hungerford DA. Chromosome studies on normal and leukemic human leukocytes. J Natl Cancer Inst. 1960;25:85–109.

Ntziachristos P, Tsirigos A, Van Vlierberghe P, Nedjic J, Trimarchi T, Flaherty MS, Ferres-Marco D, da Ros V, Tang Z, Siegle J, Asp P, Hadler M, Rigo I, de Keersmaecker K, Patel J, Huynh T, Utro F, Poglio S, Samon JB, Paietta E, Racevskis J, Rowe JM, Rabadan R, Levine RL, Brown S, Pflumio F, Dominguez M, Ferrando A, Aifantis I. Genetic inactivation of the polycomb repressive complex 2 in T cell acute lymphoblastic leukemia. Nat Med. 2012;18:298–301.

O'Neil J, Grim J, Strack P, Rao S, Tibbitts D, Winter C, Hardwick J, Welcker M, Meijerink JP, Pieters R, Draetta G, Sears R, Clurman BE, Look AT. FBW7 mutations in leukemic cells mediate NOTCH pathway activation and resistance to gamma-secretase inhibitors. J Exp Med. 2007;204:1813–24.

Olsen JH, Hahnemann JM, Borresen-Dale AL, Brondum-Nielsen K, Hammarstrom L, Kleinerman R, Kaariainen H, Lonnqvist T, Sankila R, Seersholm N, Tretli S, Yuen J, Boice Jr JD, Tucker M. Cancer in patients with ataxia-telangiectasia and in their relatives in the Nordic countries. J Natl Cancer Inst. 2001;93:121–7.

Owczarek CM, Portbury KJ, Hardy MP, O'Leary DA, Kudoh J, Shibuya K, Shimizu N, Kola I, Hertzog PJ. Detailed mapping of the ERG-ETS2 interval of human chromosome 21 and comparison with the region of conserved synteny on mouse chromosome 16. Gene. 2004;324:65–77.

Palii CG, Perez-Iratxeta C, Yao Z, Cao Y, Dai F, Davison J, Atkins H, Allan D, Dilworth FJ, Gentleman R, Tapscott SJ, Brand M. Differential genomic targeting of the transcription factor TAL1 in alternate haematopoietic lineages. EMBO J. 2011;30:494–509.

Palomero T, Sulis ML, Cortina M, Real PJ, Barnes K, Ciofani M, Caparros E, Buteau J, Brown K, Perkins SL, Bhagat G, Agarwal AM, Basso G, Castillo M, Nagase S, Cordon-Cardo C, Parsons

R, Zuniga-Pflucker JC, Dominguez M, Ferrando AA. Mutational loss of PTEN induces resistance to NOTCH1 inhibition in T-cell leukemia. Nat Med. 2007;13:1203–10.

Palomero T, Dominguez M, Ferrando AA. The role of the PTEN/AKT Pathway in NOTCH1-induced leukemia. Cell Cycle. 2008;7:965–70.

Papaemmanuil E, Hosking FJ, Vijayakrishnan J, Price A, Olver B, Sheridan E, Kinsey SE, Lightfoot T, Roman E, Irving JA, Allan JM, Tomlinson IP, Taylor M, Greaves M, Houlston RS. Loci on 7p12.2, 10q21.2 and 14q11.2 are associated with risk of childhood acute lymphoblastic leukemia. Nat Genet. 2009;41:1006–10.

Papaemmanuil E, Rapado I, Li Y, Potter NE, Wedge DC, Tubio J, Alexandrov LB, Van Loo P, Cooke SL, Marshall J, Martincorena I, Hinton J, Gundem G, Van Delft FW, Nik-Zainal S, Jones DR, Ramakrishna M, Titley I, Stebbings L, Leroy C, Menzies A, Gamble J, Robinson B, Mudie L, Raine K, O'Meara S, Teague JW, Butler AP, Cazzaniga G, Biondi A, Zuna J, Kempski H, Muschen M, Ford AM, Stratton MR, Greaves M, Campbell PJ. RAG-mediated recombination is the predominant driver of oncogenic rearrangement in ETV6-RUNX1 acute lymphoblastic leukemia. Nat Genet. 2014;46:116–25.

Parker H, An Q, Barber K, Case M, Davies T, Konn Z, Stewart A, Wright S, Griffiths M, Ross FM, Moorman AV, Hall AG, Irving JA, Harrison CJ, Strefford JC. The complex genomic profile of ETV6-RUNX1 positive acute lymphoblastic leukemia highlights a recurrent deletion of TBL1XR1. Genes Chromosomes Cancer. 2008;47:1118–25.

Patel JP, Gonen M, Figueroa ME, Fernandez H, Sun Z, Racevskis J, Van Vlierberghe P, Dolgalev I, Thomas S, Aminova O, Huberman K, Cheng J, Viale A, Socci ND, Heguy A, Cherry A, Vance G, Higgins RR, Ketterling RP, Gallagher RE, Litzow M, Van Den Brink MR, Lazarus HM, Rowe JM, Luger S, Ferrando A, Paietta E, Tallman MS, Melnick A, Abdel-Wahab O, Levine RL. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. N Engl J Med. 2012;366:1079–89.

Paulsson K, Johansson B. High hyperdiploid childhood acute lymphoblastic leukemia. Genes Chromosomes Cancer. 2009;48:637–60.

Paulsson K, Horvat A, Strombeck B, Nilsson F, Heldrup J, Behrendtz M, Forestier E, Andersson A, Fioretos T, Johansson B. Mutations of FLT3, NRAS, KRAS, and PTPN11 are frequent and possibly mutually exclusive in high hyperdiploid childhood acute lymphoblastic leukemia. Genes Chromosomes Cancer. 2008;47:26–33.

Pellagatti A, Marafioti T, Paterson JC, Malcovati L, Della Porta MG, Jadersten M, Pushkaran B, George TI, Arber DA, Killick S, Giagounidis A, Hellstrom-Lindberg E, Cazzola M, Wainscoat JS, Boultwood J. Marked downregulation of the granulopoiesis regulator LEF1 is associated with disease progression in the myelodysplastic syndromes. Br J Haematol. 2009;146:86–90.

Perez-Andreu V, Roberts KG, Harvey RC, Yang W, Cheng C, Pei D, Xu H, Gastier-Foster J, Shuyu E, Lim JY, Chen IM, Fan Y, Devidas M, Borowitz MJ, Smith C, Neale G, Burchard EG, Torgerson DG, Klussmann FA, Villagran CR, Winick NJ, Camitta BM, Raetz E, Wood B, Yue F, Carroll WL, Larsen E, Bowman WP, Loh ML, Dean M, Bhojwani D, Pui CH, Evans WE, Relling MV, Hunger SP, Willman CL, Mullighan CG, Yang JJ. Inherited GATA3 variants are associated with Ph-like childhood acute lymphoblastic leukemia and risk of relapse. Nat Genet. 2013;45:1494–8.

Perez-Andreu V, Roberts KG, Xu H, Smith C, Zhang H, Yang W, Harvey RC, Payne-Turner D, Devidas M, Cheng IM, Carroll WL, Heerema NA, Carroll AJ, Raetz EA, Gastier-Foster JM, Marcucci G, Bloomfield CD, Mrozek K, Kohlschmidt J, Stock W, Kornblau SM, Konopleva M, Paietta E, Rowe JM, Luger SM, Tallman MS, Dean M, Burchard EG, Torgerson DG, Yue F, Wang Y, Pui CH, Jeha S, Relling MV, Evans WE, Gerhard DS, Loh ML, Willman CL, Hunger SP, Mullighan CG, Yang JJ. A genome-wide association study of susceptibility to acute lymphoblastic leukemia in adolescents and young adults. Blood. 2015;125:680–6.

Petropoulos K, Arseni N, Schessl C, Stadler CR, Rawat VP, Deshpande AJ, Heilmeier B, Hiddemann W, Quintanilla-Martinez L, Bohlander SK, Feuring-Buske M, Buske C. A novel role for Lef-1, a central transcription mediator of Wnt signaling, in leukemogenesis. J Exp Med. 2008;205:515–22.

Popovic R, Riesbeck LE, Velu CS, Chaubey A, Zhang J, Achille NJ, Erfurth FE, Eaton K, Lu J, Grimes HL, Chen J, Rowley JD, Zeleznik-Le NJ. Regulation of mir-196b by MLL and its overexpression by MLL fusions contributes to immortalization. Blood. 2009;113:3314–22.

Powell BC, Jiang L, Muzny DM, Trevino LR, Dreyer ZE, Strong LC, Wheeler DA, Gibbs RA, Plon SE. Identification of TP53 as an acute lymphocytic leukemia susceptibility gene through exome sequencing. Pediatr Blood Cancer. 2013;60:E1–3.

Privitera E, Kamps MP, Hayashi Y, Inaba T, Shapiro LH, Raimondi SC, Behm F, Hendershot L, Carroll AJ, Baltimore D, et al. Different molecular consequences of the 1;19 chromosomal translocation in childhood B-cell precursor acute lymphoblastic leukemia. Blood. 1992;79:1781–8.

Pui CH, Ribeiro RC, Hancock ML, Rivera GK, Evans WE, Raimondi SC, Head DR, Behm FG, Mahmoud MH, Sandlund JT, et al. Acute myeloid leukemia in children treated with epipodophyllotoxins for acute lymphoblastic leukemia. N Engl J Med. 1991;325:1682–7.

Pui CH, Behm FG, Downing JR, Hancock ML, Shurtleff SA, Ribeiro RC, Head DR, Mahmoud HH, Sandlund JT, Furman WL, et al. 11q23/MLL rearrangement confers a poor prognosis in infants with acute lymphoblastic leukemia. J Clin Oncol. 1994;12:909–15.

Pui CH, Kane JR, Crist WM. Biology and treatment of infant leukemias. Leukemia. 1995;9:762–9.

Pylayeva-Gupta Y, Grabocka E, Bar-Sagi D. RAS oncogenes: weaving a tumorigenic web. Nat Rev Cancer. 2011;11:761–74.

Radich JP, Kopecky KJ, Boldt DH, Head D, Slovak ML, Babu R, Kirk J, Lee A, Kessler P, Appelbaum F, et al. Detection of BCR-ABL fusion genes in adult acute lymphoblastic leukemia by the polymerase chain reaction. Leukemia. 1994;8:1688–95.

Raimondi SC, Behm FG, Roberson PK, Williams DL, Pui CH, Crist WM, Look AT, Rivera GK. Cytogenetics of pre-B-cell acute lymphoblastic leukemia with emphasis on prognostic implications of the t(1;19). J Clin Oncol. 1990;8:1380–8.

Rand V, Parker H, Russell LJ, Schwab C, Ensor H, Irving J, Jones L, Masic D, Minto L, Morrison H, Ryan S, Robinson H, Sinclair P, Moorman AV, Strefford JC, Harrison CJ. Genomic characterization implicates iAMP21 as a likely primary genetic event in childhood B-cell precursor acute lymphoblastic leukemia. Blood. 2011;117:6848–55.

Raynaud S, Cave H, Baens M, Bastard C, Cacheux V, Grosgeorge J, Guidalgiroux C, Guo CY, Vilmer E, Marynen P, Grandchamp B. The 12;21 translocation involving TEL and deletion of the other TEL allele: two frequently associated alterations found in childhood acute lymphoblastic leukemia. Blood. 1996a;87:2891–9.

Raynaud S, Mauvieux L, Cayuela JM, Bastard C, Bilhounabera C, Debuire B, Bories D, Boucheix C, Charrin C, Fiere D, Gabert J. TEL/AML1 fusion gene is a rare event in adult acute lymphoblastic leukemia. Leukemia. 1996b;10:1529–30.

Ribeiro RC, Abromowitch M, Raimondi SC, Murphy SB, Behm F, Williams DL. Clinical and biologic hallmarks of the Philadelphia chromosome in childhood acute lymphoblastic leukemia. Blood. 1987;70:948–53.

Roberts KG, Morin RD, Zhang J, Hirst M, Zhao Y, Su X, Chen SC, Payne-Turner D, Churchman ML, Harvey RC, Chen X, Kasap C, Yan C, Becksfort J, Finney RP, Teachey DT, Maude SL, Tse K, Moore R, Jones S, Mungall K, Birol I, Edmonson MN, Hu Y, Buetow KE, Chen IM, Carroll WL, Wei L, Ma J, Kleppe M, Levine RL, Garcia-Manero G, Larsen E, Shah NP, Devidas M, Reaman G, Smith M, Paugh SW, Evans WE, Grupp SA, Jeha S, Pui CH, Gerhard DS, Downing JR, Willman CL, Loh M, Hunger SP, Marra MA, Mullighan CG. Genetic alterations activating kinase and cytokine receptor signaling in high-risk acute lymphoblastic leukemia. Cancer Cell. 2012;22:153–66.

Roberts KG, Li Y, Payne-Turner D, Harvey RC, Yang YL, Pei D, Mccastlain K, Ding L, Lu C, Song G, Ma J, Becksfort J, Rusch M, Chen SC, Easton J, Cheng J, Boggs K, Santiago-Morales N, Iacobucci I, Fulton RS, Wen J, Valentine M, Cheng C, Paugh SW, Devidas M, Chen IM, Reshmi S, Smith A, Hedlund E, Gupta P, Nagahawatte P, Wu G, Chen X, Yergeau D, Vadodaria B, Mulder H, Winick NJ, Larsen EC, Carroll WL, Heerema NA, Carroll AJ, Grayson G, Tasian SK, Moore AS, Keller F, Frei-Jones M, Whitlock JA, Raetz EA, White DL, Hughes TP, Guidry

Auvil JM, Smith MA, Marcucci G, Bloomfield CD, Mrozek K, Kohlschmidt J, Stock W, Kornblau SM, Konopleva M, Paietta E, Pui CH, Jeha S, Relling MV, Evans WE, Gerhard DS, Gastier-Foster JM, Mardis E, Wilson RK, Loh ML, Downing JR, Hunger SP, Willman CL, Zhang J, Mullighan CG. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. N Engl J Med. 2014;371:1005–15.

Robinson HM, Broadfield ZJ, Cheung KL, Harewood L, Harris RL, Jalali GR, Martineau M, Moorman AV, Taylor KE, Richards S, Mitchell C, Harrison CJ. Amplification of AML1 in acute lymphoblastic leukemia is associated with a poor outcome. Leukemia. 2003;17: 2249–50.

Robinson HM, Broadfield ZJ, Barber KE, Cheung KL, Stewart ARM, Wright SL, Stretford JC, Harrison CJ. Amplification of RUNX1 in acute lymphoblastic leukaemia is associated with intrachromosomal instability of the duplicated chromosome 21 – a distinct mechanism? J Med Genet. 2005;42:S20.

Robinson HM, Harrison CJ, Moorman AV, Chudoba I, Strefford JC. Intrachromosomal amplification of chromosome 21 (iAMP21) may arise from a breakage-fusion-bridge cycle. Genes Chromosomes Cancer. 2007;46:318–26.

Romana SP, Mauchauffe M, Le Coniat M, Chumakov I, Le Paslier D, Berger R, Bernard OA. The t(12;21) of acute lymphoblastic leukemia results in a tel-AML1 gene fusion. Blood. 1995a;85:3662–70.

Romana SP, Poirel H, Leconiat M, Flexor MA, Mauchauffe M, Jonveaux P, Macintyre EA, Berger R, Bernard OA. High frequency of t(12;21) in childhood B-lineage acute lymphoblastic leukemia. Blood. 1995b;86:4263–9.

Ross ME, Zhou X, Song G, Shurtleff SA, Girtman K, Williams WK, Liu HC, Mahfouz R, Raimondi SC, Lenny N, Patel A, Downing JR. Classification of pediatric acute lymphoblastic leukemia by gene expression profiling. Blood. 2003;102:2951–9.

Rowley JD. Letter: a new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature. 1973;243:290–3.

Royer-Pokora B, Loos U, Ludwig WD. TTG-2, a new gene encoding a cysteine-rich protein with the LIM motif, is overexpressed in acute T-cell leukaemia with the t(11;14)(p13;q11). Oncogene. 1991;6:1887–93.

Rubnitz JE, Link MP, Shuster JJ, Carroll AJ, Hakami N, Frankel LS, Pullen DJ, Cleary ML. Frequency and prognostic significance of HRX rearrangements in infant acute lymphoblastic leukemia: a Pediatric Oncology Group study. Blood. 1994;84:570–3.

Rubnitz JE, Camitta BM, Mahmoud H, Raimondi SC, Carroll AJ, Borowitz MJ, Shuster JJ, Link MP, Pullen DJ, Downing JR, Behm FG, Pui CH. Childhood acute lymphoblastic leukemia with the MLL-ENL fusion and t(11;19)(q23;p13.3) translocation. J Clin Oncol. 1999;17:191–6.

Russell LJ, Capasso M, Vater I, Akasaka T, Bernard OA, Calasanz MJ, Chandrasekaran T, Chapiro E, Gesk S, Griffiths M, Guttery DS, Haferlach C, Harder L, Heidenreich O, Irving J, Kearney L, Nguyen-Khac F, Machado L, Minto L, Majid A, Moorman AV, Morrison H, Rand V, Strefford JC, Schwab C, Tonnies H, Dyer MJ, Siebert R, Harrison CJ. Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukemia. Blood. 2009;114:2688–98.

Sanda T, Lawton LN, Barrasa MI, Fan ZP, Kohlhammer H, Gutierrez A, Ma W, Tatarek J, Ahn Y, Kelliher MA, Jamieson CH, Staudt LM, Young RA, Look AT. Core transcriptional regulatory circuit controlled by the TAL1 complex in human T cell acute lymphoblastic leukemia. Cancer Cell. 2012;22:209–21.

Sattler M, Griffin JD. Molecular mechanisms of transformation by the BCR-ABL oncogene. Semin Hematol. 2003;40:4–10.

Schichman SA, Caligiuri MA, Gu Y, Strout MP, Canaani E, Bloomfield CD, Croce CM. ALL-1 partial duplication in acute leukemia. Proc Natl Acad Sci U S A. 1994;91:6236–9.

Schwab CJ, Chilton L, Morrison H, Jones L, Al-Shehhi H, Erhorn A, Russell LJ, Moorman AV, Harrison CJ. Genes commonly deleted in childhood B-cell precursor acute lymphoblastic leukemia: association with cytogenetics and clinical features. Haematologica. 2013;98: 1081–8.

Seckerwalker LM, Craig JM, Hawkins JM, Hoffbrand AV. Philadelphia positive acute lymphoblastic-leukemia in adults – age distribution, Bcr breakpoint and prognostic-significance. Leukemia. 1991;5:196–9.

Secker-Walker LM, Prentice HG, Durrant J, Richards S, Hall E, Harrison G. Cytogenetics adds independent prognostic information in adults with acute lymphoblastic leukaemia on MRC trial UKALL XA. MRC Adult Leukaemia Working Party. Br J Haematol. 1997;96:601–10.

Sennanasendi H, Dastugue N, Talmant P, Bastard C, Cacheux V, Grosgeorge J, Suc A, Mechinaud F, Thyss A, Vilmer E, Raynaud SD. Deletions of the non-translocated allele of TEL (ETV6) seem to be constant in childhood B-lineage ALLs with a t(12;21). Results of a FISH study on 148 children included in the 58881 therapeutic trial from the CLCG (EORTC). Blood. 1996;88:274.

Shah S, Schrader KA, Waanders E, Timms AE, Vijai J, Miething C, Wechsler J, Yang J, Hayes J, Klein RJ, Zhang J, Wei L, Wu G, Rusch M, Nagahawatte P, Ma J, Chen SC, Song G, Cheng J, Meyers P, Bhojwani D, Jhanwar S, Maslak P, Fleisher M, Littman J, Offit L, Rau-Murthy R, Fleischut MH, Corines M, Murali R, Gao X, Manschreck C, Kitzing T, Murty VV, Raimondi SC, Kuiper RP, Simons A, Schiffman JD, Onel K, Plon SE, Wheeler DA, Ritter D, Ziegler DS, Tucker K, Sutton R, Chenevix-Trench G, Li J, Huntsman DG, Hansford S, Senz J, Walsh T, Lee M, Hahn CN, Roberts KG, King MC, Lo SM, Levine RL, Viale A, Socci ND, Nathanson KL, Scott HS, Daly M, Lipkin SM, Lowe SW, Downing JR, Altshuler D, Sandlund JT, Horwitz MS, Mullighan CG, Offit K. A recurrent germline PAX5 mutation confers susceptibility to pre-B cell acute lymphoblastic leukemia. Nat Genet. 2013;45:1226–31.

Shanmugam K, Green NC, Rambaldi I, Saragovi HU, Featherstone MS. PBX and MEIS as non-DNA-binding partners in trimeric complexes with HOX proteins. Mol Cell Biol. 1999;19: 7577–88.

Sherborne AL, Hosking FJ, Prasad RB, Kumar R, Koehler R, Vijayakrishnan J, Papaemmanuil E, Bartram CR, Stanulla M, Schrappe M, Gast A, Dobbins SE, Ma Y, Sheridan E, Taylor M, Kinsey SE, Lightfoot T, Roman E, Irving JA, Allan JM, Moorman AV, Harrison CJ, Tomlinson IP, Richards S, Zimmermann M, Szalai C, Semsei AF, Erdelyi DJ, Krajinovic M, Sinnett D, Healy J, Gonzalez Neira A, Kawamata N, Ogawa S, Koeffler HP, Hemminki K, Greaves M, Houlston RS. Variation in CDKN2A at 9p21.3 influences childhood acute lymphoblastic leukemia risk. Nat Genet. 2010;42:492–4.

Shima EA, Le Beau MM, Mckeithan TW, Minowada J, Showe LC, Mak TW, Minden MD, Rowley JD, Diaz MO. Gene encoding the alpha chain of the T-cell receptor is moved immediately downstream of c-myc in a chromosomal 8;14 translocation in a cell line from a human T-cell leukemia. Proc Natl Acad Sci U S A. 1986;83:3439–43.

Shochat C, Tal N, Bandapalli OR, Palmi C, Ganmore I, Te Kronnie G, Cario G, Cazzaniga G, Kulozik AE, Stanulla M, Schrappe M, Biondi A, Basso G, Bercovich D, Muckenthaler MU, Izraeli S. Gain-of-function mutations in interleukin-7 receptor-alpha (IL7R) in childhood acute lymphoblastic leukemias. J Exp Med. 2011;208:901–8.

Shtivelman E, Lifshitz B, Gale RP, Canaani E. Fused transcript of abl and bcr genes in chronic myelogenous leukaemia. Nature. 1985;315:550–4.

Shurtleff SA, Buijs A, Behm FG, Rubnitz JE, Raimondi SC, Hancock ML, Chan GC, Pui CH, Grosveld G, Downing JR. TEL/AML1 fusion resulting from a cryptic t(12;21) is the most common genetic lesion in pediatric ALL and defines a subgroup of patients with an excellent prognosis. Leukemia. 1995;9:1985–9.

Simon C, Chagraoui J, Krosl J, Gendron P, Wilhelm B, Lemieux S, Boucher G, Chagnon P, Drouin S, Lambert R, Rondeau C, Bilodeau A, Lavallee S, Sauvageau M, Hebert J, Sauvageau G. A key role for EZH2 and associated genes in mouse and human adult T-cell acute leukemia. Genes Dev. 2012;26:651–6.

Small D. FLT3 mutations: biology and treatment. Hematol Am Soc Hematol Educ Program. 2006; 178–84.

Smith S, Tripathi R, Goodings C, Cleveland S, Mathias E, Hardaway JA, Elliott N, Yi Y, Chen X, Downing J, Mullighan C, Swing DA, Tessarollo L, Li L, Love P, Jenkins NA, Copeland NG,

Thompson MA, Du Y, Dave UP. LIM domain only-2 (LMO2) induces T-cell leukemia by two distinct pathways. PLoS One. 2014;9:e85883.

Sneeringer CJ, Scott MP, Kuntz KW, Knutson SK, Pollock RM, Richon VM, Copeland RA. Coordinated activities of wild-type plus mutant EZH2 drive tumor-associated hypertrimethylation of lysine 27 on histone H3 (H3K27) in human B-cell lymphomas. Proc Natl Acad Sci U S A. 2010;107:20980–5.

Soler E, Andrieu-Soler C, De Boer E, Bryne JC, Thongjuea S, Stadhouders R, Palstra RJ, Stevens M, Kockx C, Van Ijcken W, Hou J, Steinhoff C, Rijkers E, Lenhard B, Grosveld F. The genome-wide dynamics of the binding of Ldb1 complexes during erythroid differentiation. Genes Dev. 2010;24:277–89.

Sorensen PH, Lessnick SL, Lopez-Terrada D, Liu XF, Triche TJ, Denny CT. A second Ewing's sarcoma translocation, t(21;22), fuses the EWS gene to another ETS-family transcription factor, ERG. Nat Genet. 1994;6:146–51.

Soulier J, Clappier E, Cayuela JM, Regnault A, Garcia-Peydro M, Dombret H, Baruchel A, Toribio ML, Sigaux F. HOXA genes are included in genetic and biologic networks defining human acute T-cell leukemia (T-ALL). Blood. 2005;106:274–86.

Stam RW, Den Boer ML, Schneider P, Nollau P, Horstmann M, Beverloo HB, Van Der Voort E, Valsecchi MG, DE Lorenzo P, Sallan SE, Armstrong SA, Pieters R. Targeting FLT3 in primary MLL-gene-rearranged infant acute lymphoblastic leukemia. Blood. 2005;106:2484–90.

Stam RW, Schneider P, Hagelstein JA, Van Der Linden MH, Stumpel DJ, DE Menezes RX, DE Lorenzo P, Valsecchi MG, Pieters R. Gene expression profiling-based dissection of MLL translocated and MLL germline acute lymphoblastic leukemia in infants. Blood. 2010;115:2835–44.

Strefford J, Van Delft F, Robinson HM, Worley H, Selzer R, Young BD, Saha V, Harrison CJ. Patients with RUNX1 (AML1) amplification exhibit consistent genomic amplification, chromosome instability and a distinct gene expression signature. J Med Genet. 2005a;42:S65.

Strefford JC, Van Delft F, Robinson HM, Worley H, Selzer R, Young BD, Saba V, Harrison CJ. Molecular characterization of AML1 (RUNX1) amplification: a poor risk chromosomal marker in acute lymphoblastic leukaemia (ALL). Chromosome Res. 2005b;13:151.

Strefford JC, Van Delft FW, Robinson HM, Worley H, Yiannikouris O, Selzer R, Richmond T, Hann I, Bellotti T, Raghavan M, Young BD, Saha V, Harrison CJ. Complex genomic alterations and gene expression in acute lymphoblastic leukemia with intrachromosomal amplification of chromosome 21. Proc Natl Acad Sci U S A. 2006;103:8167–72.

Su XY, Busson M, Della Valle V, Ballerini P, Dastugue N, Talmant P, Ferrando AA, Baudry-Bluteau D, Romana S, Berger R, Bernard OA. Various types of rearrangements target TLX3 locus in T-cell acute lymphoblastic leukemia. Genes Chromosomes Cancer. 2004;41:243–9.

Suresh S, Irvine AE. The NOTCH signaling pathway in normal and malignant blood cell production. J Cell Commun Signal. 2015;9(1):5–13.

Sykes DB, Kamps MP. E2a/Pbx1 induces the rapid proliferation of stem cell factor-dependent murine pro-T cells that cause acute T-lymphoid or myeloid leukemias in mice. Mol Cell Biol. 2004;24:1256–69.

Szczepanski T, Van Der Velden VH, Waanders E, Kuiper RP, Van Vlierberghe P, Gruhn B, Eckert C, Panzer-Grumayer R, Basso G, Cave H, Stadt UZ, Campana D, Schrauder A, Sutton R, Van Wering E, Meijerink JP, Van Dongen JJ. Late recurrence of childhood T-cell acute lymphoblastic leukemia frequently represents a second leukemia rather than a relapse: first evidence for genetic predisposition. J Clin Oncol. 2011;29:1643–9.

Tartaglia M, Martinelli S, Cazzaniga G, Cordeddu V, Iavarone I, Spinelli M, Palmi C, Carta C, Pession A, Arico M, Masera G, Basso G, Sorcini M, Gelb BD, Biondi A. Genetic evidence for lineage-related and differentiation stage-related contribution of somatic PTPN11 mutations to leukemogenesis in childhood acute leukemia. Blood. 2004;104:307–13.

Thompson BJ, Buonamici S, Sulis ML, Palomero T, Vilimas T, Basso G, Ferrando A, Aifantis I. The SCFFBW7 ubiquitin ligase complex as a tumor suppressor in T cell leukemia. J Exp Med. 2007;204:1825–35.

Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, Varambally S, Cao X, Tchinda J, Kuefer R, Lee C, Montie JE, Shah RB, Pienta KJ, Rubin MA, Chinnaiyan AM. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science. 2005;310:644–8.

Toms AV, Deshpande A, Mcnally R, Jeong Y, Rogers JM, Kim CU, Gruner SM, Ficarro SB, Marto JA, Sattler M, Griffin JD, Eck MJ. Structure of a pseudokinase-domain switch that controls oncogenic activation of Jak kinases. Nat Struct Mol Biol. 2013;20:1221–3.

Tosello V, Mansour MR, Barnes K, Paganin M, Sulis ML, Jenkinson S, Allen CG, Gale RE, Linch DC, Palomero T, Real P, Murty V, Yao X, Richards SM, Goldstone A, Rowe J, Basso G, Wiernik PH, Paietta E, Pieters R, Horstmann M, Meijerink JP, Ferrando AA. WT1 mutations in T-ALL. Blood. 2009;114:1038–45.

Treanor LM, Volanakis EJ, Zhou S, Lu T, Sherr CJ, Sorrentino BP. Functional interactions between Lmo2, the Arf tumor suppressor, and Notch1 in murine T-cell malignancies. Blood. 2011;117:5453–62.

Treanor LM, Zhou S, Janke L, Churchman ML, Ma Z, Lu T, Chen SC, Mullighan CG, Sorrentino BP. Interleukin-7 receptor mutants initiate early T cell precursor leukemia in murine thymocyte progenitors with multipotent potential. J Exp Med. 2014;211:701–13.

Trentin L, Giordan M, Dingermann T, Basso G, Te Kronnie G, Marschalek R. Two independent gene signatures in pediatric t(4;11) acute lymphoblastic leukemia patients. Eur J Haematol. 2009;83:406–19.

Trevino LR, Yang W, French D, Hunger SP, Carroll WL, Devidas M, Willman C, Neale G, Downing J, Raimondi SC, Pui CH, Evans WE, Relling MV. Germline genomic variants associated with childhood acute lymphoblastic leukemia. Nat Genet. 2009;41:1001–5.

Tripic T, Deng W, Cheng Y, Zhang Y, Vakoc CR, Gregory GD, Hardison RC, Blobel GA. SCL and associated proteins distinguish active from repressive GATA transcription factor complexes. Blood. 2009;113:2191–201.

Tsuzuki S, Seto M, Greaves M, Enver T. Modeling first-hit functions of the t(12;21) TEL-AML1 translocation in mice. Proc Natl Acad Sci U S A. 2004;101:8443–8.

Tzoneva G, Perez-Garcia A, Carpenter Z, Khiabanian H, Tosello V, Allegretta M, Paietta E, Racevskis J, Rowe JM, Tallman MS, Paganin M, Basso G, Hof J, Kirschner-Schwabe R, Palomero T, Rabadan R, Ferrando A. Activating mutations in the NT5C2 nucleotidase gene drive chemotherapy resistance in relapsed ALL. Nat Med. 2013;19:368–71.

Van Vlierberghe P, Van Grotel M, Beverloo HB, Lee C, Helgason T, Buijs-Gladdines J, Passier M, Van Wering ER, Veerman AJ, Kamps WA, Meijerink JP, Pieters R. The cryptic chromosomal deletion del(11)(p12p13) as a new activation mechanism of LMO2 in pediatric T-cell acute lymphoblastic leukemia. Blood. 2006;108:3520–9.

Van Vlierberghe P, Homminga I, Zuurbier L, Gladdines-Buijs J, Van Wering ER, Horstmann M, Beverloo HB, Pieters R, Meijerink JP. Cooperative genetic defects in TLX3 rearranged pediatric T-ALL. Leukemia. 2008a;22:762–70.

Van Vlierberghe P, Van Grotel M, Tchinda J, Lee C, Beverloo HB, Van Der Spek PJ, Stubbs A, Cools J, Nagata K, Fornerod M, Buijs-Gladdines J, Horstmann M, Van Wering ER, Soulier J, Pieters R, Meijerink JP. The recurrent SET-NUP214 fusion as a new HOXA activation mechanism in pediatric T-cell acute lymphoblastic leukemia. Blood. 2008b;111:4668–80.

Van Vlierberghe P, Palomero T, Khiabanian H, Van Der Meulen J, Castillo M, Van Roy N, De Moerloose B, Philippe J, Gonzalez-Garcia S, Toribio ML, Taghon T, Zuurbier L, Cauwelier B, Harrison CJ, Schwab C, Pisecker M, Strehl S, Langerak AW, Gecz J, Sonneveld E, Pieters R, Paietta E, Rowe JM, Wiernik PH, Benoit Y, Soulier J, Poppe B, Yao X, Cordon-Cardo C, Meijerink J, Rabadan R, Speleman F, Ferrando A. PHF6 mutations in T-cell acute lymphoblastic leukemia. Nat Genet. 2010;42:338–42.

Vanrhee F, Kasprzyk A, Jamil A, Dickinson H, Lin F, Cross NCP, Galvin MC, Goldman JM, Seckerwalker LM. Detection of the Bcr-Abl gene by reverse transcription-polymerase chain-reaction and fluorescence in-situ hybridization in a patient with Philadelphia-chromosome negative acute lymphoblastic-leukemia. Br J Haematol. 1995;90:225–8.

Vijayakrishnan J, Houlston RS. Candidate gene association studies and risk of childhood acute lymphoblastic leukemia: a systematic review and meta-analysis. Haematologica. 2010;95: 1405–14.

Waanders E, Scheijen B, Van Der Meer LT, Van Reijmersdal SV, Van Emst L, Kroeze Y, Sonneveld E, Hoogerbrugge PM, Van Kessel AG, Van Leeuwen FN, Kuiper RP. The origin and nature of tightly clustered BTG1 deletions in precursor B-cell acute lymphoblastic leukemia support a model of multiclonal evolution. PLoS Genet. 2012;8:e1002533.

Wadman I, Li J, Bash RO, Forster A, Osada H, Rabbitts TH, Baer R. Specific in vivo association between the bHLH and LIM proteins implicated in human T cell leukemia. EMBO J. 1994;13:4831–9.

Walsh KM, De Smith AJ, Chokkalingam AP, Metayer C, Roberts W, Barcellos LF, Wiemels JL, Buffler PA. GATA3 risk alleles are associated with ancestral components in Hispanic children with ALL. Blood. 2013;122:3385–7.

Wang J, Jani-Sait SN, Escalon EA, Carroll AJ, De Jong PJ, Kirsch IR, Aplan PD. The t(14;21) (q11.2;q22) chromosomal translocation associated with T-cell acute lymphoblastic leukemia activates the BHLHB1 gene. Proc Natl Acad Sci U S A. 2000;97:3497–502.

Wang H, Zang C, Taing L, Arnett KL, Wong YJ, Pear WS, Blacklow SC, Liu XS, Aster JC. NOTCH1-RBPJ complexes drive target gene expression through dynamic interactions with superenhancers. Proc Natl Acad Sci U S A. 2014;111:705–10.

Weerkamp F, Luis TC, Naber BA, Koster EE, Jeannotte L, Van Dongen JJ, Staal FJ. Identification of Notch target genes in uncommitted T-cell progenitors: no direct induction of a T-cell specific gene program. Leukemia. 2006;20:1967–77.

Wei J, Wunderlich M, Fox C, Alvarez S, Cigudosa JC, Wilhelm JS, Zheng Y, Cancelas JA, Gu Y, Jansen M, Dimartino JF, Mulloy JC. Microenvironment determines lineage fate in a human model of MLL-AF9 leukemia. Cancer Cell. 2008;13:483–95.

Weng AP, Ferrando AA, Lee W, Morris JPT, Silverman LB, Sanchez-Irizarry C, Blacklow SC, Look AT, Aster JC. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science. 2004;306:269–71.

Weng AP, Millholland JM, Yashiro-Ohtani Y, Arcangeli ML, Lau A, Wai C, Del Bianco C, Rodriguez CG, Sai H, Tobias J, Li Y, Wolfe MS, Shachaf C, Felsher D, Blacklow SC, Pear WS, Aster JC. c-Myc is an important direct target of Notch1 in T-cell acute lymphoblastic leukemia/ lymphoma. Genes Dev. 2006;20:2096–109.

Wiemels JL, Cazzaniga G, Daniotti M, Eden OB, Addison GM, Masera G, Saha V, Biondi A, Greaves MF. Prenatal origin of acute lymphoblastic leukaemia in children. Lancet. 1999a;354:1499–503.

Wiemels JL, Ford AM, Van Wering ER, Postma A, Greaves M. Protracted and variable latency of acute lymphoblastic leukemia after TEL-AML1 gene fusion in utero. Blood. 1999b;94:1057–62.

Williams RT, Roussel MF, Sherr CJ. Arf gene loss enhances oncogenicity and limits imatinib response in mouse models of Bcr-Abl-induced acute lymphoblastic leukemia. Proc Natl Acad Sci U S A. 2006;103:6688–93.

Williams RT, Den Besten W, Sherr CJ. Cytokine-dependent imatinib resistance in mouse BCR-ABL+, Arf-null lymphoblastic leukemia. Genes Dev. 2007;21:2283–7.

Wilson NK, Foster SD, Wang X, Knezevic K, Schutte J, Kaimakis P, Chilarska PM, Kinston S, Ouwehand WH, Dzierzak E, Pimanda JE, De Bruijn MF, Gottgens B. Combinatorial transcriptional control in blood stem/progenitor cells: genome-wide analysis of ten major transcriptional regulators. Cell Stem Cell. 2010;7:532–44.

Xia Y, Brown L, Yang CY, Tsan JT, Siciliano MJ, Espinosa 3rd R, Le Beau MM, Baer RJ. TAL2, a helix-loop-helix gene activated by the (7;9)(q34;q32) translocation in human T-cell leukemia. Proc Natl Acad Sci U S A. 1991;88:11416–20.

Xu H, Cheng C, Devidas M, Pei D, Fan Y, Yang W, Neale G, Scheet P, Burchard EG, Torgerson DG, Eng C, Dean M, Antillon F, Winick NJ, Martin PL, Willman CL, Camitta BM, Reaman GH, Carroll WL, Loh M, Evans WE, Pui CH, Hunger SP, Relling MV, Yang JJ. ARID5B genetic polymorphisms contribute to racial disparities in the incidence and treatment outcome of childhood acute lymphoblastic leukemia. J Clin Oncol. 2012;30:751–7.

Yamamoto JF, Goodman MT. Patterns of leukemia incidence in the United States by subtype and demographic characteristics, 1997–2002. Cancer Causes Control. 2008;19:379–90.

Yamamoto T, Isomura M, Xu Y, Liang J, Yagasaki H, Kamachi Y, Kudo K, Kiyoi H, Naoe T, Kojma S. PTPN11, RAS and FLT3 mutations in childhood acute lymphoblastic leukemia. Leuk Res. 2006;30:1085–9.

Yang JJ, Bhojwani D, Yang W, Cai X, Stocco G, Crews K, Wang J, Morrison D, Devidas M, Hunger SP, Willman CL, Raetz EA, Pui CH, Evans WE, Relling MV, Carroll WL. Genome-wide copy number profiling reveals molecular evolution from diagnosis to relapse in childhood acute lymphoblastic leukemia. Blood. 2008;112:4178–83.

Yang JJ, Cheng C, Devidas M, Cao X, Fan Y, Campana D, Yang W, Neale G, Cox NJ, Scheet P, Borowitz MJ, Winick NJ, Martin PL, Willman CL, Bowman WP, Camitta BM, Carroll A, Reaman GH, Carroll WL, Loh M, Hunger SP, Pui CH, Evans WE, Relling MV. Ancestry and pharmacogenomics of relapse in acute lymphoblastic leukemia. Nat Genet. 2011;43:237–41.

Yap DB, Chu J, Berg T, Schapira M, Cheng SW, Moradian A, Morin RD, Mungall AJ, Meissner B, Boyle M, Marquez VE, Marra MA, Gascoyne RD, Humphries RK, Arrowsmith CH, Morin GB, Aparicio SA. Somatic mutations at EZH2 Y641 act dominantly through a mechanism of selectively altered PRC2 catalytic activity, to increase H3K27 trimethylation. Blood. 2011;117:2451–9.

Zaliova M, Zimmermannova O, Dorge P, Eckert C, Moricke A, Zimmermann M, Stuchly J, Teigler-Schlegel A, Meissner B, Koehler R, Bartram CR, Karawajew L, Rhein P, Zuna J, Schrappe M, Cario G, Stanulla M. ERG deletion is associated with CD2 and attenuates the negative impact of IKZF1 deletion in childhood acute lymphoblastic leukemia. Leukemia. 2014;28:182–5.

Zeisig BB, Garcia-Cuellar MP, Winkler TH, Slany RK. The oncoprotein MLL-ENL disturbs hematopoietic lineage determination and transforms a biphenotypic lymphoid/myeloid cell. Oncogene. 2003;22:1629–37.

Zenatti PP, Ribeiro D, Li W, Zuurbier L, Silva MC, Paganin M, Tritapoe J, Hixon JA, Silveira AB, Cardoso BA, Sarmento LM, Correia N, Toribio ML, Kobarg J, Horstmann M, Pieters R, Brandalise SR, Ferrando AA, Meijerink JP, Durum SK, Yunes JA, Barata JT. Oncogenic IL7R gain-of-function mutations in childhood T-cell acute lymphoblastic leukemia. Nat Genet. 2011;43:932–9.

Zhang J, Mullighan CG, Harvey RC, Wu G, Chen X, Edmonson M, Buetow KH, Carroll WL, Chen IM, Devidas M, Gerhard DS, Loh ML, Reaman GH, Relling MV, Camitta BM, Bowman WP, Smith MA, Willman CL, Downing JR, Hunger SP. Key pathways are frequently mutated in high-risk childhood acute lymphoblastic leukemia: a report from the Children's Oncology Group. Blood. 2011;118:3080–7.

Zhang J, Ding L, Holmfeldt L, Wu G, Heatley SL, Payne-Turner D, Easton J, Chen X, Wang J, Rusch M, Lu C, Chen SC, Wei L, Collins-Underwood JR, Ma J, Roberts KG, Pounds SB, Ulyanov A, Becksfort J, Gupta P, Huether R, Kriwacki RW, Parker M, Mcgoldrick DJ, Zhao D, Alford D, Espy S, Bobba KC, Song G, Pei D, Cheng C, Roberts S, Barbato MI, Campana D, Coustan-Smith E, Shurtleff SA, Raimondi SC, Kleppe M, Cools J, Shimano KA, Hermiston ML, Doulatov S, Eppert K, Laurenti E, Notta F, Dick JE, Basso G, Hunger SP, Loh ML, Devidas M, Wood B, Winter S, Dunsmore KP, Fulton RS, Fulton LL, Hong X, Harris CC, Dooling DJ, Ochoa K, Johnson KJ, Obenauer JC, Evans WE, Pui CH, Naeve CW, Ley TJ, Mardis ER, Wilson RK, Downing JR, Mullighan CG. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. Nature. 2012;481:157–63.

Zhang MY, Churpek JE, Keel SB, Walsh T, Lee MK, Loeb KR, Gulsuner S, Pritchard CC, Sanchez-Bonilla M, Delrow JJ, Basom RS, Forouhar M, Gyurkocza B, Schwartz BS, Neistadt B, Marquez R, Mariani CJ, Coats SA, Hofmann I, Lindsley RC, Williams DA, Abkowitz JL, Horwitz MS, King MC, Godley LA, Shimamura A. Germline ETV6 mutations in familial thrombocytopenia and hematologic malignancy. Nat Genet. 2015;47:180–5.

Zuurbier L, Petricoin 3rd EF, Vuerhard MJ, Calvert V, Kooi C, Buijs-Gladdines JG, Smits WK, Sonneveld E, Veerman AJ, Kamps WA, Horstmann M, Pieters R, Meijerink JP. The significance of PTEN and AKT aberrations in pediatric T-cell acute lymphoblastic leukemia. Haematologica. 2012;97:1405–13.

Chapter 8
Environmental Factors and Exposure Time Windows Related to the Etiology of Acute Lymphoblastic Leukemia in Children

Maria Luisa Pérez-Saldivar, A. Rangel-López, A. Fajardo-Gutiérrez, and Juan Manuel Mejía-Aranguré

Abstract Our objective in this chapter is to highlight recent advances and provide a perspective on the current understanding of the environmental factors related to the etiology of acute leukemia (AL) in children. Cancer is a major cause of infant mortality and is one of the main public health problems at a global level. AL is the most frequent type of cancer among children younger than 15 years of age. The causes of most forms of leukemia are unknown; only a few types of exposure have been established as risk factors. Nowadays, AL in children is considered to be the result of the interaction of different environmental factors with a genetic susceptibility to the disease. Environmental risk factors may play an important role in the development of childhood acute lymphoblastic leukemia (ALL). A "multi-stage" model for this disease has been proposed when the first "hit" occurs, possibly before conception or in the prenatal stage, and the second hit, called genetic susceptibility, occurs in the postnatal window through environmental exposure to a particular agent. We review the published studies of risk factors associated with the development of childhood ALL in recent years and identified these "windows of exposure" – preconception,

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prenatal, and postnatal – that may be critical to the development of the disease. The results are summarized in tables and discussed. Additionally, we discuss the use of ALL-associated fusion genes and genetic polymorphisms, together or separately, as indicators of ALL susceptibility and increased risk.

Keywords Childhood acute lymphoblastic leukemia • Risk factors • Epidemiology and review

Introduction

The people have benefited in many respects from industrialization and modernization, but this has also led to drastic changes in lifestyle and environment that have had a significant impact on the pattern of disease in the population (Suk et al. 2003). Such is the case of childhood cancer, which is one of the leading causes of death worldwide; in the US population aged between 1 and 19 years, cancer is the second most frequent cause of death, surpassed only by accidents. In Mexico, cancer in children moved from the 13th position in 1971 to the second place in 2000 among the population aged 1–14 years (Woodruff et al. 2004; Juárez-Ocaña et al. 2003).

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer and has been reported to be the result of environmental exposures and genetic susceptibility. So far the cause of ALL is unknown only exposure to radiation in utero and Down Syndrome have been recognized as risk factors associated with the disease (Eden 2010); yet, these account for a very small fraction of cases. Identifying the relevant risk factors and genetic conditions that make children more susceptible to developing the disease is considered a major challenge, which has prompted the exploration of different risk factors associated with the development of ALL through epidemiological (mostly case–control) studies. Some factors have been studied more extensively than others, and some have implications for public interest, but the search for risk factors has yielded controversial results because they are not reproducible or they lack biological plausibility, which may have led to an absence of further etiological clarification. This review is aimed at identifying risk factors associated with childhood ALL that have been studied throughout the world during the past decade and any findings, in addition to new proposals that might reveal the causation of childhood ALL.

Epidemiology

Acute leukemias (ALs) are a heterogeneous group of conditions characterized by the disordered proliferation of a clone of hematopoietic cells (Ruíz Argüelles 2009). ALs are classified by morphology, immunophenotype, and cytogenetics; morphologically, ALL is the most common type of leukemia (~80 %), followed by acute myelogenous leukemia (AML) with ~20 % (Pui and Evans 2013).

Table 8.1 Childhood acute lymphoblastic leukemia (ALL) incidence in different countries

Incidence rate × 10^6	Age group	Period	Country (reference)
Americas			
43.1	0–14 years	1981–1996	Costa Rica (Monge et al. 2002)
43.4	0–14 years	1996–2006	Mexico City (Bernaldez-Rios et al. 2008)
44.9	0–14 years	1996–2000	Mexico City (Mejía-Aranguré et al. 2005)
34.2	0–11 years	1996–2000	San Salvador (Mejía-Aranguré et al. 2005)
49.5	0–14 years	2006–2007	Mexico City (Pérez-Saldivar et al. 2011)
40.9	0–19 years	1992–2004	SEER/Spanish/Hispanic (Linabery and Ross 2008)
35.5	0–14 years	1992–1994	Uruguay (Castillo et al. 2001)
44.0	0–14 years	1988–1994	USA (Hispanic children) (Glazer et al. 1999)
Europe			
44.0	0–14 years	1991–2004	Eastern Germany (Spix et al. 2008)
40.3	0–14 years	1996–2006	Greece (Petridou et al. 2008)
35.7	0–14 years	1994–2000	Ireland (Stack et al. 2007)
35.9	0–14 years	1998–2001	Nordic countries (Hjalgrim et al. 2003a)
28.2	0–14 years	1954–1998	Northwest England (McNally et al. 2001)
48.3	0–14 years	1995–1998	Northwest Italy (Magnani et al. 2003)
25.0	0–14 years	1995–1997	Yorkshire, UK (Feltbower et al. 2001)
Oceania			
40.8	0–14 years	1997–2006	Australia (Baade et al. 2010)

ALs constitute the main type of childhood cancer worldwide, representing 30–35 % of all cancers among children aged <15 years (Fajardo-Gutiérrez et al. 1999). The incidence varies in different parts of the world; for example, high incidence rates have been reported in developed countries such as the USA, UK, Canada, Hong Kong, and Japan, whereas low incidence rates have been reported for countries in Africa (Parkin et al. 1988; Stiller 2004). This has led to the belief that the economic development of countries could be causing a high incidence of the disease; however, these differences may lie in the lack of population-based cancer registries and a lack of medical and technological resources for diagnosis in developing countries. Thus the true incidence is not known. However, studies published by developing countries report very high incidences of childhood ALL, as in the case of Mexico City and Costa Rica, in addition to the Hispanic populations of California, Texas, and Florida, where the highest incidence rates of childhood ALL have been found (Mejía-Aranguré et al. 2005; Glazer et al. 1999; Monge et al. 2002; Wilkinson et al. 2001). In general, there is a worldwide variation in childhood ALL. Table 8.1 shows the incidence rates of childhood ALL reported by different countries. It has been identified that whites are at a higher risk of developing the disease because there is a higher rate of childhood ALL compared with blacks, in whom a significantly lower incidence of this disease has been reported. There is also a slight male predominance in developed countries. These inherent ethnic and gender variables are thus causing differences in the worldwide incidence rates (Eden 2010).

A peak incidence in the development of childhood ALL has been found at between 2 and 5 years of age for most common types of leukemia, childhood ALL or precursor B-cell leukemia, in developed countries (Ramot and Magrath 1982; Pratt et al. 1988). Although an age peak has not been reported in developing countries (Greaves et al. 1993), one study of childhood ALs in Mexico City found two incidence peaks in childhood ALL: one was observed at 1–6 years of age and the other at 9–10 years, similar to those reported for US Hispanics (Pérez-Saldivar et al. 2011; de Souza Reis et al. 2011).

The seasonal variation in the birth and diagnosis of children with ALL may provide some evidence of an infectious etiology for childhood ALL, as seasonal climatic changes lead to respiratory infections or gastrointestinal infections in winter, spring, and summer. It may also indicate the presence of environmental factors such as pesticides, which are applied consistently in rural areas. Also, spatiotemporal clusters occur when an excess of childhood ALL cases is observed in a small geographical area at certain points in time compared with other areas and other times. Tables 8.3a, 8.3b and 8.3c presents studies published on this topic. In a study of ALL cases close in date and place of birth in the 4- to 14-year age group, the proportions of expected and observed cases were 14.9 and 25, respectively ($p=0.01$), finding a spatiotemporal clustering (Gustafsson and Carstensen 2000). In the UK, a spatiotemporal grouping was also found with the nearest neighbor threshold (NNT) and geographic distance ($I=29.76$); NNT was 23.6 expected cases and 35 observed cases in the group aged 18–54 months, and precursor B cell ALL ($p=0.016$) (McNally et al. 2002). In Hungary, a correlation was also observed in the spatial incidence of ALL in the group aged 0–4 years for the period 1981–2000 ($I=0.18$; $p=0.0012$) for both sexes, and for the period when the Chernobyl accident occurred (1986–1990; $I=0.1334$; $p=0.005$) (Nyari et al. 2013). Regarding seasonality, the results obtained in Denmark show a seasonal variation in the month of birth and the diagnosis of childhood ALL. The ratio of cases born with a higher peak in the month compared with other months was $R=1.4$ (1.0–2.0), with a peak in April, and the ratio of cases per month of diagnosis with a higher peak in the month compared with other months was $R=1.6$ (1.2–2.0), with a peak in October (Sørensen et al. 2001). Another study from the UK showed two peaks of births of patients diagnosed with ALL, in the months February to August, and the other in May and November ($p=0.027$) (Nyari et al. 2006). Gao et al., in an analysis of 24 studies in different countries, found a peak for the diagnosis of ALL in the UK in the 0–14 age group between May and October, and for the USA in the age group 0–19 years, there was a peak in the summer between April and August (Gao et al. 2007). Seasonal peaks with regard to date of birth reported for Hungary took place in February and August, peak by the date of diagnosis was not found, and by gender, February and August for boys and November and May for girls (Nyári et al. 2008). In France, three peaks in the date of diagnosis were reported in April, August, and December; the standardized incidence ratio (ISR) in the group aged 1–6 years in children with B-cell precursor was 1.11 (1.04–1.18) (Goujon-Bellec et al. 2013). Finally, India also showed three peaks in the time of diagnosis of childhood ALL: April to July, August to November, and December to March, presenting more cases between August and November ($n=181$, $p=0.046$) (Kulkarni and Marwaha 2013).

"Multi-step" Model and Windows of Exposition

The proposal that the onset of childhood ALL occurs in utero is supported by studies conducted in monozygotic twins in the UK, as it was observed that both children developed the disease. These studies analyzed fusion gene breakpoints and sequences in more than ten sets of twins and found that each pair shares the same breakpoint in fusion genes, resulting in a chromosomal translocation associated with a non-inherited gene, called *TEL-AML1*, also known as *ETV6-RUNX1* (Greaves et al. 2003; Greaves 2003; Zelent et al. 2004). Taking into account that the breaking points of the same gene fusion observed among patients with ALL are highly variable, the detection of the same breakpoint diagnosis in twin pairs studied elucidated the prenatal origin of leukemia, i.e., starting in a single cell cloned in utero in the fetus of one twin followed by transfer of the clonal progeny to other twin via intraplacental anastomosis (Greaves 2003; Greaves et al. 2003). These breakpoints of *TEL-AML1* were detected in both twins at birth and at diagnosis in one of the twins, thanks to the neonatal blood sample that was taken and stored for each patient. Likewise, in another study of twins, it was found that a pair of twins who had the same fusion gene, *TEL-AML1*, generated prenatally, had different deletions in *TEL*; thus, it was thought that these deletions occurred independently, as the second event was required for the development of the disease in the other twin. In addition, deletions occurring in *TEL* were detected in over 50 % of cases of *TEL-AML1*-positive childhood ALL, which supports the proposal that these deletions occur after the *TEL-AML1* fusion. These findings allowed Greaves et al. to propose the hypothesis that postnatal ALL in twins might require a second event for the disease to develop and manifest frankly (Greaves 2003; Greaves et al. 2003). In addition, deletions occurring in *TEL* were detected in over 50 % of cases of *TEL-AML1*-positive childhood ALL, which supports the proposal that these deletions might occur after the *TEL-AML1* fusion. As noted, the age peak in which there has been an increased incidence of childhood ALL is in 2–5 years, although it is diagnosed at later, before the 14 years old, so it would considering a longer response time. This was also observed in another study on twins who had the same genetic fusion, *TEL-AML1*, but with different deletions of *TEL* and a difference in diagnosis of ALL of 9 years between one twin and the other. This range clearly reflects the difference in the times at which postnatal secondary events can occur (Wiemels et al. 1999). The contribution of these twin studies has been invaluable and indicates that the multi-stage model proposed by Greaves, with a primary event initiated prenatally, applies to most, if not all, cases of childhood ALL. An event occurring prenatally and a second postnatal event or events are necessary for ALL manifestation. Added to this, experiments have been conducted with transgenic mice fitted with a transgene containing *TEL-AML1*, the fusion of which is not alone sufficient to induce leukemia, because in the absence of any additional environmental exposure, no blood disease develops (Andreasson et al. 2001). This observation also supports the multistage model of Greaves.

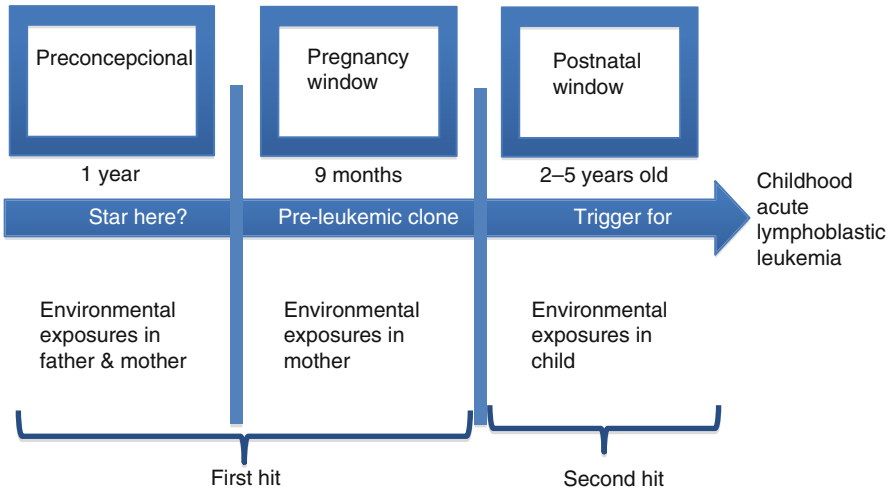

Fig. 8.1 Illustration of a multistage model of childhood acute lymphoblastic leukemia (ALL)

The proposal of this "multi-stage" model involves a primary event during the prenatal stage (preconceptional/prenatal) and a second event during the postnatal stage, providing us with the establishment of three possible exposure windows: preconception, pregnancy, and postnatally; this is of great importance in the development of childhood leukemia because critical exposure to environmental agents may be giving them the "hits" required for the development of leukemia, which according to the model starts with a first "hit" during one of the two "windows" or both to carry out the fusion of genes (genetic susceptibility) and the second "hit" occurring after birth through exposure of the child to an environmental factor or several factors that trigger leukemogenesis.

It is very important to identify which window or windows of exposure are critical and which environmental factors play an important role in the development of this disease (Fig. 8.1).

Preconceptional Window

The intended effect occurs in the preconception stage and possibly generates fusion genes, which would be the first event, the "first hit" or initiating event that cannot be discounted in the development of childhood ALL. The probable mechanism by which cancer development occurs via parents to their offspring is by carcinogenic or mutagenic damage to the germ cells (ovum and/or sperm) by exposure to carcinogens (Fabia and Thuy 1974). A review was performed in PubMed of published articles focusing on the study of risk factors during the preconception window and its specific association with childhood ALL in the past 14 years. These studies are presented in Tables 8.2a and 8.3a. The risk factors studied are: medications, X-rays,

Table 8.2a Risk factors and childhood acute lymphoblastic leukemia

Exposure	Principal results, OR (95 % CI)	Sample size Ca/Co	Annotation	Place/reference	Conclusions
Parental medication use and ALL risk		1842/1986	The CCG completed a large-scale case–control study to evaluate the potential risk factors for childhood ALL. As part of this study, they report the results of the analyses addressing parental medication use and the risk of ALL	USA/(Wen et al. 2002)	The findings of this study suggest that certain parental medication use immediately before and during the index pregnancy might influence the risk of ALL in offspring
Mothers					
Vitamins	0.7 (0.5–1.0)				
Iron supplements	0.9 (0.7–1.0)				
Antihistamines or allergy remedies	1.3 (1.0–1.8)				
Mind-altering drugs	1.5 (1.0–2.1)				
Fathers					
Amphetamines or diet pills	2.2 (1.0–4.6)				
Mind-altering drugs	1.3 (1.0–1.8)				
Mothers and fathers combined					
Amphetamines or diet pills	2.8 (0.5–15.6)				
Mind-altering drugs	1.8 (1.1–3.0)				

(continued)

Table 8.2a (continued)

Exposure	Principal results, OR (95 % CI)	Sample size Ca/Co	Annotation	Place/reference	Conclusions
Parental diagnostic X-ray and ultrasound, and risk of ALL		1842/1986	Cases were institutionally based and identified through the member institutions of the CCG, one of two large cooperative pediatric clinical trial groups in the USA that treat 93% of childhood cancer in the US	USA/(Shu et al. 2002)	Diagnostic ultrasound tests were not linked to the risk of childhood ALL before pregnancy
Maternal lower abdominal X-ray					
Total ALL	0.9 (0.8–1.2)				
T-cell ALL	1.2 (0.7–2.1)				
Early pre-B cell	0.8 (0.6–1.1)				
Pre-B cell	1.0 (0.6–1.8)				
Paternal lower abdominal X-ray					
Total ALL	1.1 (0.8–1.4)				
T-cell ALL	1.3 (0.5–3.0)				
Early pre-B cell	1.2 (0.8–1.7)				
Pre-B cell	0.9 (0.5–1.8)				

Maternal reproductive history and ALL risk		1842/1986	Data were stratified by immunophenotype	USA/(Ou et al. 2002)	The authors found that birth characteristics and maternal reproductive factors were related to subgroups of leukemia as determined by immunophenotype. Involvement of hormonal factors in the development of childhood ALL has been repeatedly implicated and needs to be further studied in other populations
ALL					
Any birth control	1.2 (1.0–1.3)				
Methods of birth control					
Oral contraceptive use	1.2 (1.0–1.4)				
Rhythm method	1.2 (1.0–1.4)				
Miscarriage before index pregnancy					
Ever	1.1 (1.0–1.3)				
One miscarriage	1.1 (1.0–1.4)				
Induced abortion before index pregnancy					
Ever	1.2 (1.0–1.4)				
One abortion	1.2 (1.0–1.5)				
T-cell ALL					
Ever	1.8 (1.0-3-3)				
One abortion	1.9 (1.0–3.6)				
Induced abortion before index pregnancy					
Ever	2.2 (1.1–4.3)				
One abortion	2.1 (1.0–4.3)				
Early pre-B cell ALL					
Methods of birth control					
Rhythm method	1.2 (1.0–1.6)				

(continued)

Table 8.2a (continued)

Exposure	Principal results, OR (95 % CI)	Sample size Ca/Co	Annotation	Place/reference	Conclusions
Exposure to alcohol and ALL risk		491/491	They also conducted a case-only study design for estimating the interaction parameter between genotype and an environmental exposure	Canada/(Infante-Rivard et al. 2002)	This study suggested a moderately increased risk with paternal preconceptional consumption of alcohol
Maternal yes/no	0.8 (0.6–1.1)				
Paternal yes/no	1.4 (1.0–2.0)				
<1/day	1.4 (1.0–2.0)				
1 <3/day	1.6 (1.1–2.5)				
≥3/day	1.7 (1.1–2.7)				
Parental occupational exposure and ALL risk (compared with non-exposed)	5.05 (1.52–16.73)	112/112	Data obtained were analyzed with regard to the type of exposure and were categorized as chemicals and physical exposures	Israel/(Abadi-Korek et al. 2006)	This study supports the relationship between parental occupational exposures, especially to solvents and pesticides and the risk of ALL in children
Maternal exposure X-rays and ALL risk		389/876	A meta-analysis of their findings in relation to paternal X-rays before conception with the published findings of previous studies was also conducted	Australia/(Bailey et al. 2010)	There was no evidence of an increased risk with maternal abdominal X-rays before pregnancy with the index child. There was some evidence of an increased risk of ALL in the offspring if the father had more than one abdominal X-ray before conception or had ever had an intravenous pyelogram
Any diagnostic X-rays	0.73 (0.55–0.95)				
Paternal exposure X-rays					
Any diagnostic X-rays	1.17 (0.88–1.55)				
Number of X-rays					
>1	1.47 (0.98–2.21)				
Any intravenous pyelograms	3.56 (1.59–7.98)				

Maternal use of folate, iron, and vitamins associated with ALL		416/1361	A meta-analysis including this and two other studies was conducted	Australia/(Milne et al. 2010)	There was some evidence of a protective effect of folate supplements taken before pregnancy
First 3 months of pregnancy					
Any folate	0.88 (0.66–1.16)				
Any iron	1.21 (0.82–1.79)				
Any vitamins	0.89 (0.68–1.16)				
Folate with iron	1.31 (0.88–1.95)				
Folate without iron	0.71 (0.51–0.98)				
Exposure to professional pest control around the home		388/870	The authors assessed whether the association between ALL and professional pest control treatments varied according to the timing of the exposure, the location or frequency of the treatment, or the type of pest being treated	Australia/(Bailey et al. 2011b)	Study provides some evidence of a modest increased risk of ALL associated with professional pest control treatments
Any pest control	1.19 (0.83–1.69)				
By cytogenetic subtype					
ETV6-Runx-1 t (12;21)	2.18 (1.03–4.60)				
Any painting done inside home	1.08 (0.80–1.45)	389/876	The Aus-ALL was a national study of the genetic, dietary, and environmental causes of childhood ALL	Australia/(Bailey et al. 2011a)	Study found some evidence of an increased risk of ALL associated with house painting before/during the pregnancy; when oil-based paints were used indoors; when someone other than the parents painted indoors; and when the mother painted the outside of the house with oil-based paint before pregnancy
Use of oil-based paint	1.45 (0.98–2.15)				
By someone other than parents					
Painting inside home	2.37 (1.30–4.30)				
Use of oil-based paint	3.50 (1.35–9.03)				
Parents painted outside					
Any painting	1.08 (0.59–1.98)				
Use of oil-based paint	2.97 (1.06–8.33)				

(continued)

Table 8.2a (continued)

Exposure	Principal results, OR (95 % CI)	Sample size Ca/Co	Annotation	Place/reference	Conclusions
Mother refueling vehicle		389/876	Aus-ALL was a national, population-based, multicenter case–control study that recruited 416 cases and 1361 controls aged younger than 15 years from mid-2003 to early 2007	Australia/(Bailey et al. 2011c)	They found no evidence that refueling a vehicle with petrol in the year before or during pregnancy increased the risk of ALL in the offspring. There was some suggestion that burning wood in a closed burner could increase the risk, but there was no dose–response relationship and chance may explain the finding
No vs. any	0.82 (0.57–1.20)				
Petrol					
No vs. any	1.00 (0.71–1.41)				
Diesel					
No vs. any	1.01 (0.56–1.83)				
LPG[a]					
No vs. any	0.63 (0.32–1.23)				
Father refueling vehicle					
No vs. any	1.56 (0.65–3.77)				
Petrol					
No vs. any	0.90 (0.54–1.51)				
Diesel					
No vs. any	1.14 (0.81–1.61)				
LPG					
No vs. any	1.07 (0.67–1.69)				
Burning of wood to heat the home					
No vs. any	1.23 (0.94–1.62)				
Closed burner					
Low vs. high	1.41 (1.02–1.94)				

Parental occupational exposure to hydrocarbons and ALL		85/85	A job-exposure matrix was used to classify parental exposure to hydrocarbons on the basis of the main industrial activity of each workplace where parents worked before (both parents) or during the index pregnancy (mother only)	Colombia/ (Castro-Jiménez and Orozco-Vargas 2011)	These findings suggest an association between childhood ALL and parental occupational exposure to carcinogenic and probably carcinogenic hydrocarbons before conception
Maternal					
Mineral oils	3.14 (1.34–7.35)				
Aliphatic	3.50 (1.41–8.67)				
Aromatics	3.50 (1.41–8.67)				
Trichloroethylene	3.50 (1.41–8.67)				
Benzene	3.00 (1.27–7.05)				
Epichlorohydrin	3.66 (1.02–13.14)				
Ethylene oxide	2.85 (1.20–6.75)				
Diesel engine exhaust	3.66 (1.48–90.4)				
Petroleum	2.18 (1.07–4.45)				
Paternal					
Mineral oils	2.15 (1.12–4.16)				
Trichloroethylene	2.15 (1.12–4.16)				
Active smoking in father	1.93 (1.06–3.54)				

(continued)

Table 8.2a (continued)

Exposure	Principal results, OR (95 % CI)	Sample size Ca/Co	Annotation	Place/reference	Conclusions
Risk of ALL and parental occupational exposure to ELF magnetic fields		379/854 mothers 328/748 fathers	This study examines the risk of ALL in the children of parents with preconceptional, periconceptional, gestational, and post-natal occupational exposure to ELF fields determined by expert assessment	Australia/(Reid et al. 2011)	In this study no increased risk of ALL was found in the offspring of parents with occupational exposure to ELF
Mother					
Occupational exposure 2 years before birth					
Any exposure	1.13 (0.87–1.48)				
Father					
Occupational exposure 1 year before birth					
Any exposure	1.33 (0.88–1.99)				
Paternal smoking and ALL risk	1.16 (1.03–1.31)	18 case–control studies were included	Systematic review and meta-analysis	USA/(Liu et al. 2011)	Evidence from the current meta-analysis strongly suggests a positive association between paternal smoking and childhood ALL. Given the high prevalence of smoking among males (35 % in developed countries and 50 % in developing countries, the association with ALL is of great relevance to public health
With the highest exposure index	1.37 (1.13–1.66)				
With paternal smoking 1 or 3 months before pregnancy	1.13 (0.98–1.29)				

Maternal smoking and ALL risk		388/868	Meta-analyses of paternal smoking, including results from Aus-ALL and those of previous studies were carried out	Australia/(Milne et al. 2012)	Study results suggest that heavier paternal smoking around the time of conception might be a risk factor for childhood ALL
Any	1.07 (0.81–1.42)				
≥15 CPD	0.97 (0.67–1.40)				
Paternal smoking					
Any	1.22 (0.92–1.61)				
≥15 CPD	1.35 (0.98–1.86)				
0–1 age group					
≥15 CPD	5.73 (1.49–22.09)				
2–4 age group					
≥15 CPD	1.36 (0.84–2.21)				
5–9 age group					
≥15 CPD	1.46 (0.80–2.66)				
10–15 age group					
≥15 CPD	0.81 (0.32–2.03)				
Meta-analyses paternal smoking	1.44 (1.24–1.68)				
Parental occupational to pesticides and risk of ALL		378/854 mothers	Organophosphate insecticides, organochlorines, phenoxy herbicides, other herbicides, and other pesticides were considered	Australia/(Glass et al. 2012)	Results did not show an increased risk of acute lymphoblastic leukemia in the offspring of fathers with occupational exposure to pesticides. However, a small over-all ALL risk cannot be ruled out from the current study results. The prevalence of maternal occupational exposure to pesticides was too low to draw any conclusions
Paternal		327/748 fathers			
Any exposure	1.06 (0.73–1.55)				
Maternal					
Any exposure	1.00 (0.09–11.2)				
1 year before birth					
Any exposure	0.66 (0.07–6.38)				

(continued)

Table 8.2a (continued)

Exposure	Principal results, OR (95 % CI)	Sample size Ca/Co	Annotation	Place/reference	Conclusions
Parental alcohol consumption and risk of ALL		393/1249	They also analyzed brain tumors and separated their results	Australia/(Milne et al. 2013b)	They found no evidence that maternal alcohol use before or during pregnancy was associated with an increased risk of ALL; rather, there was evidence of inverse associations, particularly with wine. Their findings suggest that both men and women should limit their alcohol intake when planning a pregnancy
Maternal					
Any alcohol	0.50 (0.38–0.66)				
>7 days/week	0.38 (0.26–0.55)				
Any beer	0.83 (0.58–1.19)				
Any wine	0.64 (0.49–0.84)				
Any spirits/mixers	0.82 (0.61–1.10)				
Paternal					
Any alcohol	0.72 (0.48–1.08)				
≥28 days/week	1.20 (0.79–1.83)				
Any beer	0.87 (0.63–1.19)				
Any wine	0.69 (0.52–0.94)				
Any spirits	1.11 (0.84–1.48)				
Paternal smoking		602/918	Study of the Etiology of Childhood Lympho-hematopoietic Malignancies is a population-based case–control study conducted in Italy	Italy/(Farioli et al. 2014)	This study does not support the hypothesis that parental active smoking might be associated with ALL
1–10 cigarettes/day	0.99 (0.69–1.41)				
≥11 cigarettes/day	0.93 (0.69–1.27)				

[a]Liquefied petroleum gas

Table 8.2b Parental characteristics and risk of a childhood ALL

Exposure	Principal results	Sample size	Annotation	Place/authors	Conclusions
Family history of neoplasms and ALL risk	SIR	6,994,345/1925 cases ALL Population based-cohort study	Data from the Swedish family-cancer database were used	Germany/(Couto et al. 2005)	This population-based cohort study indicates that children with first-degree relatives diagnosed with leukemia are at a higher risk of contracting ALL. However, this increase in risk seems to exist only when the affected relative is a twin, confirming a prenatal origin of childhood ALL
ALL in:					
Any first-degree relatives	3.80 (2.08–6.38)				
Cancer in parents only					
Testicular	3.12 (1.50–5.75)				
Teratoma	4.10 (1.29–9.64)				
Parental age and risk of ALL	OR 95 % (CI)	229/630,000	They used data from the population-based CCRP, Italy, and the ISTAT	Italy/(Maule et al. 2007)	Maternal age was associated with ALL
Maternal age					
≤24 years	0.66 (0.43–1.00)				
30–34 years	1.46 (1.07–1.99)				
35–39 years	1.40 (0.91–2.15)				
≥40 years	1.63 (0.71–3.74)				
5-year increase	1.30 (1.15–1.47)				
Paternal age					
≤24 years	0.85 (0.46–1.60)				
30–34 years	1.29 (0.91–1.82)				
35–39 years	1.67 (1.15–2.43)				
≥40 years	1.39 (0.85–2.28)				
5-year increase	1.15 (1.03–1.27)				

(continued)

Table 8.2b (continued)

Exposure	Principal results	Sample size	Annotation	Place/authors	Conclusions
Family characteristics and risk of ALL	OR 95 % (CI)	425/3350	This study was based on combined population-based data, obtained by linking the SCCR with census records	Swiss/(Feller et al. 2010)	Increasing maternal, but not paternal, age was associated with risk of ALL. They found only a weak association with the number of older siblings, suggesting a delay in disease manifestation
Maternal age at birth (years)					
25–29	1.14 (0.84–1.54)				
30–34	1.38 (1.02–1.89)				
≥35	1.58 (1.10–2.29)				
Paternal age at birth (years)					
25–29	0.75 (0.49–1.14)				
30–34	0.90 (0.59–1.35)				
≥35	1.15 (0.76–1.74)				
Number of older siblings					
One older sibling	1.03 (0.83–1.29)				
≥2 older siblings	0.98 (0.72–1.33)				

Family history of cancer, medical history and risk of ALL	OR 95 % (CI)	1842/1986	Dichotomous variables were created to designate any or no family history of cancer and non-malignant disease. Categorical variables were created to assess dose–response for the number of relatives with cancer and selected non-malignant diseases	USA/(Zierhut et al. 2012)	There were no associations between family history of any autoimmune diseases, immunodeficiencies, birth defects, thyroid diseases and risk of childhood ALL. These results show no association between an overall family history of cancer and childhood ALL, although they provide additional evidence for an inverse association with a family history of allergic disease. Two potentially new associations between ALL and family history of esophageal cancer and rheumatoid arthritis require confirmation in other studies and validation with medical records
Any relative with cancer					
Parents	0.98 (0.93–1.00)				
Grandparents	0.85 (0.56–1.29)				
Any relative developing cancer at age <40 years	0.91 (0.77–1.07)				
Esophageal cancer	0.92 (0.78–2.31)				
Food and drug allergies	0.22 (0.07–0.80)				
Allergic diseases	0.83 (0.73–0.95)				
Maternal side	0.86 (0.76–0.98)				
Rheumatoid arthritis	0.79 (0.65–0.96)				

alcohol, smoke, occupational and household exposure to petroleum, and the reproductive history of the mother.

In summary, the factors positively associated with the development of acute leukemia in children in the preconception window are medications used by the mother as antihistamines or allergy remedies, with an odds ratio (OR) of 1.3 (95 % confidence intervals [95 % CI] 1.0–1.8), and mind-altering drugs with an OR of 1.5 (95 % CI 1.0–2.1) and the father's use of amphetamines or diet pills and mind-altering drugs, with an OR of 2.8 (95 % CI 0.5–15.6) and 1.8 (95 % CI 1.1–3.0) respectively (Wen et al. 2002). Shu et al. found no risks from exposure to X-rays and ultrasound by parents in this preconception window (Shu et al. 2002), and the Australian group (Bailey et al. 2010) did not find any risk from the same factor in their study in 2010 either, with the exception of intravenous pyelography being performed in the father, with an OR of 3.56 (95 % CI 1.59–7.98). Regarding the reproductive history of the mother, contraceptive use, OR 1.2 (95 % CI 1.0–1.3), and abortions before the index pregnancy showed a slight association with ALL, OR 1.1 (95 % CI 1.0–1.3), and a slightly stronger association with T-cell, OR 1.8 (95 % CI 1.0–3.3) (Ou et al. 2002). Findings from the Canadian group on alcohol consumption by the mother before pregnancy show a slightly protective effect for the risk of ALL, OR 0.8 (95 % CI 0.6–1.1), as does alcohol consumption by the father, OR 1.4 (95 % CI 1.0–2.0), which increased when drinking by the father is multiplied by more than three times a week, OR 1.7 (95 % CI 1.1–2.7) (Infante-Rivard et al. 2002). Milne et al. also found alcohol to be a protective factor for ALL, OR 0.50 (95 % CI 0.38–0.66), and specifically when wine was consumed by the mother, OR 0.64 (95 % CI 0.49–0.84), and the father, OR 0.69 (95 % CI 0.52–0.94) during the preconception window (Milne et al. 2013). The findings on occupational exposure in an Israeli study showed a very high risk, OR 5.05 (95 % CI 1.52–16.73). This occupational exposure indicated the use of solvents and pesticides (Abadi-Korek et al. 2006) and is in agreement with the findings of a Colombian group that found a very high risk for maternal occupational exposure to hydrocarbons, such as petroleum, OR 2.18 (95 % CI 1.07–4.45), benzene, OR 3.50 (95 % CI 1.41–8.67), diesel engine exhaust, OR 3.66 (95 % CI 1.48–90.4), and others. Paternal occupational exposure was also associated with ALL and mineral oils, OR 2.15 (95 % CI 1.12–4.16) and trichloroethylene, OR 2.15 (95 % CI 1.12–4.16), at this stage (Castro-Jiménez and Orozco-Vargas 2011). Another group of Australian researchers also studied occupational exposure to pesticides by the parents, finding no increased risk for ALL in their offspring (Glass et al. 2012). Occupational exposure to magnetic fields of extremely low frequency (ELF-MFs) was also sought in this window, finding small and non-significant risks to the mother, OR 1.13 (95 % CI 0.87–1.48), and to the father, OR 1.33 (95 % CI 0.88–1.99) (Reid et al. 2011). The same Australian group also explored the maternal use of vitamins, iron, and folate from preconception, finding that folate was slightly inversely associated with the development of ALL, OR 0.88 (95 % CI 0.66–1.16) (Milne et al. 2010). With regard to smoking a meta-analysis of paternal smoking in the preconception window was conducted. Eighteen studies were assessed and OR of 1.13 was found (95 % CI 0.98–1.29) when the father smoked for 1–3 months before the pregnancy and with the highest exposure, OR 1.37 (95 % CI 1.13, 1.66) (Liu et al. 2011). Meanwhile, Milne et al. found no risk from maternal smoking with

regard to ALL, OR 1.07 (95 % CI 0.81–1.42), but they did find that a risk from paternal smoking before pregnancy was associated with the development of ALL in the age group 0–1, OR 5.73 (95 % CI 1.49–22.09), when the father smoked more than 15 cigarettes a day; in their small meta-analysis of similar studies performed, OR of 1.44 (95 % CI 1.24–1.68) was found, coinciding with the results of the 2011 meta-analysis conducted by Liu et al. (Milne et al. 2012).

Other environmental factors inside and around the home have also been studied in this preconception window, such as exposure to pesticides around the home, but only a modest risk has been found, OR 1.19 (95 % CI 0.83–1.69), which increases with the presence of the rearrangement of *ETV6-RUNX1* t(12;21), OR 2.18 (95 % CI 1.03–4.60) (Bailey et al. 2011). The use of paint in the home was also studied, finding a significant risk when person other than the parents painted the interior of the house, OR 2.37 (95 % CI 1.30–4.30), and when oil-based paint was used, OR 3.50 (95 % CI 1.35–9.03), exterior painting OR 2.97 (95 % CI 1.06–8.33) (Bailey et al. 2011). When exposure of parents in the home to vehicle fuel and burning wood was studied, the only risk was found in burning wood to heat the home, OR 1.23 (95 % CI 0.94–1.62) (Bailey et al. 2011).

Additionally, the authors have been studying some of the biological characteristics of the parents, such as age at pregnancy and a family history of cancer associated with leukemia. The results of a cohort study in Germany found that the standardized incidence ratio (SIR) for a family history of neoplasms in first-degree relatives was 3.80 (95 % CI 2.08–6.38), and for testicular cancer and teratoma in parents, the SIR was 3.12 (95 % CI 1.50–5.75) and 4.10 (95 % CI 1.29–9.64), respectively (Couto et al. 2005). Zierhut et al. did not find any association between ALL and a family history of cancer reported in the medical records, but found an inverse association with a family history of allergies, rheumatoid arthritis, and allergies in the mother, OR 0.83 (95 % CI 0.73–0.95), OR 0.79 (95 % CI 0.65–0.96), and OR 0.86 (95 % CI 0.76–0.98) respectively (Zierhut et al. 2012). The age of the parents was also studied in Italy, finding a risk of ALL with a maternal age of 30–34 years, OR 1.46 (95 % CI 1.07–1.99), and an increase every 5 years after the age of 40 years, OR 1.30 (95 % CI 1.15–1.47). Regarding the father's age, the risk of ALL in the group aged 35–39 years was OR 1.67 (95 % CI 1.15–2.43), and OR 1.15 (95 % CI 1.03–1.27) with an increase every 5 years after age of 40 years (Maule et al. 2007). These results agree with those from Feller et al. for maternal age 30–34 years, OR 1.38 (95 % CI 1.02–1.89), and OR 1.58 (95 % CI 1.10–2.29) for maternal age over 35 years. For the father no increased risk was observed for age and ALL in the offspring (Feller et al. 2010).

Pregnancy Window

Exposures taking place during this window may play a major role in generating the first blow to the development of ALL. During this window, it is proposed that translocations, gene fusion, and genetic polymorphisms might occur as a result of an error in the repair of breaking the strands of DNA may be occurring replication

errors. One possible mechanism for this "second hit" is by transplacental transmission to the fetus of any mutagenic damage incurred during development due to exposure to a carcinogenic agent (Autrup 1993). The most important childhood ALL translocations involve genes encoding transcription factors. These translocations produce pairs of deregulated genes or chimeric fusion protein with altered transcriptional regulation or altered constitutive kinase (Look 1997; Greaves and Wiemels 2003), generating a loss of control of the cell cycle and cell proliferation without differentiation. Translocations, fusion genes, and polymorphisms may be responsible for an increased susceptibility gene in these children, which requires the second event, or "second hit" for the development of the disease.

Tables 8.3a, 8.3b and 8.3c shows the risk factors studied in this window: occupational exposure and exposure in the home of the parents to paints, solvents, petroleum, and pesticides; alcohol, smoking actively and passively, supplements taken by the mother such as vitamins, iron, and folate; diet during pregnancy, X-ray, ultrasound, and viruses. Occupational exposure of the parents to ELF-MFs, birth weight, gestational age, birth order, and parental age at pregnancy have also been explored, in addition to other variables such as socioeconomic status, seasonality, and clusters of childhood ALL with the dates of birth and diagnosis, population-mixing, among others. Risk factors positively associated with childhood ALL during this window for the past 14 years include the use of paints at home when more than four rooms were painted, OR 1.7 (95 % CI 1.1–2.7) (Freedman et al. 2001). Bailey et al. found a high risk when the mother painted using oil paints, OR 4.05 (95 % CI 0.91–17.92) during pregnancy, and a reduced risk in the case of paternal exposure, OR 1.26(95 % CI 0.78–2.03) (Bailey et al. 2011). Also, the use of pesticides by professionals around the house during pregnancy produced a modest increase in the development of ALL, OR 1.30 (95 % CI 0.86–1.97), and the risk increased with the presence of the rearrangement *ETV6-RUNX1* t(12;21), OR 2.73 (95 % CI 1.21–6.15) (Bailey et al. 2011). On the other hand, in a meta-analysis that included 28 case–control studies and one cohort study analyzing the maternal association between exposure to benzene during pregnancy and the risk of ALL in descendants found for the use of solvents an OR of 1.25 (95 % CI 1.09–1.45), petroleum 1.42 (95 % CI 1.10–1.84), and paint 1.23 (95 % CI 1.02–1.47) (Zhou et al. 2014). In addition, it is found that the use or storage of some chemicals increased the risk of childhood ALL, OR 2.20 (95 % CI 1.04–4.64) (Castro-Jiménez and Orozco-Vargas 2011).

The maternal exposure to X-rays and ultrasound during pregnancy was studied and was not linked to the development of ALL in offspring, OR 0.9 (95 % CI 0.8–1.1) (Shu et al. 2002). These results agree with those found by the Australian group regarding the connection between exposure to X-rays during pregnancy and ALL, OR 0.46 (95 % CI 0.15–1.57) (Bailey et al. 2010). Some medications, such as antihistamines or allergy remedies and mind-altering drugs taken by the mother, have been studied, showing an OR of 1.3 (95 % CI 1.0–1.8) and an OR of 1.5 (95 % CI 1.0–2.1) when used during pregnancy (Wen et al. 2002). Interestingly, the Australian group evaluated the use of supplements, such as iron and folate taken by the mother during this stage. Initially, they found an inverse relationship with childhood ALL, OR 0.37 (95 % CI 0.21–0.65), for iron/folate, and OR 0.40 (95 % CI 0.21–0.73), for

Table 8.3a Risk factors during pregnancy and childhood acute leukemia

Exposure	Principal results (95 % CI)	Sample size Ca/Co	Annotation	Place/reference	Conclusions
House painting and ALL risk		640/640	Exposure was classified by the total number of rooms painted (1–2, 3–4, >4 rooms), and the frequency (1–2, 3–5, >5 times since birth)	USA/(Freedman et al. 2001)	This study found important risks for childhood ALL associated with substantial pre-birth exposure to indoor house painting
Yes vs. no	1.2 (0.9–1.5)				
Number of rooms painted vs. never					
>4	1.7 (1.1–2.7)				
Supplements used by mother and ALL risk		83/166	They analyzed other paternal exposures in the 5 years before the child's birth, such as paints or pigments, agricultural chemicals, solvents, and ionizing radiation	Australia/ (Thompson et al. 2001)	The results and related evidence lend support to the hypothesis that folate supplementation in pregnancy might reduce the risk of childhood ALL
Iron or folate	0.37 (0.21–0.65)				
Iron and folate	0.41 (0.22–0.75)				
Folate with or without iron	0.40 (0.21–0.73)				
Iron alone	0.75 (0.37–1.51)				

(continued)

Table 8.3a (continued)

Exposure	Principal results (95 % CI)	Sample size Ca/Co	Annotation	Place/reference	Conclusions
Maternal diagnostic X-ray, ultrasound and risk of ALL		1842/1986	Cases were institutionally based and identified through the member institutions of the CCG, one of two large cooperative pediatric clinical trial groups in the USA that treat 93% of childhood cancers in the USA	USA/(Shu et al. 2002)	In utero diagnostic ultrasound tests were not linked to the risk of childhood ALL. We found little consistent evidence that in utero diagnostic ultrasound tests or X-rays were linked to an increased risk of childhood ALL
Total ALL					
Ever had ultrasound	0.9 (0.8–1.1)				
Ever had X-ray	1.0 (0.8–1.3)				
Ever had pelvimetric X-ray	1.2 (0.8–1.7)				
T-cell ALL					
Ever had ultrasound	1.2 (0.7–1.9)				
Ever had X-ray	1.0 (0.5–2.3)				
Ever had pelvimetric X-ray	2.2 (0.6–7.6)				
Early pre-B cell					
Ever had ultrasound	0.9 (0.7–1.1)				
Ever had X-ray	0.9 (0.6–1.3)				
Ever had pelvimetric X-ray	1.2 (0.7–2.2)				
Pre-B cell					
Ever had ultrasound	1.2 (0.8–2.0)				
Ever had X-ray	1.1 (0.5–2.4)				
Ever had pelvimetric X-ray	0.7 (0.2–2.3)				

Parental medication use and ALL risk		1842/1986	The CCG completed a large-scale case–control study to evaluate the potential risk factors for childhood ALL. As part of this study, they report the results of the analyses addressing parental medication use and the risk of ALL	USA/(Wen et al. 2002)	Maternal use of vitamins and iron supplements during pregnancy was associated with a reduced risk of childhood ALL. Parental use of amphetamines or diet pills and mind-altering drugs, may influence the risk of childhood ALL
Mothers					
Vitamins	0.7 (0.5–1.0)				
Iron supplements	0.8 (0.7–1.0)				
Antihistamines or allergy remedies	1.3 (1.0–1.8)				
Mind-altering drugs	1.5 (1.0–2.1)				
Mothers and fathers combined					
Amphetamines or diet pills	2.8 (0.5–15.6)				
Mind-altering drugs	1.8 (1.1–3.0)				
Exposure to alcohol and ALL risk		491/491	They also conducted case-only study design for estimating the interaction parameter between genotype and an environmental exposure	Canada/(Infante-Rivard et al. 2002)	This study suggested protective effects of the maternal consumption of alcohol during pregnancy. The presence of variants in GSTM1 and CYP2E1 could modify the risk associated with prenatal exposure
Maternal yes/no	0.7 (0.5–0.9)				
<1/day	0.7 (0.5–1.0)				
≥1/day	0.8 (0.5–1.6)				
Genotypes and maternal consumption of alcohol					
GSTM1 null					
1st trimester	1.3 (0.6–2.5)				
2nd trimester	2.3 (1.0–5.1)				
3rd trimester	2.4 (1.1–5.4)				
CYP2E1*5					
1st trimester	1.2 (0.3–3.8)				
2nd trimester	2.8 (0.9–8.6)				
3rd trimester	2.6 (0.8–8.1)				

(continued)

Table 8.3a (continued)

Exposure	Principal results (95 % CI)	Sample size Ca/Co	Annotation	Place/reference	Conclusions
ALL associated with maternal EBV		342/1216	To identify maternal infection or offspring susceptibility to perinatal infection, IgM and IgG antibodies to three human herpesviruses cytomegalovirus, EBV, and human herpesvirus 6 were determined	Nordic countries/ (Lehtinen et al. 2003)	An epidemiological association between maternal EBV infection and the risk of the subsequent development of childhood ALL has been documented
IgG	1.9 (0.8–4.4)				
IgM	2.9 (1.5–5.8)				
Maternal diet and risk of ALL		138/138	The time frame covered was the year before the index pregnancy. This time period was chosen because it would represent the probable state of nutritional adequacy at the start of the pregnancy	USA/(Jensen et al. 2004)	They found maternal intake of vegetables, fruits, and protein sources to be inversely associated with childhood ALL
Vegetables	0.53 (0.33–0.85)				
Protein sources	0.40 (0.18–0.90)				
Fruits	0.71 (0.49–1.04)				
Maternal consumption of food and risk of ALL		131/131	An attempt was made to match each ALL case with one control of the same gender and similar age (±6 months)	Greece/(Petridou et al. 2005)	Results found evidence that young children of women who during their index pregnancy tended to consume what is currently considered to be a "healthy" diet, which is a diet high in vegetables, fruits, fish, and seafood and low in meat and meat products, sugars, and syrups, are at a lower risk of ALL
Fish and seafood	0.72 (0.59–0.89)				
Fruits	0.72 (0.57–0.91)				
Vegetables	0.76 (0.60–0.95)				
Meat and products	1.25 (1.00–1.57)				
Sugars and syrups	1.32 (1.05–1.67)				

Parental occupational exposure and risk of ALL (compared with non-exposed)	4.90 (0.94–25.53)	112/112	Data obtained were analyzed with regard to the type of exposure and were categorized as: chemicals (general and unspecified chemicals, pesticides, and metals), physical–chemical (wood and sawdust), and physical (radiation and high temperature)	Israel/(Abadi-Korek et al. 2006)	This study supports the relationship between parental occupational exposures, especially to solvents and pesticides, and risk of ALL in children
ALL and intake of vitamin and mineral supplements by the mother		97/303	They also studied the same variables, but in the children with/without ALL	New Zealand/ (Dockerty et al. 2007)	This study, of similar size to the Australian study, does not support the hypothesis of a protective effect of folate on childhood ALL
Folic acid (any, with or without iron)	1.1 (0.5–2.7)				
Iron (any, with or without folic acid)	1.2 (0.7–2.1)				
Iron without folic acid	1.3 (0.8–2.3)				
Multivitamins	0.8 (0.2–3.1)				
Other supplements	1.5 (0.7–3.1)				
Maternal diet and risk of ALL		282/359	This is the second phase of a previous report of 138 case–control pairs from phase 1 of the NCCLS	USA/(Kwan et al. 2009)	These data suggest that it might be prudent for women to consume a diet rich in vegetables and adequate in protein before and during pregnancy as a possible means of reducing childhood ALL risk in their offspring
Vegetables	0.65 (0.50–0.84)				
Protein sources	0.55 (0.32–0.96)				
Fruit	0.81 (0.65–1.00)				
Legume food groups	0.75 (0.59–0.95)				

(continued)

Table 8.3a (continued)

Exposure	Principal results (95 % CI)	Sample size Ca/Co	Annotation	Place/reference	Conclusions
Maternal use of folate, iron, and vitamins associated with ALL		416/1361	A meta-analysis including this and two other studies was conducted	Australia/(Milne et al. 2010)	Neither the results of this new case–control study nor those of a meta-analysis of these results with those of other comparable studies support the hypothesis that the maternal use of folate supplements during pregnancy might protect against the risk of childhood ALL. Meta-analysis suggests that taking vitamin supplements in general during pregnancy might protect against childhood ALL, but, with the current evidence, this effect is unlikely to be large or, even real, due specifically to folate
First 3 months of pregnancy					
Any folate	0.95 (0.72–1.26)				
Any iron	0.95 0.68–1.34)				
Any vitamins	0.99 (0.75–1.31)				
Folate with iron	0.90 (0.64–1.28)				
Folate without iron	1.05 (0.76–1.46)				
Last 6 months of pregnancy					
Any folate	1.00 (0.76–1.29)				
Any iron	0.94 (0.71–1.24)				
Any vitamins	1.02 (0.79–1.31)				
Folate with iron	0.91 (0.67–1.22)				
Folate without iron	1.25 (0.86–1.81)				
Maternal exposure to X-rays and risk of ALL		389/876	A meta-analysis of their findings in relation to paternal X-rays before conception with the published findings of previous studies was also conducted	Australia/(Bailey et al. 2010)	There was no evidence of an increased risk with maternal abdominal X-rays before the birth of the index child
Any diagnostic X-rays	0.46 (0.15–1.57)				

Maternal consumption of coffee and tea and ALL		337/697	Aus-ALL is a national, population-based, multicenter case–control study that recruited 416 children with ALL and 870 controls over 4 years	Australia/(Milne et al. 2011)	The observed increased risk associated with coffee and tea consumption may be confined to ALL with translocations. These associations should be explored further in large international consortia
Coffee consumption	0.89 (0.61–1.30)				
2 + cups/day	1.12 (0.72–1.74)				
Tea consumption	0.82 (0.56–1.18)				
2 + cups/day	0.82 (0.54–1.23)				
Cases with translocations					
Coffee consumption	1.18 (0.60–2.33)				
2 + cups/day	1.48 (0.69–3.20)				
Tea consumption	1.32 (0.69–2.51)				
2 + cups/day	1.52 (0.76–3.04)				
Exposure to professional pest control around the home and risk of ALL		388/870	They assessed whether the association between ALL and professional pest control treatments varied according to the timing of the exposure, the location or the frequency of the treatment, or the type of pest being treated	Australia/(Bailey et al. 2011b)	Study provides some evidence of a modest increased risk of ALL associated with professional pest control treatments, particularly if termite treatment was carried out before or during the index pregnancy
Any pest control	1.30 (0.86–1.97)				
Any genetic feature	1.64 (1.02–2.65)				
ETV6-Runx-1 t (12;21)	2.73 (1.21–6.15)				

(continued)

Table 8.3a (continued)

Exposure	Principal results (95 % CI)	Sample size Ca/Co	Annotation	Place/reference	Conclusions
Any painting done inside the home associated with ALL		389/876	The Aus-ALL was a national study of the genetic, dietary, and environmental causes of childhood ALL	Australia/(Bailey et al. 2011a)	Some evidence of an increased risk of ALL associated with house painting during the pregnancy and when the mother painted the outside of the house with oil-based paint during the pregnancy
Use of oil-based paint	1.11 (0.80–1.53)				
By someone other than parents	1.20 (0.76–1.92)				
Painting inside home					
Use of oil-based paint	1.58 (0.83–2.99)				
Mother (±father or other)	0.97 (0.35–2.64)				
Any painting	1.00 (0.49–2.07)				
Use of oil-based paint	4.05 (0.91–17.92)				
Father (±mother or other)					
Any painting	0.97 (0.72–1.31)				
Use of oil-based paint	1.26 (0.78–2.03)				
Paternal smoking and risk of ALL	1.19 (1.07,1.32)	18 case–control studies were included	Systematic review and meta-analysis	USA/(Liu et al. 2011)	Evidence from the current meta-analysis strongly suggests a positive association between paternal smoking and childhood ALL
With the highest exposure index	1.34 (1.02, 1.77)				
Maternal passive smoking and risk of ALL	2.00 (1.07–3.71)	85/85	An occupational exposure matrix was used to classify parental exposure to hydrocarbons on the basis of the main industrial activity of each workplace where parents worked during the index pregnancy (mother only)	Colombia/ (Castro-Jiménez and Orozco-Vargas 2011)	These findings suggest an association between childhood ALL and parental occupational exposure to carcinogenic and probably carcinogenic hydrocarbons before conception
Use or storage of any chemical product	2.20 (1.04–4.64)				
Petroleum	2.80 (1.01–7.77)				

Risk of ALL and parental occupational exposure to ELF magnetic fields and risk of ALL		379/854 mothers	This study examines the risk of ALL in the children of parents with preconceptional, periconceptional, gestational, and post-natal occupational exposure to ELF fields determined by expert assessment	Australia/(Reid et al. 2011)	In this study no increased risk of ALL was found in the offspring of parents with occupational exposure to ELF
Mother		328/748 fathers			
Occupational exposure in the 1 year before birth					
Any exposure	1.11 (0.86–1.44)				
Any time before birth	0.96 (0.74–1.25)				
Father					
Occupational exposure in the 1 year before birth					
Any exposure	1.33 (0.88–1.99)				
Any time before birth	0.78 (0.56–1.09)				
Maternal smoking associated with ALL		388/868	Meta-analyses of paternal smoking, including results from the Aus-ALL and those of previous studies were carried out	Australia/(Milne et al. 2012)	Study results suggest that heavier paternal smoking around the time of conception might be a risk factor for childhood ALL
Any	1.02 (0.76–1.37)				
≥15 CPD	0.98 (0.67–1.44)				
Paternal smoking					
Any	1.28 (0.97–1.70)				
≥15 CPD	1.46 (1.05–2.01)				

(continued)

Table 8.3a (continued)

Exposure	Principal results (95 % CI)	Sample size Ca/Co	Annotation	Place/reference	Conclusions
Parental occupational exposure to pesticides and risk of ALL		378/854 mothers 327/748 fathers	Organophosphate insecticides, organochlorines, phenoxy herbicides, other herbicides, and other pesticides were considered	Australia/(Glass et al. 2012)	Results did not show an increased risk of acute lymphoblastic leukemia in the offspring of fathers with occupational exposure to pesticides. However, a small overall risk of ALL cannot be ruled out according to the current study results. The prevalence of maternal occupational exposure to pesticides was too low to draw any conclusions
Paternal					
Any exposure	1.06 (0.73–1.55)				
Maternal					
Any exposure	0.66 (0.07–6.38)				
Any time before birth	0.64 (0.21–1.96)				

Maternal dietary intake of folate, vitamins B6 and B12 associated with ALL		333/695	They obtained the information through FFQs and the nutrient intake was quantified using a customized computer software package that merged data from Australian food composition databases for folate and energy and for B6 and B12	Australia/(Bailey et al. 2012)	Results are consistent with a modest protective effect of a higher dietary intake of folate and vitamin B12 against ALL in the offspring
Any folate	1.05 (0.75–1.48)				
Any B6 or B12	1.22 (0.87–1.71)				
Energy-adjusted dietary folate (mcg)					
>524–624	0.44 (0.27–0.71)				
>624	0.70 (0.44–1.12)				
Energy-adjusted dietary B6 (mcg)					
>1.67–1.85	1.28 (0.82–2.00)				
>1.85	1.60 (1.02–2.51)				
Energy-adjusted dietary B12 (mcg)					
>4.27–5.34	0.85 (0.56–1.31)				
>5.34	0.49 (0.31–0.77)				

(continued)

Table 8.3a (continued)

Exposure	Principal results (95 % CI)	Sample size Ca/Co	Annotation	Place/reference	Conclusions
Maternal alcohol and risk of ALL		393/1249	The authors also analyzed brain tumors and separated their results	Australia/(Milne et al. 2013b)	The authors found no evidence that maternal alcohol use before or during pregnancy was associated with an increased risk of either cancer; rather, there was evidence of inverse associations, particularly with wine. Their findings suggest that both men and women should limit their alcohol intake when planning a pregnancy
Any alcohol	0.62 (0.48–0.81)				
>2 days/week	0.56 (0.37–0.86)				
Any beer	0.71 (0.43–1.19)				
Any wine	0.66 (0.49–0.89)				
Any spirits/mixers	0.74 (0.47–1.17)				
Parental smoking and ALL risk		767/1139	This study evaluated the risk of childhood ALL by cytogenetic type in children	USA/(Metayer et al. 2013b)	The authors' data suggest that combined exposures to tobacco smoking before the child's birth including during the preconception period and after birth might be involved in the etiology of childhood ALL, consistent with the two-step model for leukemogenesis
Maternal smoking					
Yes vs. no	0.83 (0.56–1.24)				
Paternal smoking					
Yes vs. no	1.17 (0.91–1.50)				
Joint effect of prenatal smoking:					
Maternal	0.60 (0.27–1.35)				
Paternal	0.91 (0.68–1.22)				
Paternal prenatal smoking + child's passive smoking and ALL t (12;21)	2.08 (1.04–4.16)				

Paternal smoking and risk of ALL		602/918	SETIL, a study on the etiology of child-hood lympho-hematopoietic malignancies), is a population-based case–control study conducted in Italy	Italy/(Farioli et al. 2014)	This study does not support the hypothesis that parental active smoking is associated with ALL. Maternal exposure to second-hand smoke was associated with ALL
1–10 CPD	0.81 (0.56–1.19)				
≥11 CPD	1.01 (0.74–1.39)				
Maternal smoking 1st trimester pregnancy					
1–10 CPD	1.06 (0.71–1.59)				
≥11 CPD	1.64 (0.67–4.03)				
Maternal second-hand smoke					
<2 h/day	1.09 (0.75–1.61)				
2–4 h/day	1.81 (1.20–2.73)				
>4 h/day	1.83 (1.23–2.71)				
Maternal benzene exposure and risk of ALL		28 case–control studies and one cohort study (16,695/1,472,786)	A meta-analysis of epidemiological studies	China/(Zhou et al. 2014)	Based on the present meta-analysis, they concluded that maternal solvent, paint, and petroleum exposure during pregnancy are associated with ALL. Avoidance of maternal benzene exposure during pregnancy may contribute to a decrease in the risk of childhood ALL
Solvent	1.25 (1.09–1.45)				
Petroleum	1.42 (1.10–1.84)				
Paint	1.23 (1.02–1.47)				
Maternal smoking	0.99 (0.93–1.06)				

(continued)

Table 8.3a (continued)

Exposure	Principal results (95 % CI)	Sample size Ca/Co	Annotation	Place/reference	Conclusions
Virus infection and risk of ALL		70 pretreatment patients (66 with B cell ALL and 4 with T-cell ALL)	Viral genomes were screened in 70 bone marrow samples from ALL patients through standard and a more sensitive nested PCR	Mexico/(Morales-Sánchez et al. 2014)	Results argue that viral genomes were not present in all leukemic cells, and, hence, infection most likely was not part of the initial genetic lesions leading to ALL. The high statistical power of the study suggested that these agents might not be involved in the genesis of ALL in Mexican children
EBV+	0.43 (0.12–1.57)				
HCMV+	0.72 (0.22–2.32)				
HHV6+	2.29 (0.4–15.32)				
Any coinfection	1.12 (0.24–5.2)				

Table 8.3b Life factors, birth characteristics and childhood ALL

Exposure	Principal results (95% CI)	Sample size Ca/Co	Annotation	Place/authors	Conclusions
ALL		1842/1986	Data were stratified by immunophenotype	USA/(Ou et al. 2002)	This study suggests that the association of ALL with birth characteristics and maternal reproductive factors varies with the immunophenotype of the ALL
Maternal age					
<20	1.4 (1.1–1.9)				
Paternal age					
<25	1.2 (1.0–1.4)				
40/over	1.4 (1.0–1.9)				
Birth order					
2nd	1.3 (1.1–1.6)				
3rd	1.5 (1.2–2.0)				
4th/over	2.0 (1.3–3.0)				
Birth weight					
>4000 g	1.4 (1.1–1.8)				
T-cell ALL					
>4000 g	2.4 (1.1–5.5)				
Early pre-B cell ALL					
Paternal age					
<25	1.3 (1.0–1.6)				
40/over	1.7 (1.1–2.7)				
Birth order					
2nd	1.3 (1.0–1.6)				
3rd	1.5 (1.0–2.2)				
4th/over	2.0 (1.1–3.6)				
Pre-B cell ALL					
Maternal age					
<20	3.4 ((1.4–8.4)				
35/over	2.6 (1.1–5.9)				
Birth order					
2nd	1.9 (1.1–3.3)				

(continued)

Table 8.3b (continued)

Exposure	Principal results (95% CI)	Sample size Ca/Co	Annotation	Place/authors	Conclusions
Paternal age ≥35 years	1.49 (0.96–2.31)	188/434,745	Historical cohort study	UK/(Murray et al. 2002)	This population-based cohort study is the first such study to demonstrate that being born into a household of high socio- economic position or low household density is a risk factor for the development of childhood ALL. There is also evidence that being born into an extended family may be protective. These findings offer support for the theory that modulation of the immune system by early exposure to infection is important in reducing future risk of ALL
History of miscarriage (1 or more vs. none)	0.86 (0.78–0.96)				
Gestational age ≥40 weeks	0.67 (0.48–0.94)				
High birth weight (≥3500 g)	1.66 (1.18–2.33)				
Three or more adults in the household	0.58 (0.21–1.61)				
Household density (≥1 person per room)	0.56 (0.35–0.91)				
High birth weight (>4000 g)	1.26 (1.17–1.37)	18 case–control studies (10,282 cases)	Meta-analysis, data were analyzed by ALL and AML separately	Denmark/(Hjalgrim et al. 2003b)	The association between birth weight and leukemia risk was observed consistently in studies. High birth weight may result from high levels of growth factors in utero, and these growth factors may increase the risk of ALL by inducing proliferative stress on the bone marrow

Low birth weight (≤3000 g)	$p=0.01$	32	Transversal study. Guthrie cards were analyzed for the presence of preleukemic cells. Positive screening cards were strongly associated with lower birth weight	Germany/(Gruhn et al. 2008)	Positive screening cards were strongly associated with lower birth weight. Only large preleukemic cell clone populations persist, as detected in the Guthrie cards of the lower birth weight patients
1-standard deviation increase in POB	1.18 (1.04–1.35)	347/762	Variable was constructed POB the ratio of observed birth weight to "optimal birth weight" at the estimated gestational age at birth	Australia/(Milne et al. 2009)	Results confirm a positive association between the rapidity of fetal growth and the risk of childhood ALL and were consistent with a biologically plausible mechanism of accelerated growth associated with higher levels of circulating IGF-I in the fetus
Taller at diagnosis than population control		207/1481	Biometric parameters before the induction of chemotherapy were available	Australia/(Davis et al. 2011)	Results suggest that children diagnosed with ALL in Western Australia are slightly taller than controls in the general population. It is consistent with the notion that growth factors might play a role in the pathogenesis of ALL
Male	0.67 cm (0.21–1.54)				
Female	0.30 cm (0.68–1.28)				
Fetal growth with IGF1 gene non-Hispanic and Hispanics	$p=0.002$	377/448	Eight genes involved in fetal growth and body size were evaluated	USA/(Chokkalingam et al. 2012)	Genes regulating fetal growth and body size (LGF) were significantly associated with risk of childhood ALL
IGF2 in Hispanics	$p=0.040$				
IGF2R in Hispanics	$p=0.051$				
IGF2R in non-Hispanics	$p=0.009$				

(continued)

Table 8.3b (continued)

Exposure	Principal results (95% CI)	Sample size Ca/Co	Annotation	Place/authors	Conclusions
High birth weight (≥4500 g)	1.2 (1.1–13)	4075/12,065	Pooled data from three of the largest childhood cancer case–control studies ever conducted	USA, UK, Germany/ (Roman et al. 2013)	There were increased risks of ALL in high birth-weight babies and those who are LGA and those with an increased proportion of optimal weights
Risk per kg increase at birth					
≤1500 g	0.2 (0.1–0.7)				
≥4500 g	1.2 (0.9–1.6)				
<10th centile	0.8 (0.7–0.9)				
≥90th centile	1.3 (1.1–1.4)				
Large gestational age relative to appropriate for gestational age	1.24 (1.13–1.36)	12 case–control studies (7348/12,489)	Pooling of original data from 12 case–control studies participating in the Childhood Leukemia International Consortium	International/(Milne et al. 2013a)	12 case–control studies internationally reveal strong evidence of an association observed in children whose birth weight was less than 4000 g, suggesting that accelerated fetal growth might be associated with an increased risk of ALL in the absence of high birth weight
1 standard deviation increase in POB	1.16 (1.09–1.24)				
Small-for-gestational-age	0.86 (0.78–0.96)				

Table 8.3c Seasonal variations and space-time clustering

Exposure	Principal results	Sample size	Annotation	Place/authors	Conclusions
Spatiotemporal clustering and ALL		1020 ALL	Close-pair method of Knox was used	Sweden/(Gustafsson and Carstensen 2000)	Results strengthen the evidence of spatiotemporal clustering around the birth date in children who later developed ALL and support the hypothesis that pre- or perinatal infections might induce a process leading to ALL
4- to 14-year age group case-pair with ALL observed close in date and place of birth	Expected = 14.9 Observed = 25 $p = 0.01$				
Seasonal variation in the month of birth and diagnosis in childhood ALL		458 ALL	Periodic regression was used	Denmark/(Sørensen et al. 2001)	Data provide some evidence that the occurrence of ALL in childhood may result, in part, from exposure to one or more infectious agents in utero or postnatally
ALL case births at the peak month/case births at the trough month	Ratio = 1.4 (95 % CI, 1.0–2.0) peak April				
ALL case diagnosis at the peak month/case diagnosis at the trough month	Ratio = 1.6 (95 % CI, 1.2–2.0) peak October				

(continued)

Table 8.3c (continued)

Exposure	Principal results	Sample size	Annotation	Place/authors	Conclusions
Spatiotemporal clustering of ALL by immunophenotype		512 ALL	NNT and geographical distance were applied for Knox test and K-function analysis	UK/(McNally et al. 2002)	The clustering was only apparent among spatiotemporal pairs that involved at least one female case. The current evidence would suggest that there might be greater exposure to the etiological agent at an earlier age, or a greater prevalence of any agent
Analyses by disease group and age precursor B cell ALL:	NNT $I = 29.76$ $p = 0.005$		I = proportion of pairs whose distance apart is ≤ t in time and ≤ s in space		
Aged 18–54 months					
Analysis of precursor B-cell ALL aged 18–54 months by gender and population density:	NNT Expected = 23.6 Observed = 35				
Female, any clustering pairs	Strength = 48.6 % $p = 0.016$				
Seasonality of birth for ALL	Two peaks, one in early February and early August and two through May and November (amplitude 0.34 $p = 0.027$)	121 ALL	Walter–Elwood's method and logistic regression model were applied	UK/(Nyari et al. 2006)	Both peaks reflected the seasonality of infectious diseases in temperate climates: respiratory virus infections (e.g., influenza) show marked seasonality occurring in the winter months and gastrointestinal infections peak in the summer months
Six-month period					

Seasonality for ALL	Peak reported	24 articles from 11 countries (27,000 cases)	Data were reanalyzed using an angular methodology and the von Mises distribution	UK/(Gao et al. 2007)	In general terms, no consistent evidence for the seasonality of ALL has been established. Thus, despite extensive study, neither the presence nor the absence of a seasonal component to ALL has been firmly established
0–14 years old	May–October (England)				
0–19 years old	Summer April–August (USA)				
Seasonality related to date of diagnosis	Not found	121 ALL	Logistic regression with harmonic components was applied	Hungary/(Nyári et al. 2008)	Results provide some evidence that male-specific immune responses to infections around the time of birth may explain the male predominance in the incidence of ALL
Date of birth and ALL	February and August				
Seasonality by gender related to month of birth					
Male	February, August				
Female	November, May				
Spatial autocorrelation incidence of ALL 1981–2000	$I=0.18$ $p=0.0012$	134 ALL	Group aged 0–4 years was studied. The Potthoff–Whittinghill and Moran I autocorrelation methods were used to test for spatial clustering	Hungary/(Nyari et al. 2013)	Study is consistent with an environmental etiology for ALL in children associated with environmental factors in small geographical areas. Although a possible effect of the Chernobyl accident was found in the autocorrelation analysis, the role of chance cannot be excluded
By gender:					
Male	$I=0.14$ $p=0.028$				
Female	$I=0.04$ $p=0.16$				
By periods:					
1986–1990 (Chernobyl accident)	$I=0.1334$ $p=0.005$				
1991–1995	$I=0.054$ $p=0.018$				

(continued)

Table 8.3c (continued)

Exposure	Principal results	Sample size	Annotation	Place/authors	Conclusions
Seasonal variations	Standardized incidence ratio = 1.11 (1.04–1.18) Three peaks: April, August and December	6686 ALL	The seasonality of ALL was tested with Poisson regression models on ALL patients aged 0–14 years, and specifically on the B-cell precursor of ALL	France/(Goujon-Bellec et al. 2013)	The study also suggests seasonal variations in the month of diagnosis for boys aged 1–6 years
1–6 years old boys B-cell precursor					
Seasonality in diagnosis of ALL	Total patients diagnosed with ALL	446 SDI	Only patients with SDI <8 weeks were included in the study to minimize the bias generated due to delayed presentation on seasonality	India/(Kulkarni and Marwaha 2013)	This is the first study from Asia suggesting the presence of seasonality in the diagnosis of ALL
Summer (April–July)	142				
Monsoon (August–November)	181				
Winter (December–March)	123				
	$p = 0.046$				

folate with or without iron (Thompson et al. 2001). In New Zealand it was subsequently attempted to evaluate the use of these supplements and their association with ALL. The authors found no association between folic acid, OR 1.1 (95 % CI 0.5–2.7), or iron, OR 1.2 (95 % CI 0.7–2.1), and ALL (Dockerty et al. 2007). After the same Australian group tried to replicate their study with a larger sized case–control sample, however, they found no risk related to folate intake, OR 0.95 (95 % CI 0.72–1.26); iron, OR 0.95 (95 % CI 0.68–1.34); or vitamins, OR 0.99 (95 % CI 0.75–1.31), taken alone or in combination during the first and second semesters of pregnancy (Milne et al. 2010). In 2012, the authors returned to conduct a study with fewer cases and controls, but they found a modest protective effect for folate in doses of 524–624 mcg, OR 0.44 (95 % CI 0.27–0.71), vitamins B12 in doses >5.34 μg, OR 0.49 (95 % CI 0.31–0.77), but the risk for the consumption of vitamin B6 greater than (>) 1.85 mcg with regard to the development of childhood ALL with OR 1.60 (95 % CI 1.02–2.51) (Bailey et al. 2012). Active and passive smoking during pregnancy is one of the more consistent risk factors associated with leukemia. A meta-analysis conducted by Liu et al. including 18 case–control studies on ALL associated with paternal exposure to smoke during the pregnancy showed an OR of 1.19 (95 % CI 1.07, 1.32) (Liu et al. 2011); maternal passive smoking in Colombia showed a significant risk with an OR of 2.00 (95 % CI 1.07–3.71) (Castro-Jiménez and Orozco-Vargas 2011). Australians found no risk for the active exposure of the mother to smoking, OR 1.02 (95 % CI 0.76–1.37), but a positive risk for the father, OR 1.28 (95 % CI 0.97–1.70), coinciding with the results of Metayer et al. on maternal smoking, OR 0.83 (95 % CI 0.56–1.24), and on paternal smoking, OR 1.17 (95 % CI 0.91–1.50) (Metayer et al. 2013). The Italians found only maternal passive smoking to be associated with ALL, OR 1.81 (95 % CI 1.20–2.73), but not active smoking, OR 1.06 (95 % CI 0.71–1.59) or paternal smoking, OR 0.81 (95 % CI 0.56–1.19), during the pregnancy (Farioli et al. 2014). Alcohol consumption by the mother during pregnancy has been evaluated and an inverse association was found, OR 0.7 (95 % CI 0.5–0.9); however, when some genotypes were studied, such as GSTM1 null and CYP2E1*5, only an association with GSTM1 null and alcohol consumption in the second and third trimesters was found, OR 2.3 (95 % CI 1.0–5.1) and OR 2.4 (95 % CI 1.1–5.4), respectively (Infante-Rivard et al. 2002). Another study supports the notion that earlier alcohol consumption and specifically wine drinking by the mother during pregnancy resulted in protection from ALL, OR 0.62 (95 % CI 0.48–0.81) and OR 0.62 (95 % CI 0.48–0.81), respectively; however, these inverse associations are probably explained by confounders (Milne et al. 2013).

Moreover, maternal exposure to certain viruses by the mother during pregnancy and its association with ALL has been carried out in two studies, with varying results. IgM and Epstein–Barr virus (EBV), OR 2.9 (95 % CI 1.5–5.8), have been associated with childhood ALL (Lehtinen et al. 2003), while Morales-Sanchez et al. did not find involvement of these viruses in the development of ALL, OR 0.43 (95 % CI 0.12–1.57), after the pregnancy (Morales-Sánchez et al. 2014). Occupational exposure from parents during pregnancy has also attracted significant attention, such as the Israeli study that found a strong association in occupations involving the use of solvents and pesticides, OR 4.90 (95 % CI 0.94–25.53). Another

study looking occupational exposure to pesticides found no risk, OR 0.66 (95 % CI 0.07–6.38), with regard to childhood ALL (Glass et al. 2012). The occupational ELF-MFs in parents were also evaluated during this stage; however, slight non-significant risks were found, OR 1.11 (95 % CI 0.86–1.44) with maternal exposure and OR 1.33 (95 % CI 0.88–1.99) with paternal exposure (Reid et al. 2011). Coffee and tea consumption by the mother during pregnancy has also been evaluated, with no increased risk of childhood ALL caused by drinking coffee, OR 0.89 (95 % CI 0.61–1.30), and tea, OR 0.82 (95 % CI 0.56–1.18) (Milne et al. 2011). Finally, the study of maternal diet during pregnancy has provided encouraging results, with the use of rich vegetables, fruits, and proteins studied by one of the US groups, who found the following results: OR 0.53 (95 % CI 0.33–0.85) for vegetables, OR 0.40 (95 % CI 0.18–0.90) for fruits, and OR 0.71 (95 % CI 0.49–1.04) for proteins related to childhood ALL (Jensen et al. 2004). These results were also found by the Greeks, with a protective effect caused by eating seafood, OR 0.72 (95 % CI 0.59–0.89), fruits, OR 0.72 (95 % CI 0.57–0.91), vegetables, OR 0.76 (95 % CI 0.60–0.95), but there is a risk with meat consumption, OR 1.25 (95 % CI 1.00–1.57), and sugars and syrups, OR 1.32 (95 % CI 1.05–1.67) (Petridou et al. 2005). The same US group conducted a second phase on maternal diet in pregnancy with a larger sample size and again confirmed the protective effect of vegetable consumption, OR 0.65 (95 % CI 0.50–0.84), proteins, OR 0.55 (95 % CI 0.32–0.96), fruits, OR 0.81 (95 % CI 0.65–1.00), and legumes, OR 0.75 (95 % CI 0.59–0.95) (Kwan et al. 2009).

The factors as age of parents during pregnancy, birth order, previous abortions, birth weight, and gestational age have been studied extensively. One of the studies associated maternal age <20 years with the risk of development of childhood ALL, OR 1.4 (95 % CI 1.1–1.9), for paternal age <25 and >40 years, OR 1.2 (95 % CI 1.0–1.4) and OR 1.4 (95 % CI 1.0–1.9) respectively. When analyzed according to immunophenotyping, it was found that maternal age <20 years and >35 years were positively associated with pre-B cell, OR 3.4 (95 % CI 1.4–8.4) and OR 2.6 (95 % CI 1.1–5.9), respectively (Ou et al. 2002). In a historical cohort study, authors from the UK also found that paternal age ≥35 years posed a significant risk to ALL,OR 1.49 (95 % CI 0.96–2.31) (Murray et al. 2002). On the other hand, Ou et al. found an increased risk for the birth order of the index child, OR 2.0 (95 % CI 1.3–3.0), to the highest order; the same was observed in early pre-B cell ALL, OR 2.0 (95 % CI 1.1–3.6); birth weight >4000 g, OR 1.4 (95 % CI 1.1–1.8); and T-cell ALL, OR 2.4 (95 % CI 1.1–5.5) (Ou et al. 2002). Regarding a gestational age of ≥40 weeks, Murray et al. found an OR of 0.67 (95 % CI 0.48–0.94), and the risk of birth weight ≥3500 g had an OR of 1.66 (95 % CI 1.18–2.33) with regard to childhood ALL (Murray et al. 2002). In Denmark, a meta-analysis of 18 case–control studies was performed to evaluate the association between high birth weight (>4000 g) and the development of ALL; the risk reported had an OR of 1.26 (95 % CI 1.17–1.37) (Hjalgrim et al. 2003). An Australian group found a risk of OR of 1.18 (95 % CI 1.04–1.35), by a one standard deviation increase in the proportion of optimal weight (POB) (Milne et al. 2009). Pooled data from birth weight ≥4500 g reported in studies from the USA, the UK, and Germany found an OR of 1.2 (95 % CI 1.1–13) (Roman et al. 2013). The Childhood Leukemia International Consortium (CLIC)

pooled data and carried out a meta-analysis considering fetal growth weight for gestational age and the proportion of optimal birth weight (POBW), finding that for advanced gestational age OR was 1.24 (95 % CI 1.13–1.36), and for a one standard deviation increase in (POB), the OR was 1.16 (95 % CI 1.09–1.24). When reported, small-for-gestational-age had an OR of 0.86 (95 % CI 0.78–0.96) (Milne et al. 2013). Only a cross-sectional study in which the presence of a pre-leukemic clone on Guthrie cards and birth weight recorded in the medical records showed a relationship with low birth weight, $p = 0.01$ (Gruhn et al. 2008). Another group wanted to know the height of children diagnosed with ALL and they were found to be taller than controls: boys 0.67 cm (95 % CI 0.21–1.54) and girls 0.30 cm (95 % CI 0.68–1.28) (Davis et al. 2011). Also, the relationship of some genes involved insulin-like growth factors (*IGF*) in fetal growth and for body size a statistically significant association was found in the presence of *IGF2* and *IGF2R* in Hispanic and non-Hispanics (Chokkalingam et al. 2012).

Postnatal Window

At this stage it is assumed that the second postnatal event, or "second hit," in the development of childhood ALL occurs, according to the model proposed by Greaves. At this stage, we consider the exposures of children from an early stage of life up to the diagnosis of the disease. Greaves describes ALL starting in utero with the "first hit," which generates a long-latency pre-leukemic clone, until the "second hit" occurs with a second mutation at 2–4 years, which precipitates the onset of leukemia. Greaves' proposal that one or several environmental exposures may mount a challenge to the immune system, leading to an abnormal response of the immune system, which increases the number of pre-leukemic cells, in turn leading to leukemia. The environmental factors that Greaves proposed might be able to produce an immune response of this nature are childhood infections. However, there are other environmental factors that have been studied in the postnatal window that have been proposed to generate the "second hit" necessary for leukemia.

Tables 8.4a and 8.4b shows that in the last 14 years, different environmental factors explored in the postnatal window include residential exposure to ELF-MFs, the use of solvents and paints at home, the passive exposure of children to smoke, supplements taken by the child such as vitamins and iron, infections, X-rays, occupational exposures in parents, and smoking, which could be exposing the child indirectly. Factors positively associated with the development of childhood ALL include exposure to solvents in the home when artwork is carried out, with an OR of 4.1 (95 % CI 1.1–15.1) (Freedman et al. 2001). Also, when some painting work indoors was carried out by a person other than the parents, the OR was 1.64 (95 % CI 1.08–2.49), and when the paint used was oil-based, the OR was 2.07 (95 % CI 1.11–3.83) (Bailey et al. 2011).

The diagnostic X-ray carrying the child study to determine whether there was any association with the risk of developing ALL; however, a risk of ALL was

Table 8.4a Exposure to environmental risk factors (electromagnetic fields [EMFs], paints, solvents, pesticides, medical procedures, etc.) and childhood ALL

Exposure	Principal results (95 % CI)	Sample size Ca/Co	Annotation	Place/reference	Conclusions
Distance to		408/408	Exposure index of distance and strength of multiple power lines was created	USA/(Kleinerman et al. 2000)	They found no suggestion that any of those variables are related to risk, and no evidence that children living near high-voltage power lines are at an increased risk of acute lymphoblastic leukemia
Closest power line and ALL risk					
0–14 m	0.79 (0.46–1.34)				
Closest transmission line					
0–23 m	0.57 (0.15–2.13)				
Closest distribution line					
0–14 m	0.75 (0.43–1.30)				
Household organic solvents and ALL risk		640/640	The authors categorized the frequency of exposure as low (<1 time/month), medium (1–4 times/month), and high (>4 times/month)	USA/(Freedman et al. 2001)	Participation by household members in some common household activities that involve organic solvents was associated with elevated risks of childhood ALL
Model building					
High vs. low frequency	1.9 (0.7–5.8)				
Artwork					
High vs. low frequency	4.1 (1.1–15.1)				
Auto/truck maintenance					
High vs. low	1.5 (0.8–2.7)				

X-ray exposure and risk of ALL		1842/1986	Cases were institutionally based and identified through the member institutions of the CCG, one of two large cooperative pediatric clinical trials groups in the USA that treat 93% of childhood cancers in the USA	USA/(Shu et al. 2002)	The results of this large case–control investigation suggest that ALL might not be linked to exposure to X-rays. The absence of biological evidence linking specific immunophenotypes of childhood leukemia with low-level ionizing radiation exposures. Further progress in understanding these relationships may require in vitro and in vivo studies
Total ALL	1.1 (0.9–1.2)				
T-cell ALL	1.1 (0.7–1.7)				
Early pre-B cell	1.1 (0.8–1.3)				
Pre-B cell	1.7 (1.1–2.7)				
Parental occupational exposure and ALL risk (compared with non-exposed)	4.48 (1.78–11.26)	112/112	Data obtained were analyzed with regard to the type of exposure and were categorized as: chemicals (general and unspecified chemicals, pesticides, and metals), physical–chemical (wood and sawdust), and physical (radiation and high temperature)	Israel/(Abadi-Korek et al. 2006)	This study supports the relationship between parental occupational exposures, especially to solvents and pesticides and risk of ALL in children
Paternal exposure only	8.18 (1.48–45.21)				
Maternal exposure only	3.66 (1.28–10.47)				
Both parents exposed	7.93 (2.06–30.56)				
Organic solvents	2.11 (1.10–4.20)				
Pesticides	2.35 (1.10–5.0)				
Other hazardous	1.70 (1.14–2.44)				

(continued)

Table 8.4a (continued)

Exposure	Principal results (95 % CI)	Sample size Ca/Co	Annotation	Place/reference	Conclusions
ALL and intake of vitamin and mineral supplements by the child		97/303	Child's use of a supplement for 5 or more days before the reference date	New Zealand/ (Dockerty et al. 2007)	This study, of similar size to the Australian study, does not support the hypothesis of a protective effect of folate on childhood ALL
Folic acid (any, with or without iron)	1.0 (0.4–2.8)				
Iron (any, with or without folic acid)	1.1 (0.4–2.8)				
Iron without folic acid	1.6 (0.1–19.3)				
Multivitamins	1.0 (0.4–2.8)				
Other supplements	1.6 (0.8–3.4)				
Insecticides in home	33 % vs. 14 %	41/41	Environmental exposures were determined by questionnaire and by urinalysis of pesticide metabolites using isotope dilution gas chromatography–high-resolution mass spectrometry	USA/(Soldin et al. 2009)	Reported use of pesticides using the questionnaire did not correlate with the pesticide concentrations that were measured in the urine of either the mothers or the children. The association between ALL risk and pesticide exposure merits further studies
Pesticides levels					
Higher Ca/Co	$p<0.02$				
Organophosphate metabolites in urine Ca/Co DETP and DEDTP	$p<0.05$				
	$p<0.03$				
	$p<0.05$				
Agricultural pesticides in child's lifetime and risk of ALL		213/268	Insecticides, herbicides, fungicides, plant growth regulators were analyzed, but only fumigants were significant	USA/(Rull et al. 2009)	Results detected a modest increase in ALL risk with residential proximity to moderate levels of the agricultural use of several types of pesticides, but not at higher levels of use
Fumigants					
Low exposure	1.0				
Moderate exposure	1.7 (1.0–3.1)				
High exposure	0.8 (0.4–1.4)				

ELF and ALL risk wire code		10 case–control studies	A systematic review. Selected studies that evaluated ALL separately	Brazil/(Pelissari et al. 2009)	An association may exist between exposure to low-frequency magnetic fields and acute lymphoblastic leukemia in children, but this association is weak, preventing the observation of consistency in the findings. Future studies from a wider range of geographic regions should focus on the analysis of acute lymphoblastic leukemia, which is the subtype with the greatest impact on the increasing overall incidence of childhood leukemia
VH vs. UG	2.75 (0.90–8.44) 2.11 (0.70–6.36)				
VHCC vs. VLCC	0.88 (0.48–1.63)				
HCC vs. LCC	1.04 (0.65–1.66)				
Measurement (μT):					
≥0.13	2.83 (0.83–9.62)				
≥0.15	2.86 (0.88–9.29)				
≥0.2	3.32 (1.27–8.68)				
≥0.4	1.53 (0.91–2.56)				
	1.19 (0.77–1.84)				
	4.67 (1.15–19.00)				
Estimated ELF (μT):					
≥0.2	0.51 (0.11–2.33)				
≥0.4	0.33 (0.04–2.72)				
Distance (m):					
≤50	3.06 (1.31–7.13)				
51–100	1.61 (0.88–2.95)				
Living near high-voltage power lines and ALL risk		300/300	Data included children aged 1–18 years	Iran/(Sohrabi et al. 2010)	This study emphasizes the risk of ALL of living close to overhead high-voltage power lines. Authorities should consider legal limitations for building constructions being at least 600 m from these power lines. Overhead power lines should be changed to underground lines in existing risky neighborhoods
<600 m	2.61 (1.73–3.94)				
123 kV	9.93 (3.47–28.5)				
230 kV	10.78 (3.75–31)				
400 kV	2.98 (0.93–9.54)				

(continued)

Table 8.4a (continued)

Exposure	Principal results (95 % CI)	Sample size Ca/Co	Annotation	Place/reference	Conclusions
Exposure of child to diagnostic X-rays and ALL risk		389/876	A meta-analysis of the authors' findings in relation to paternal X-rays before conception with the published findings of previous studies was also conducted	Australia/(Bailey et al. 2010)	There was little evidence of any increased risk of childhood ALL associated with X-rays of the child or with the child having any X-rays more than 6 months before the censoring date
Before reference date					
Any diagnostic X-rays	1.21 (0.93–1.57)				
Any plain chest X-ray	1.26 (0.89–1.77)				
Any plain arm X-ray	1.49 (1.00–2.22)				
Any plain leg X-ray	1.20 (0.74–1.96)				
>6 months before reference date					
Any diagnostic X-rays	1.15 (0.88–1.51)				
Any plain chest X-ray	1.24 (0.86–1.78)				
Any plain arm X-ray	1.21 (0.77–1.91)				
Any plain leg X-ray	0.76 (0.42–1.36)				
ELF associated with ALL 24-h measurement:		162/565	–	Brazil/(Wünsch-Filho et al. 2011)	Even though these results are consistent with the small risks reported in other studies on ELF-MFs and leukemia in children, overall this results do not support an association between magnetic fields and childhood leukemia, but small numbers and likely biases weaken the strength of this conclusion
≥0.3 μT	1.09 (0.33–3.61)				
Nighttime ≥0.3 μT	1.52 (0.46–5.01)				
Distance (m):					
≤200	1.67 (0.49–5.75)				
≤50	3.57 (0.41–31.44)				

Childhood ALL and parental occupational exposure to hydrocarbons		85/85	A occupational exposure matrix was used to classify parental exposure to hydrocarbons on the basis of the main industrial activity of each workplace where the parents worked before (both parents) or during the index pregnancy (mother only)	Colombia/ (Castro-Jiménez and Orozco-Vargas 2011)	The findings showed a significantly higher risk of ALL among children whose parents were occupationally exposed to carcinogenic and probably carcinogenic hydrocarbons before the child's conception. These results also suggest that maternal exposure to these chemicals might be more important than paternal exposure
Father only					
Mineral oils	2.92 (1.16–7.36)				
1,3-Butadiene	4.18 (1.47–11.88)				
Trichloroethylene	2.76 (1.09–7.06)				
Mother only					
Mineral oils	6.68 (1.59–28.08)				
1,3-Butadiene	11.67 (1.74–78.05)				
Trichloroethylene	7.41 (1.66–33.07)				
Both parents					
Mineral oils	13.68 (3.58–52.22)				
1,3-Butadiene	2.05 (0.12–29.27)				
Trichloroethylene	17.56 (4.12–74.81)				
Risk of ALL and parental occupational exposure to ELF magnetic fields		379/854 mothers	This study examines the risk of ALL in the children of parents with preconceptional, periconceptional, gestational, and post-natal occupational exposure to ELF fields determined by expert assessment	Australia/(Reid et al. 2011)	In this study no increased risk of ALL was found in the offspring of parents with occupational exposure to ELF
Mother		328/748 fathers			
Occupational exposure 1 year after birth					
Any exposure	1.34 (0.94–1.91)				

(continued)

Table 8.4a (continued)

Exposure	Principal results (95 % CI)	Sample size Ca/Co	Annotation	Place/reference	Conclusions
Exposure to professional pest control around the home and risk of ALL		388/870	They assessed whether the association between ALL and professional pest control treatments varied according to the timing of the exposure, the location or frequency of the treatment, or the type of pest being treated	Australia/(Bailey et al. 2011b)	The study provides some evidence of a modest increased risk of ALL associated with professional pest control treatments, particularly when the child was aged between 2 and 3 years
Any pest control	1.24 (0.93–1.65)				
Pre-B cell	1.39 (1.03–1.87)				
Any genetic feature	1.46 (1.05–2.04)				
Chromosomal deletions	1.83 (1.00–3.35)				
ETV6-Runx-1 t (12;21)	2.39 (1.25–4.55)				
Any painting done inside the home		389/876	The Aus-ALL was a national study of the genetic, dietary, and environmental causes of childhood ALL	Australia/(Bailey et al. 2011a)	There was little evidence of an increased risk of childhood ALL associated with any painting inside the house after the child's birth
Use of oil-based paint	1.08 (0.81–1.45)				
By someone other than the parents	1.32 (0.89–1.97)				
Painting inside the home	1.64 (1.08–2.49)				
Use of oil-based paint	2.07 (1.11–3.83)				
Paternal smoking and ALL risk	1.20 (0.97, 1.49)	18 case–control studies were included	Systematic review and meta-analysis	USA/(Liu et al. 2011)	Evidence from the current meta-analysis strongly suggests a positive association between paternal smoking and childhood ALL
With the highest exposure index	1.35 (1.06, 1.72)				
Pyrethroid pesticide exposure and risk of ALL		176/180	Urinary metabolite levels were adjusted for creatinine (μg/g). Total metabolites means the sum of three metabolites (cis-DCCA, trans-DCCA, and 3-PBA)	China/(Ding et al. 2012)	They observed an increased risk of childhood ALL associated with increased urinary metabolite levels of pyrethroid pesticides
Total urinary pyrethroid metabolites	2.75 (1.43–5.29)				
Cis-DCCA	2.21 (1.16–4.19)				
Trans-DCCA	2.33 (1.23–4.41)				
3-PBA	1.84 (1.00–3.38)				

Exposure to herbicides in house dust and risk of ALL		269/333	Dust from carpets and rugs is a reservoir for a variety of chemicals known to be persistent (e.g., organochlorine compounds, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and nicotine)	USA/(Metayer et al. 2013a)	There were no apparent associations between the current dust levels of most herbicides analyzed, except for chlorthal and possibly alachlor. Although these compounds or added impurities have carcinogenic properties, these findings need to be replicated in an independent series
Dust levels of chlorthal					
First tertile	1.49 (0.82–2.72)				
Second tertile	1.49 (0.83–2.67)				
Third tertile	1.57 (0.90–2.73)				
Child's passive smoking at home	1.20 (0.84–1.72)	767/1139	This study examined the risk of childhood ALL according to cytogenetic type in children	USA/(Metayer et al. 2013b)	The author's data suggest that combined exposures to tobacco smoking before the child's birth, including during the preconception period and after birth, are involved in the etiology of childhood ALL, consistent with the two-step model for leukemogenesis
Joint effect of maternal prenatal smoking and child's passive smoking:					
Maternal prenatal	0.60 (0.27–1.35)				
Child's passive	1.11 (0.75–1.65)				
Exposure of both periods	1.04 (0.74–1.47)				
Joint effect of paternal prenatal smoking and child's passive smoking:					
Paternal prenatal	0.91 (0.68–1.22)				
Child's passive	0.80 (0.51–1.09)				
Exposure of both periods	1.50 (1.01–2.23)				

(continued)

Table 8.4a (continued)

Exposure	Principal results (95 % CI)	Sample size Ca/Co	Annotation	Place/reference	Conclusions
Exposure of PAH[c] and risk of ALL from bagless vacuums:		251/306	They collected carpet dust samples using a high volume small surface sampler (HVS3) and dust from the home vacuum cleaner	USA/(Deziel et al. 2014)	The increased ALL risk among participants with vacuum dust suggests that PAH exposure might increase the risk of childhood ALL; however, reasons for the different results based on HVS3 dust samples deserve further study
Benzo[a]pyrene	1.42 (0.95–2.12)				
Dibenzo[a,h]anthracene	1.98 (1.11–3.55)				
Benzo[k]fluoroanthene	1.71 (0.91–3.22)				
Indeno[1,2,3-cd]pyrene	1.81 (1.04–3.16)				
PAH toxic equivalence	2.35 (1.18–4.69)				
Exposure to PBDE[d] and risk of ALL		167/214	Carpet dust samples were collected primarily using a specialized vacuum, the high-volume small-surface sampler (HVS3; Envirometrics, Inc.), in the room where the child spent the most time while awake	USA/(Ward et al. 2014)	They found no association with ALL for most common PBDEs in residential dust, but observed positive associations for specific octa- and nonaBDEs
Summed					
pentaBDEs	0.7 (0.4–1.3)				
octaBDEs	1.3 (0.7–2.3)				
decaBDEs	1.0 (0.6–1.8)				
Highest concentrations for specific PBDEs					
BDE-196	2.1 (1.1–3.8)				
BDE-203	2.0 (1.1–3.6)				
BDE-206	2.1 (1.1–3.9)				
BDE-207	2.0 (1.03–3.8)				
Child's passive smoking and ALL risk		602/918	SETIL, a study on the etiology of childhood lymphohematopoietic malignancies) is a population-based case–control study conducted in Italy	Italy/(Farioli et al. 2014)	They found very weak evidence for an increased risk for ALL for children exposed to second-hand smoke
<1000 cigarettes	0.94 (0.61–1.45)				
1000–6999 cigarettes	1.07 (0.67–1.72)				
≥7000 cigarettes	1.36 (0.91–2.04)				

Table 8.4b Population mixing, socioeconomic status, etc.

Exposure	Principal results	Sample size	Annotation	Place/authors	Conclusions
Incidence of ALL	1.5-fold increase ($p=0.01$) (mean annual increase:0.11 cases/10^5)	1806 ALL	The authors discussed possible underlying socioeconomic factors, including infant care and breast-feeding, hygiene, birth order, industry, and pollution	Czech Republic/ (Hrusák et al. 2002)	Incidence of ALL increase in children 1–4 years of age correlating with socioeconomic and political changes. This increase was more prominent in girls. Data support the view that a considerable proportion of preschool ALL is caused by exogenous factors
1980–1998					
Incidence of ALL					
Age 1–4 years					
Males	Slope 0.13 $p=0.03$				
Females	Slope 0.09 $p>0.05$				
Population mixing	RR (95 % CI)	121 ALL	Poisson regression was used to investigate the relationship between risk of ALL and the population-mixing index based on the number of incomers	Hungary/(Nyári et al. 2006)	This study supports Kinlen's population-mixing hypothesis around the time of birth as a general risk factor for ALL in children
All children					
Age under 5 years	2.07 (0.91–4.75)				
All incomers	2.13 (1.02–4.44)				
Boys					
Age under 5 years	2.24 (0.71–7.09)				
All incomers	3.10 (1.13–8.51)				
Social stratum		507 ALL	They evaluated socioeconomic status as an ecological variable	Brazil/(Ribeiro et al. 2008)	Results support the hypothesis of an infectious etiology for childhood ALL
Poorest vs. richest	RR=0.34 (0.28–0.44)				
Households with 7 or more persons low % vs. high %	RR=0.32 (0.25–0.43)				

(continued)

Table 8.4b (continued)

Exposure	Principal results	Sample size	Annotation	Place/authors	Conclusions
Population mixing		3150 ALL	Poisson regression methods were used	England and Wales/ (Stiller et al. 2008)	The positive association with diversity of incomers into rural areas is also consistent with the higher incidence of leukemia predicted by Kinlen, where population-mixing results in below-average herd immunity to an infectious agent
Age 1–4 years old					
Rural	IRR = 1.26 $p = 0.05$				
Mixed	IRR = 1.07 $p = 0.05$				

[a]Diethyldithiophosphate
[b]Diethylthiophosphate
[c]Polycyclic aromatic hydrocarbons
[d]Polybrominated Diphenyl Ethers
VH very high, *UG* underground, *VHCC* very high current configuration, *VLCC* very low current configuration, *HCC* high current configuration, *LCC* low current configuration, *DETP* diethylthiophosphate, *DEDTP* diethyldithiophosphate.

observed with an OR of 1.1 (95 % CI 0.9–1.2), but this risk increased when analyzing the pre-B cell immunophenotype, OR 1.7 (95 % CI 1.1–2.7); thus, it is possible that some other factor might be confounding the association (Shu et al. 2002). This prompted the Australian group to conduct a study analyzing X-ray exposure in children 6 months before the date of diagnosis, but they found no significant risk, OR 1.21 (95 % CI 0.93–1.57), and when the arm was exposed to X-rays the OR was 1.49 (95 % CI 1.00–2.22) (Bailey et al. 2010). Another way to expose children to carcinogens is through occupational exposure of fathers, which indirectly expose their children through breast milk, work clothes or breath (Lowengart et al. 1987). An Israeli group conducted a study on occupational exposure and the risk of ALL and found very high risks associated with the use of solvents and pesticides in occupations, OR 4.48 (95 % CI 1.78–11.26), when the father was exposed, OR 8.18 (95 % CI 1.48–45.21), when the mother was exposed, OR 3.66 (95 % CI 1.28–10.47), and when both were exposed, OR 7.93 (95 % CI 2.06–30.56). Also, when organic solvents were used, the OR was 2.11 (95 % CI 1.10–4.20); when pesticides were used, the OR was 2.35 (95 % CI 1.10–5.0); and when hazardous substances were used, the OR was 1.70 (95 % CI 1.14–2.44) (Abadi-Korek et al. 2006). In Colombia, occupational exposure to hydrocarbons and their association with childhood ALL were also studied, and they found that when the father used mineral oil, the OR was 2.92 (95 % CI 1.16–7.36), when he used 1,3-butadieno the OR was 4.18 (95 % CI 1.47–11.88), and with the use of trichloroethylene the OR was 2.76 (95 % CI 1.09–7.06). In addition, the risks were very high with maternal occupational exposure, OR 6.68 (95 % CI 1.59–28.08), OR 11.67 (95 % CI 1.74–78.05), and OR 7.41 (95 % CI 1.66–33.07), respectively. Similar results were observed when both parents were exposed to mineral oils, OR 13.68 (95 % CI 3.58–52.22), and trichloroethylene, OR 17.56 (95 % CI 4.12–74.81) (Castro-Jiménez and Orozco-Vargas 2011). Another factor of occupational exposure that caught the attention of researchers was ELF-MF to which the mother was exposed at work, but there was a non-significant risk of her baby developing childhood ALL, with an OR of 1.34 (95 % CI 0.94–1.91) (Reid et al. 2011).

The intake of vitamins, iron, and folic acid by the child was also evaluated to discover if it acted as a protective factor for ALL and finding no effect on the results (Dockerty et al. 2007). Another factor studied was the exposure to insecticides and pesticides used in the home and its association with the development of ALL measured directly in urine. A higher concentration was found in cases than in controls (33 % vs. 14 %; $p < 0.02$) (Soldin et al. 2009). Meanwhile the Chinese also evaluated pyrethroid pesticides in the urine of patients with high-risk ALL and found the presence of total metabolites in urine and pyrethroids, OR 2.75 (95 % CI 1.43–5.29) (Ding et al. 2012). Furthermore, Rull et al. evaluated the use of agricultural pesticides professionally near the child's home, finding a modest risk of ALL in the moderate category, but not for high exposure, OR 1.7 (95 % CI 1.0–3.1) and OR 0.8 (95 % CI 0.4–1.4) (Rull et al. 2009). These results agree with those from the Australian group, who found that the application of professional pesticides near the child's home had an OR of 1.24 (95 % CI 0.93–1.65). When cases with a genetic feature, chromosomal deletions, and rearrangements of ETV6-RUNX1 t(12;21)

were analyzed, the OR were 1.46 (95 % CI 1.05–2.04), 1.83 (95 % CI 1.00–3.35), and 2.39 (95 % CI 1.25–4.55), respectively (Bailey et al. 2011).

Residential exposure to ELF-MFs has been one of the most frequently studied factors in childhood leukemia. It has been evaluated with different measurement variables, from coding wiring distance energy distributors and pylons, with spot measurements and meters for 24 h in children's rooms. There have been three studies on ELF-MF exposure specifically associated with ALL over 14 years and a systematic review by a Brazilian group. Only one study found significant risks associated with the development of ALL when the child's residence was at a distance <600 m from power lines, OR 2.61 (95 % CI 1.73–3.94), and these risks increase with a voltage of 123 kV, OR 9.93 (95 % CI 3.47–28.5), of 230 kV, OR 10.78 (95 % CI 3.75–31), and of 400 kV, OR 2.98 (95 % CI 0.93–9.54) (Sohrabi et al. 2010). Regarding the systematic review conducted by a Brazilian group of ten case–control studies with different methods of measuring exposure, one study reported a risk of childhood ALL with an OR of 3.32 (95 % CI 1.27–8.68) when the exposure was ≥0.15 μT and a risk with an OR of 4.67 (95 % CI 1.15–19.00) when exposure was ≥0.4 μT. In another study that measured the distance of residence of the child from the power source found an OR of 3.06 (95 % CI 1.31–7.13) with a distance of ≤50 m (Pelissari et al. 2009). The other studies found no association with childhood ALL (Kleinerman et al. 2000; Wünsch-Filho et al. 2011).

Smoking has also been a factor associated with childhood ALL development as children are exposed to smoking passively. The meta-analysis conducted by Liu et al. found that paternal smoking after the child's birth was associated positively, OR 1.20 (95 % CI 0.97, 1.49), with the highest exposure having an OR of 1.35 (95 % CI 1.06, 1.72) (Liu et al. 2011). The North American group also assessed the child's passive smoking and found a very slight risk, OR 1.20 (95 % CI 0.84–1.72), associated with the development of ALL, and when the effect of the mother having smoked since before conception was combined, the OR was 1.11 (95 % CI 0.75–1.65) (Metayer et al. 2013), while the Italians found no association of passive exposure to smoke by the child, OR 0.94 (95 % CI 0.61–1.45) (Farioli et al. 2014). Another proposal to study exposure to carcinogens in children is by the chemical analysis of dust in the carpets of the house where the children resided, with special sampling pumps. When herbicides were sought, no significant risks were found, OR 1.57 (95 % CI 0.90–2.73) in the third tertile (Metayer et al. 2013). When the authors sought polycyclic aromatic hydrocarbons (PAH) in these samples of dust that they continued to obtain from the child's homes, they found no association, but when the vacuum bags were obtained, they found an OR of 1.98 (95 % CI 1.11–3.55) for dibenzo[a,h]anthracene and an OR of 1.81 (95 % CI 1.04–3.16) for indeno[1,2,3-cd]pyrene. Toxic equivalence to PAH was associated with the development of childhood ALL, OR 2.35 (95 % CI 1.18–4.69); however, the results should be viewed with caution (Deziel et al. 2014). In addition, the authors also sought polybrominated diphenyl ethers (PBDEs) in dust samples and found no association, but when they sought specific PBDEs such as BDE-196, -203, -206, and -207, the OR were 2.1 (95 % CI 1.1–3.8), 2.0 (95 % CI 1.1–3.6), 2.1 (95 % CI 1.1–3.9), and 2.0 (95 % CI 1.03–3.8), respectively (Ward et al. 2014).

Another hypothesis regarding the development of childhood leukemia was proposed by Kinlen (1988), who states that an excess of childhood leukemia could be seen in places that had an unusual mixture of populations. The agent has not recognized that predisposition should be mild, as infections could lead to a rare response to the infectious agent, causing leukemia in relatively isolated communities with a sudden high population density exposed to unknown infectious agents. The rapid influx of new arrivals to a previously isolated community begins to cause increased contact between newcomers and original residents who are susceptible to infection. The risk of leukemia is thus increased in both long-term residents and newcomers (Kinlen 1995). This phenomenon occurs in wartime population growth with newcomers arriving at the community centers in search of work, community medical services, and tourism (McNally and Eden 2004). Studies investigating mixed populations may be ecological and seek an association between socioeconomic status and deprivation, to discover the exposure to some of the infectious agents proposed by Greaves and Kinlen. In the review, a study on the Czech Republic evaluated the incidence of ALL in the age group 1–4 years during the period in which the political and socio-economic transition occurred in the communist countries of Central Europe, finding a higher incidence of ALL of 1.5-fold in the period 1980–1998 ($p=0.01$) and an annual average increase 0.11 cases per 100 mil people (Hrusák et al. 2002). In Hungary, the population mix in some communities was assessed, and it was found that the rate ratio (RR) for all incoming children was 2.13 (95 % CI 1.02–4.44); for children aged <5 years it was 2.07 (95 % CI 0.91–4.75). When analyzed according to sex, in incoming boys the RR was 3.10 (95 % CI 1.13–8.51), and in incoming boys aged <5 years old the RR was 2.24 (95 % CI 0.71–7.09) (Nyári et al. 2006). Subsequently, the population mixing was analyzed in England and Wales for the group aged 1–4 years and the incidence rate ratio (IRR) found in the rural area was 1.26 ($p=0.05$); in urban/rural areas the IRR was 1.07 ($p=0.05$) (Stiller et al. 2008). Ribeiro et al. wanted to find out the relationship between socioeconomic status in some of the communities in Brazil and the development of ALL, obtaining data from the Social Exclusion Index to compare the group of rich and poor, obtaining an RR of 0.34 (95 % CI 0.28–0.44). When comparing the number of people in household with seven or more persons the RR was 0.32 (95 % CI 0.25–0.43) (Ribeiro et al. 2008).

Finally, Greaves described "late infection" and its association with the development of childhood AL, particularly for ALL (highest peak incidence in B cell precursors), postulating that infections may promote the "second hit" because of a belated abnormal immune response in children exposed to these infections (Greaves 1988), which has led to numerous studies on postnatal infections in children and their association with the development of childhood ALL, providing evidence to support or not support Greaves' hypothesis. Researchers have sought different measures of exposure to infections according to proxy variables such as immunizations, breastfeeding, day-care attendance by children, hospitalization for infections, social contact, etc. The results found in this review of studies published in the past 14 years found an inverse relationship between day-care attendance and ALL development, with a decreased risk with an OR of 0.49 (95 % CI 0.31–0.77), in the age group

<4 years (Infante-Rivard et al. 2000). Another study conducted by a group of US researchers found that going to preschool for more than 50,000 h the child was protected against the development of ALL, OR 0.64 (95 % CI 0.45–0.95) (Ma et al. 2002). In 2005, the authors confirmed these findings with a sample size that included Hispanic children, for whom no association was found, but if one non-Hispanic white children, OR 0.42 (95 % CI 0.18–0.99), >5000 h compared with children who did not attend day care (Ma et al. 2005). In England, similar results were also found on children attending formal or informal childcare, OR 0.48 (95 % CI 0.37–0.62) and OR 0.62 (95 % CI 0.51–0.75), respectively (Gilham et al. 2005). The results are also consistent with a study from Denmark in which it was found that day-care attendance protects against the development of leukemia, OR 0.68 (95 % CI 0.48–0.95) (Kamper-Jørgensen et al. 2008). Urayama et al. decided to perform a meta-analysis to add further evidence to support this inverse relationship to the development of ALL with a review of 14 studies of cases and controls and found that when the child went to kindergarten and if he/she went in the first 2 years of life, the OR was 0.76 (95 % CI 0.67–0.87) (Urayama et al. 2010). Subsequently, Urayama et al. conducted a study in the USA including Hispanics and non-Hispanic whites to find out the association among day-care attendance, birth order, and common childhood infections in these two ethnic groups. When the three variables were assessed separately, for preschool attendance at the age of 6 months per 1000 h the OR was 0.90 (95 % CI 0.82–1.00) and for those with an older sibling the OR was 0.68 (95 % CI 0.50–0.92) in non-Hispanic whites, while having had an ear infection before 6 months of age was protective in both ethnic groups. When variables were analyzed simultaneously, it proved that for attending preschool the OR was 0.83 (95 % CI 0.73–0.94) and for having an older sibling the OR was 0.59 (95 % CI 0.43–0.83) in Non-Hispanic whites. Regarding Hispanic children, a significant reduction in the risk of developing ALL was found when they had an ear infection, OR 0.45 (95 % CI 0.25–0.79) (Urayama et al. 2011). Another study, in which the history of infection was analyzed in children, discovered that when roseola or fever and rash were reported, there was an inverse relationship, OR 0.33 (95 % CI 0.16–0.68), but there was risk when tonsillitis was reported, OR 2.56 (95 % CI 1.22–5.38) (Chan et al. 2002). Other studies showed that infections were reported to incur risks of developing ALL. One study conducted in the UK with a very large sample size evaluated infections in the first year of life with regard to childhood cancer in general, but stratified ALL in the group aged 2–5 years, OR 1.4 (95 % CI 1.1–1.9) (Roman et al. 2007). The findings of Chang et al. are consistent with these results, with an OR of 3.2 (95 % CI 2.2–4.7) when an infection was reported in the first year of life, and when asked if there had been any infection 1 year before diagnosis, OR was 3.9 (95 % CI 2.6–5.8) (Chang et al. 2012). A more modest risk was found by UK authors when infectious disease was reported, OR 1.04 (95 % CI 1.01–1.07), and when the child was not being breastfed, OR 1.17 (95 % CI 0.94–1.44) (Crouch et al. 2012). In Canada, it was found that breastfeeding was protective against ALL, with an OR of 0.68 (95 % CI 0.49–0.95); contrary to this result, one of the US groups found no significant risk when the child was

breastfed, OR 1.49 (95 % CI 0.83–2.65) (Kwan et al. 2005). Social contact has also been evaluated; for example, changing their place of residence in the first year of life was shown to be a protective factor for ALL, OR 0.47 (95 % CI 0.23–0.98) (Chan et al. 2002). Chang et al. then evaluated the level of social contact of the resident US population according to occupation and observed no statistical association when the parent had a high level of social contact; when the father or mother had a high level of contact, OR was 0.92 (95 % CI 0.61–1.39) and 1.14 (95 % CI 0.81–1.59), respectively (Chang et al. 2007).

Allergic diseases were also studied to understand their association with childhood ALL. For asthma, hay fever, food allergy, and eczema an inverse relationship was reported: OR 0.8 (95 % CI 0.6–1.0) for asthma, OR 0.6 (95 % CI 0.5–0.8) for hay fever, OR 0.7 (95 % CI 0.6–0.8) for food allergy, and OR 0.7 (95 % CI 0.5–0.9) for eczema (Wen et al. 2000). These results are also confirmed by another study in the USA: allergies were reported to protect against the development of childhood ALL, OR 0.58 (95 % CI 0.38–0.88) (Rosenbaum et al. 2005). In Greece, a decreased risk was found, OR 0.49 (95 % CI 0.34–0.72), when an allergic disease was reported, but biological samples of patients' blood were also obtained to measure levels of allergen-specific (IgE) antibodies and confirm allergies in patients. The risk for laboratory-confirmed allergy had an OR of 0.43 (95 % CI 0.22–0.84) and that of food allergies had an OR of 0.39 (0.18–0.83) (Lariou et al. 2013), the authors agreeing that having allergies is a protective factor against ALL. However, for the Taiwanese, allergies did constitute a risk for childhood ALL when the allergy was reported before 1 year of age and when the allergy occurred after 1 year of age, OR 1.7 (95 % CI 1.5–2.0) and OR 1.3 (95 % CI 1.1–1.5) respectively (Chang et al. 2012).

Epidemiological studies focusing on the etiology of childhood ALL over 14 years of study help to identify the different environmental factors that have been associated with the development of childhood lymphoblastic leukemia, and the importance of identifying these within three critical windows, not only supporting the model proposed by Greaves, but the effects on every window. This model indicates that leukemia may be explained by environmental exposure, which causes the "first hit," called genetic susceptibility, and re-exposure to some of the environmental factors gives the "second hit" provoking overt leukemia. Although this model does not account for 100 % of patients with childhood ALL, it is assumed in most cases of childhood ALL that it is important to recognize these exposure windows, which may be critical in identifying the effects associated with exposure to the environmental factor(s) that can cause these "two hits".

Figure 8.2 summarize the risk factors studied in each window of exposure for ALL development. Apart from infection being the most consistent risk factor in the postnatal window, no other environmental risk factor that occurs during only one window of exposure, such as smoking, occupational exposure to hydrocarbons, pesticides, X-rays, etc., has been associated with an increased risk of childhood ALL during the three windows of exposure (Tables 8.2a, 8.2b, 8.3a, 8.3b, 8.3c, 8.4a, 8.4b and 8.5).

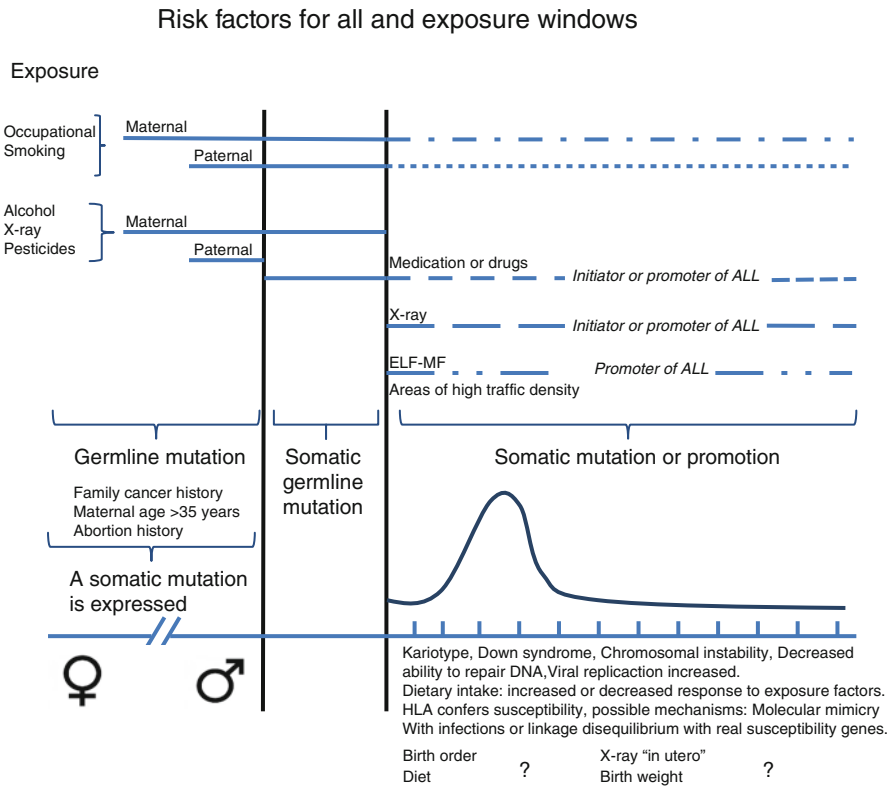

Fig. 8.2 Risk factors for childhood ALL and windows of exposure. There are three periods that are involved in the development of ALL. If in one window there are many components to the causes of ALL, in the other windows fewer components are necessary for that child to develop ALL

Susceptibility and Genetic Polymorphisms

Although the role of environmental exposure is still currently undefined, it is likely that carcinogenesis due to environmental exposure is influenced by the co-inheritance of multiple low-risk variants, such as single nucleotide polymorphisms (SNPs) in susceptible genes (Mehta et al. 2006).

These variants can be identified by comparing the frequency of polymorphic genotypes in cases and controls. In most childhood leukemia cases, characteristic genetic alterations are observed, including numerical and structural chromosomal changes such as hyperdiploidy (>46 chromosomes) or translocations, in addition to the more subtle changes in the form of point mutations and gene deletions (Wiemels 2012).

Somatically acquired genetic aberrations in ALL lymphoblasts are prognostic and can guide risk-directed therapy. The extent to which germline variation contributes to ALL susceptibility, however, is less clear and is the subject of current research.

Table 8.5 Childhood infections and risk of ALL

Exposure	Principal results (95 % CI)	Sample size Ca/Co	Annotation	Place/reference	Conclusions
Day-care utilization	0.96 (0.75–1.24)	633/687	Data were analyzed stratified by common ALL (2–5 years)	USA/(Neglia et al. 2000)	With one exception (ear infections), these data do not support the hypothesis that a decrease in the occurrence of common childhood infection increases the risk of ALL
Day-care attendance	0.49 (0.31–0.77)	491/491	Data were analyzed stratified by <4, >4 years old	Canada/(Infante-Rivard et al. 2000)	The current results support the view that infection may be involved in the etiology of childhood leukemia and that the timing of exposure is important
Breastfeeding	0.68 (0.49–0.95)				
Older siblings at diagnosis*	4.54 (2.27–9.07)				
Older siblings in 1st year**	0.46 (0.22–0.97)				
Day-care histories		255/760	Data were collected on experiences before kindergarten or first grade (<6 years old)	USA/(Rosenbaum et al. 2000)	These findings do not support the hypothesis that infrequent contact with peers during early childhood might delay exposure to infectious diseases and increase the risk of ALL
1–18 months	1.74 (0.89–3.42)				
19–36 months	1.32 (0.64–2.71)				
Stay at home	1.32 (0.70–2.52)				

(continued)

Table 8.5 (continued)

Exposure	Principal results (95 % CI)	Sample size Ca/Co	Annotation	Place/reference	Conclusions
Allergic disorders and childhood ALL		1842/1986	Cases were identified through the member institutions of the CCG throughout the USA	USA/(Wen et al. 2000)	The results from this study, in agreement with most previous studies on adult cancer, suggest that allergic disorders might be associated with a reduced risk of childhood ALL
Asthma	0.8 (0.6–1.0)				
Hay fever	0.6 (0.5–0.8)				
Food and drug allergies	0.7 (0.6–0.8)				
Eczema	0.7 (0.5–0.9)				
Hives	0.9 (0.7–1.2)				
Any of the above conditions	0.7 (0.6–0.8)				
Number of those suffering from any of the above disorders					
One condition	0.8 (0.7–0.9)				
Two or more	0.6 (0.5–0.7)				
Allergic disorders among subject's siblings					
Hay fever	0.7 (0.6–0.9)				
Any sibling with any condition	0.9 (0.8–1.0)				

Day-care attendance		140/140	Calculated the change in risk associated with each unit of change in day care	USA/(Ma et al. 2002)	These findings support the hypothesis that delayed exposure to common infections might play an important role in the etiology of childhood acute lymphoblastic leukemia, and suggest that extensive contact with other children in a day-care setting might be associated with a reduced risk of ALL
Total child-hours (thousands) or	0.99 (0.98–0.99)				
A child with 50,000 child-hours	0.64 (0.45–0.95)				
1st year of life		98/228	Day-care attendance was analyzed too	Hong Kong/(Chan et al. 2002)	These results provide support for the delayed exposure hypothesis in an affluent geographical setting in which population exposure to infectious agents is quite distinct from the settings of previous case–control studies
Change of area of residence	0.47 (0.23–0.98)				
Roseola and/or fever and rash reported	0.33 (0.16–0.68)				
Year before diagnosis					
Tonsillitis reported	2.56 (1.22–5.38)				
Any social activity	0.73 (0.62–0.87)	3140/6305	Data included several types of childhood cancer, but the analysis was ALL vs. controls stratified for group aged 2–5 years with similar results to those for children <14 years old	UK/(Gilham et al. 2005)	These results support the hypothesis that reduced exposure to infection in the first few months of life increases the risk of developing ALL
Informal day care	0.62 (0.51–0.75)				
Formal day care	0.48 (0.37–0.62)				

(continued)

Table 8.5 (continued)

Exposure	Principal results (95 % CI)	Sample size Ca/Co	Annotation	Place/reference	Conclusions
Breastfeeding patterns		311/400	Data were analyzed stratified by 2–5 years	USA/(Kwan et al. 2005)	This study did not support the hypothesis that breastfeeding protects against the risk of childhood ALL
Ever vs. never	1.49 (0.83–2.65)				
Allergy		255/760	Data included early childhood infections. Were stratified 2–5 years old	USA/(Rosenbaum et al. 2005)	A positive allergy history in the index child before leukemia diagnosis was associated with a significantly reduced risk of ALL
Any positive allergy	0.58 (0.38–0.88)				
Day-care attendance		294/376	Data were analyzed as non-Hispanic white and Hispanic, in Hispanic children no association was observed	USA/(Ma et al. 2005)	In Hispanic children, no association was observed among day-care attendance, early infections, and risk of childhood ALL or c-ALL. These results offer indirect yet strong support for the infectious disease hypothesis in the etiology of ALL in non-Hispanic white children and highlight an important ethnic difference
>5,000 child-hours ALL	0.42 (0.18–0.99)				
>5,000 child-hours c-ALL	0.33 (0.11–1.01)				
Self-reported ear infection and C-ALL	0.32 (0.14–0.74)				
Infections in the 1st year of life	1.4 (1.1–1.9)	1713/3417	Data included all childhood cancers were stratified by ALL in children aged 2–5 years	UK/(Roman et al. 2007)	These findings support the hypothesis that a dysregulated immune response to infection in the first few months of life might promote transition to overt ALL later in childhood

Parental SC and risk of ALL		376/376	Data were collected from parents interviewed on employment history (and duration) of each job held after the child's birth up to the age of 3 years for those children diagnosed from age 3 to 14.9 or up to the date of leukemia diagnosis	USA/(Chang et al. 2007)	Results indicated that including information of the duration of parental occupation may be important when studying the association between parental occupational social contact and childhood leukemia
Paternal					
Low vs. high	0.92 (0.61–1.39)				
Maternal					
Low vs. high	1.14 (0.81–1.59)				
Combined parental low vs. high	1.21 (0.84–1.73)				
Rural status paternal SC and ALL	2.28 (0.76–6.85)				
Rural status paternal SC and c-ALL	3.53 (0.86–14.57)				
Childcare attendance	0.68 (0.48–0.95)	559/5590	Data were analyzed, also case-matched controls	Denmark/(Kamper-Jørgensen et al. 2008)	Childcare attendance during the first 2 years of life was associated with a reduced risk of childhood ALL and supports the "delayed infection hypothesis"
<6 months	0.72 (0.42–1.25)				
6–11 months	0.64 (0.41–1.02)				
12–23 months	0.68 (0.46–1.01)				
Day-care attendance	0.76 (0.67–0.87)	14 case–control studies (6,108 cases)	Meta-analysis	USA/(Urayama et al. 2010)	This analysis provides strong support for an association between exposure to common infections in early childhood and a reduced risk of ALL
≤2 years of age	0.79 (0.65–0.95)				
Anytime before diagnosis	0.81 (0.70–0.94)				

(continued)

Table 8.5 (continued)

Exposure	Principal results (95 % CI)	Sample size Ca/Co	Annotation	Place/reference	Conclusions
Day-care attendance at 6 months old		669/977	Data were analyzed in non-Hispanic whites and Hispanic separately	USA/(Urayama et al. 2011)	The reduced risks associated with ear infection early in life, a direct indicator of exposure to infection, in non-Hispanic whites and Hispanics provide evidence that the "delayed infection" hypothesis may be operative in both ethnic populations
Non-Hispanic white	0.90 (0.82–1.00) for each thousand child-hours of exposure				
Older sibling	0.68 (0.50–0.92)				
Day-care attendance	0.83 (0.73–0.94)				
Older sibling	0.59 (0.43–0.83)				
Hispanic					
Ear infections	0.45 (0.25–0.79)				
Allergy <1 year before diagnosis	1.7 (1.5–2.0)	846/3374	Data were analyzed and stratified by age 2–5 years	Taiwan/(Chang et al. 2012a)	The present study suggests that the pathogenesis of childhood ALL and allergies might share a common biological mechanism
>1 year before diagnosis	1.3 (1.1–1.5)				
Before 1 year old	1.4 (1.1–1.7)				
Any infection before 1 year of age	3.2 (2.2–4.7)	846/3374	Data were analyzed by ALL and AML separately	Taiwan/(Chang et al. 2012b)	Children with leukemia may have a dysregulated immune function present at an early age, resulting in more episodes of symptomatic infections compared with healthy controls
1 year before diagnosis	3.9 (2.6–5.8)				

Infection illness	1.04 (1.01–1.07)	756/1171	Combined data from medical records, parental interview, and population census	UK/(Crouch et al. 2012)	Infectious diagnoses in the first year of life were significantly increased in children who developed leukemia between 2 and 14 years of age, as well as in those who had birth orders >1, were not breastfed, lived in deprived areas, or were diagnosed with eczema
Birth order >1	0.96 (0.79–1.19)				
Eczema	0.91 (0.71–1.16)				
Deprivation high	1.17 (0.87–1.57)				
Never breastfed	1.17 (0.94–1.44)				
Hospitalization for infectious previous to diagnosis	IRR (incidence rate ratio) 95%CI	1,778,129 (815 ALL)	Cohort study	Denmark/(Vestergaard et al. 2013)	The absence of an association between hospitalization for infections and risk of childhood ALL directs future investigations into the role of infections in the development of childhood ALL toward the exploration of less severe infections
Hospitalization for infectious disease at/after 2 years of age	0.92 (0.78–1.07)				
	1.04 (0.81–1.32)				
Allergic history	0.49 (0.34–0.72)	252/294	Blood samples were available where allergen-specific IgE was determined	Greece/(Lariou et al. 2013)	Beyond the already established inverse association of allergic history with childhood ALL, an association of the same magnitude is evident when serological markers of allergic predisposition are used as an alternative measure of allergy
Serum IgE	0.43 (0.22–0.84)				
Food IgE	0.39 (0.18–0.83)				

*Diagnosis at <4 years old
**Diagnosis at >=4 years old

Direct evidence for a genetic predisposition to ALL is provided by the high risk associated with Bloom syndrome, neurofibromatosis, ataxia telangiectasia, and Down's syndrome. The heritable basis of susceptibility to ALL is further supported by a recent candidate gene (CGAS), the advent of high-resolution genome-wide analyses (GWAS) of gene expression, DNA copy number alterations, epigenetic changes, and more recently, next-generation whole genome and transcriptome sequencing have provided new insights into leukemogenesis, drug resistance, and host pharmacogenomics, suggesting that the co-inheritance of multiple germline variants might contribute to disease risk (Pui et al. 2011; Downing et al. 2012).

Five GWAS have been performed so far, with populations between 50/50 and 3275/4817 ALL cases/healthy controls (Papaemmanuil et al. 2009; Treviño et al. 2009; Han et al. 2010; Sherborne et al. 2010; Ellinghaus et al. 2012). These studies identified several risk loci with allelic odds ratios (OR) of the disease-related allele between 1.34 and 9.99. Enciso-Mora et al. (2012) calculated that 25 % of the total variation in B-ALL risk is accounted for by common genetic variation. On the other hand, previous GWAS-identified loci (IKZF1, CDKN2A, ARID5B, and CEBPE) explain only 8 % of this total. The data provide the rationale for the continued investigation of additional susceptibility loci that were likely missed by previous GWAS. Although GWAS represent a powerful approach to the identification of disease loci, the p value requirement for defining a significant association may in turn increase the probability of missing a true association (Wesolowska et al. 2011).

The assertion that ALL may have a genetic basis has long been pursued through association studies based on candidates for childhood ALL susceptibility genes, which have been categorized into those coding for carcinogen metabolism enzymes involved in xenobiotic metabolism (Krajinovic et al. 1999), oxidative stress response (Krajinovic et al. 2002), DNA repair proteins (Batar et al. 2009), folate metabolism enzymes (Petra et al. 2007), and cell–cycle regulation (Healy et al. 2007) and others have been associated with ALL (Vijayakrishnan and Houlston 2010).

One of the most important initiating events is thought to result from the misrepair of double-strand DNA breaks (DSBs) during nonhomologous end-joining (NHEJ) (Kim et al. 2006; Hassanzadeh et al. 2011; Emerenciano et al. 2007). The DNA repair system plays an important role in maintaining genome integrity and stability through the reversal of DNA damage. If accumulated mutations occur in corresponding DNA repair genes, their reversal capacity could be damaged, substantially increasing the risk of cancer. SNPs in common DNA repair genes have been identified and demonstrated to be linked to sporadic carcinogenesis (Roberts et al. 2011; Shiraishi et al. 2010).

Considering roles in DSB repair, the X-ray repair cross-complementing group 1 (XRCC1) gene is one of the most important DNA repair genes (Caldecott 2003; Lee et al. 2009). X-ray repair cross-complementing group 1 (XRCC1), located on chromosome 19q13.2–13.3, at 33 kb in length, is one of the most important proteins in base excision repair (BER) (Chou et al. 2008). BER is also the predominant DNA damage repair pathway for the processing of small base lesions derived from oxidation and alkylation damage (Lan et al. 2004).

XRCC1 also participates in the single-strand DNA break (SSB) repair pathway for the repair of DNA destruction, which occurs very frequently in mammals, and

the BER pathway, which operates on small lesions usually caused by endogenous substances or xenobiotics. Moreover, it is reported that the DNA repair function may be modified by genetic polymorphisms. Genetic instability and even carcinogenesis may be caused if the capacity for DNA repair is deficient (Goode et al. 2002; Jiang et al. 2009). Also reported by relevant studies is the disruption of XRCC1 in mice, which results in early embryonic lethality in the BER pathway (Tebbs et al. 1999; Horton et al. 2008), and an excess of deletions found among induced mutations in EM-C11 (one cell line identified as defective in XRCC1 function), perhaps resulting from the reduced ligation efficiency of SSB (Op het Veld et al. 1998). Therefore, the size of the DNA repair capacity modified by *XRCC1* gene polymorphisms gives different hereditary susceptibility to ALL to different populations. In other words, *XRCC1* gene polymorphisms may be associated with childhood ALL. More than 300 validated SNPs in the *XRCC1* gene have been reported in the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP). Nevertheless, only three common SNP sites in the *XRCC1* gene encoding region have been extensively studied: codon 399 (extron 10, G → A, Arg → Gln), codon 194 (extron 6, C → T, Arg → Trp), and codon 280 (extron 9, G → A, Arg → His). Molecular epidemiological studies on the association between the genetic predisposition of children to ALL and XRCC1 polymorphisms have presented some contradictory results (Joseph et al. 2005; Pakakasama et al. 2007; Batar et al. 2009; Meza-Espinoza et al. 2009; Tumer et al. 2010; Canalle et al. 2011; Stanczyk et al. 2011). However, these inconsistent results fail to clarify this complicated genetic relationship because of the small sample size and low statistical power. More research is needed to investigate the relationship between polymorphisms in DNA repair genes and childhood ALL.

Conclusions

Epidemiological studies evaluating risk factors associated with childhood ALL during the three windows suggest that various factors might interact in combination to develop the disease. So far, Greaves' hypothesis that childhood ALL might be an abnormal immune system response to infections in children born with a pre-leukemic clone is the most plausible. Whether or not other environmental factors play an important role in the development of childhood ALL is still unknown; however, the published epidemiological studies provide the possibility to make some recommendations, such as that regarding paternal smoking (it is best to stop smoking or at least avoid to smoke during the preconception stage, pregnancy, and the early years of their children's lives to prevent children from being exposed passively) and paternal consumption of alcohol during the preconception stage. Regarding occupational exposure involving petroleum products, parents should use personal protective equipment and be very careful when handling these substances, avoiding bringing them home on the clothes or skin. The handling of chemicals at home such as oil paints and pesticides should be avoided, so that their children are not exposed. For the mother, a diet rich in vegetables, proteins, fruits, and sea food

is recommended. More studies contributing to the etiology of childhood ALL are definitely needed to identify factors that may participate in each window, but we need to think about the theoretical and epidemiological aspects of childhood ALL to develop explanatory theories about the disease to protect children against these agents that cause this distressing disease.

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Bibliography

Abadi-Korek I, et al. Parental occupational exposure and the risk of acute lymphoblastic leukemia in offspring in Israel. J Occup Environ Med Am Coll Occup Environ Med. 2006;48(2):165–74. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16474265. Accessed 16 Nov 2014.

Andreasson P, et al. The expression of ETV6/CBFA2 (TEL/AML1) is not sufficient for the transformation of hematopoietic cell lines in vitro or the induction of hematologic disease in vivo. Cancer Genet Cytogenet. 2001;130(2):93–104. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11675129. Accessed 21 Nov 2014.

Autrup H. Transplacental transfer of genotoxins and transplacental carcinogenesis. Environ Health Perspect. 1993;101(Suppl):33–8. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1519962&tool=pmcentrez&rendertype=abstract. Accessed 20 Nov 2014.

Baade PD, et al. Trends in incidence of childhood cancer in Australia, 1983–2006. Br J Cancer. 2010;102(3):620–6. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2822940&tool=pmcentrez&rendertype=abstract. Accessed 21 Nov 2014.

Bailey HD, et al. Exposure to diagnostic radiological procedures and the risk of childhood acute lymphoblastic leukemia. Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cospons Am Soc Prev Oncol. 2010;19(11):2897–909. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20861400. Accessed 16 Nov 2014.

Bailey HD, Milne E, et al. Exposure to house painting and the use of floor treatments and the risk of childhood acute lymphoblastic leukemia. Int J Cancer. 2011a;128(10):2405–14. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20658537. Accessed 16 Nov 2014.

Bailey HD, Armstrong BK, et al. Exposure to professional pest control treatments and the risk of childhood acute lymphoblastic leukemia. Int J Cancer. 2011b;129(7):1678–88. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21080443. Accessed 17 Nov 2014.

Bailey HD, de Klerk NH, et al. Refuelling of vehicles, the use of wood burners and the risk of acute lymphoblastic leukaemia in childhood. Paediatr Perinat Epidemiol. 2011c;25(6):528–39. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21980942. Accessed 17 Nov 2014.

Bailey HD, et al. Maternal dietary intake of folate and vitamins B6 and B12 during pregnancy and the risk of childhood acute lymphoblastic leukemia. Nutr Cancer. 2012;64(7):1122–30. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22966944. Accessed 18 Nov 2014.

Batar B, et al. DNA repair gene XPD and XRCC1 polymorphisms and the risk of childhood acute lymphoblastic leukemia. Leuk Res. 2009;33(6):759–63. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19101034. Accessed 21 Nov 2014.

Bernaldez-Rios R, et al. The age incidence of childhood B-cell precursor acute lymphoblastic leukemia in Mexico City. J Pediatr Hematol Oncol. 2008;30(3):199–203. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18376281. Accessed 21 Nov 2014.

Caldecott KW. XRCC1 and DNA strand break repair. DNA Repair. 2003;2(9):955–69. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12967653. Accessed 21 Nov 2014.

Canalle R, et al. Impact of thymidylate synthase promoter and DNA repair gene polymorphisms on susceptibility to childhood acute lymphoblastic leukemia. Leuk Lymphoma. 2011;52(6):1118–26. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21463130. Accessed 21 Nov 2014.

Castillo L, et al. Childhood cancer in Uruguay: 1992–1994. Incidence and mortality. Med Pediatr Oncol. 2001;37(4):400–4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11568906. Accessed 21 Nov 2014.

Castro-Jiménez MÁ, Orozco-Vargas LC. Parental exposure to carcinogens and risk for childhood acute lymphoblastic leukemia, Colombia, 2000–2005. Prev Chronic Dis. 2011;8(5):A106. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3181179&tool=pmcentrez&rendertype=abstract. Accessed 17 Nov 2014.

Chan LC, et al. Is the timing of exposure to infection a major determinant of acute lymphoblastic leukaemia in Hong Kong? Paediatr Perinat Epidemiol. 2002;16(2):154–65. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12060313. Accessed 17 Nov 2014.

Chang JS, et al. Parental social contact in the work place and the risk of childhood acute lymphoblastic leukaemia. Br J Cancer. 2007;97(9):1315–21. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2360459&tool=pmcentrez&rendertype=abstract. Accessed 18 Nov 2014.

Chang JS, Tsai Y-W, et al. Allergy and risk of childhood acute lymphoblastic leukemia: a population-based and record-based study. Am J Epidemiol. 2012a;176(11):970–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23171876. Accessed 18 Nov 2014.

Chang JS, Tsai C-R, et al. Medically diagnosed infections and risk of childhood leukaemia: a population-based case-control study. Int J Epidemiol. 2012b;41(4):1050–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22836110. Accessed 18 Nov 2014.

Chokkalingam AP, et al. Fetal growth and body size genes and risk of childhood acute lymphoblastic leukemia. Cancer Causes Control CCC. 2012;23(9):1577–85. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3415610&tool=pmcentrez&rendertype=abstract. Accessed 17 Nov 2014.

Chou W-C, et al. Chk2-dependent phosphorylation of XRCC1 in the DNA damage response promotes base excision repair. EMBO J. 2008;27(23):3140–50. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2599873&tool=pmcentrez&rendertype=abstract. Accessed 21 Nov 2014.

Couto E, Chen B, Hemminki K. Association of childhood acute lymphoblastic leukaemia with cancers in family members. Br J Cancer. 2005;93(11):1307–9. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2361521&tool=pmcentrez&rendertype=abstract. Accessed 17 Nov 2014.

Crouch S, et al. Infectious illness in children subsequently diagnosed with acute lymphoblastic leukemia: modeling the trends from birth to diagnosis. Am J Epidemiol. 2012;176(5):402–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22899827. Accessed 18 Nov 2014.

Davis E, et al. Western Australian children with acute lymphoblastic leukemia are taller at diagnosis than unaffected children of the same age and sex. Pediatr Blood Cancer. 2011;56(5):767–70. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21246704. Accessed 17 Nov 2014.

De Souza Reis R, et al. Childhood leukemia incidence in Brazil according to different geographical regions. Pediatr Blood Cancer. 2011;56(1):58–64. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21108440. Accessed 18 Nov 2014.

Deziel NC, et al. Polycyclic aromatic hydrocarbons in residential dust and risk of childhood acute lymphoblastic leukemia. Environ Res. 2014;133:388–95. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24948546. Accessed 17 Nov 2014.

Ding G, et al. Pyrethroid pesticide exposure and risk of childhood acute lymphocytic leukemia in Shanghai. Environ Sci Technol. 2012;46(24):13480–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23153377. Accessed 17 Nov 2014.

Dockerty JD, et al. Vitamin and mineral supplements in pregnancy and the risk of childhood acute lymphoblastic leukaemia: a case-control study. BMC Public Health. 2007;7:136. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1925082&tool=pmcentrez&rendertype=abstract. Accessed 18 Nov 2014.

Downing JR, et al. The pediatric cancer genome project. Nat Genet. 2012;44(6):619–22. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3619412&tool=pmcentrez&rendertype=abstract. Accessed 21 Nov 2014.

Eden T. Aetiology of childhood leukaemia. Cancer Treat Rev. 2010;36(4):286–97. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20223594. Accessed 5 Nov 2014.

Ellinghaus E, et al. Identification of germline susceptibility loci in ETV6-RUNX1-rearranged childhood acute lymphoblastic leukemia. Leukemia. 2012;26(5):902–9. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3356560&tool=pmcentrez&rendertype=abstract. Accessed 21 Nov 2014.

Emerenciano M, Koifman S, Pombo-de-Oliveira MS. Acute leukemia in early childhood. Braz J Med Biol Res Rev Bras Pesqui Médicas Biológicas/Soc Brasileira Biofísica … [et al]. 2007;40(6):749–60. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17581672. Accessed 21 Nov 2014.

Enciso-Mora V, et al. Common genetic variation contributes significantly to the risk of childhood B-cell precursor acute lymphoblastic leukemia. Leukemia. 2012;26(10):2212–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22456626. Accessed 21 Nov 2014.

Fabia J, Thuy TD. Occupation of father at time of birth of children dying of malignant diseases. Br J Prev Soc Med. 1974;28(2):98–100. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=478845&tool=pmcentrez&rendertype=abstract. Accessed 19 Nov 2014.

Fajardo-Gutiérrez A, et al. Descriptive epidemiology of malignant tumors in children. Rev Panam Salud Públ Pan Am J Public Health. 1999;6(2):75–88. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10574008. Accessed 20 Nov 2014.

Farioli A, et al. Tobacco smoke and risk of childhood acute lymphoblastic leukemia: findings from the SETIL case-control study. Cancer Causes Control CCC. 2014;25(6):683–92. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24699944. Accessed 17 Nov 2014.

Feller M, et al. Family characteristics as risk factors for childhood acute lymphoblastic leukemia: a population-based case-control study. PLoS One. 2010;5(10). Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2949397&tool=pmcentrez&rendertype=abstract. Accessed 17 Nov 2014.

Feltbower RG, et al. Incidence of childhood acute lymphoblastic leukaemia in Yorkshire, UK. Lancet. 2001;358(9279):385–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11502322. Accessed 21 Nov 2014.

Freedman DM, et al. Household solvent exposures and childhood acute lymphoblastic leukemia. Am J Public Health. 2001;91(4):564–7. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1446651&tool=pmcentrez&rendertype=abstract. Accessed 17 Nov 2014.

Gao F, Chia K-S, Machin D. On the evidence for seasonal variation in the onset of acute lymphoblastic leukemia (ALL). Leuk Res. 2007;31(10):1327–38. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17481728. Accessed 17 Nov 2014.

Gilham C, et al. Day care in infancy and risk of childhood acute lymphoblastic leukaemia: findings from UK case-control study. BMJ (Clin Res Ed). 2005;330(7503):1294. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=558199&tool=pmcentrez&rendertype=abstract. Accessed 17 Nov 2014.

Glass DC, et al. Risk of childhood acute lymphoblastic leukaemia following parental occupational exposure to pesticides. Occup Environ Med. 2012;69(11):846–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22718709. Accessed 17 Nov 2014.

Glazer ER, et al. Cancer among Hispanic children in California, 1988–1994: comparison with non-Hispanic white children. Cancer. 1999;86(6):1070–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10491536. Accessed 20 Nov 2014.

Goode EL, Ulrich CM, Potter JD. Polymorphisms in DNA repair genes and associations with cancer risk. Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cospons Am Soc Prev Oncol. 2002;11(12):1513–30. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12496039. Accessed 21 Nov 2014.

Goujon-Bellec S, et al. Time trends and seasonal variations in the diagnosis of childhood acute lymphoblastic leukaemia in France. Cancer Epidemiol. 2013;37(3):255–61. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23352628. Accessed 17 Nov 2014.

Greaves MF. Speculations on the cause of childhood acute lymphoblastic leukemia. Leukemia. 1988;2(2):120–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3278171. Accessed 20 Nov 2014.

Greaves M. Pre-natal origins of childhood leukemia. Rev Clin Exp Hematol. 2003;7(3):233–45. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15024967. Accessed 21 Nov 2014.

Greaves MF, Wiemels J. Origins of chromosome translocations in childhood leukaemia. Nat Rev Cancer. 2003;3(9):639–49. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12951583. Accessed 21 Nov 2014.

Greaves MF, et al. Geographical distribution of acute lymphoblastic leukaemia subtypes: second report of the collaborative group study. Leukemia. 1993;7(1):27–34. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8418376. Accessed 20 Nov 2014.

Greaves MF, et al. Leukemia in twins: lessons in natural history. Blood. 2003;102(7):2321–33. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12791663. Accessed 21 Nov 2014.

Gruhn B, et al. Prenatal origin of childhood acute lymphoblastic leukemia, association with birth weight and hyperdiploidy. Leukemia. 2008;22(9):1692–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18548099. Accessed 17 Nov 2014.

Gustafsson B, Carstensen J. Space-time clustering of childhood lymphatic leukaemias and non-Hodgkin's lymphomas in Sweden. Eur J Epidemiol. 2000;16(12):1111–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11484799. Accessed 17 Nov 2014.

Han S, et al. Genome-wide association study of childhood acute lymphoblastic leukemia in Korea. Leuk Res. 2010;34(10):1271–4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20189245. Accessed 21 Nov 2014.

Hassanzadeh J, et al. Maternal and prenatal risk factors for childhood leukemia in southern of Iran. Iran Red Crescent Med J. 2011;13(6):398–403. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3371935&tool=pmcentrez&rendertype=abstract. Accessed 21 Nov 2014.

Healy J, et al. Promoter SNPs in G1/S checkpoint regulators and their impact on the susceptibility to childhood leukemia. Blood. 2007;109(2):683–92. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17008550. Accessed 21 Nov 2014.

Hjalgrim LL, Rostgaard K, et al. Age- and sex-specific incidence of childhood leukemia by immunophenotype in the Nordic countries. J Natl Cancer Inst. 2003a;95(20):1539–44. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14559876. Accessed 21 Nov 2014.

Hjalgrim LL, Westergaard T, et al. Birth weight as a risk factor for childhood leukemia: a meta-analysis of 18 epidemiologic studies. Am J Epidemiol. 2003b;158(8):724–35. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14561661. Accessed 17 Nov 2014.

Horton JK, et al. XRCC1 and DNA polymerase beta in cellular protection against cytotoxic DNA single-strand breaks. Cell Res. 2008;18(1):48–63. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2366203&tool=pmcentrez&rendertype=abstract. Accessed 21 Nov 2014.

Hrusák O, et al. Acute lymphoblastic leukemia incidence during socioeconomic transition: selective increase in children from 1 to 4 years. Leukemia. 2002;16(4):720–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11960355. Accessed 17 Nov 2014.

Infante-Rivard C, Fortier I, Olson E. Markers of infection, breast-feeding and childhood acute lymphoblastic leukaemia. Br J Cancer. 2000;83(11):1559–64. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2363437&tool=pmcentrez&rendertype=abstract. Accessed 17 Nov 2014.

Infante-Rivard C, et al. Childhood acute lymphoblastic leukemia associated with parental alcohol consumption and polymorphisms of carcinogen-metabolizing genes. Epidemiology (Cambridge, MA). 2002;13(3):277–81. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11964928. Accessed 16 Nov 2014.

Jensen CD, et al. Maternal dietary risk factors in childhood acute lymphoblastic leukemia (United States). Cancer Causes Control CCC. 2004;15(6):559–70. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15280635. Accessed 18 Nov 2014.

Jiang J, et al. Polymorphisms of DNA repair genes: ADPRT, XRCC1, and XPD and cancer risk in genetic epidemiology. Methods Mol Biol (Clifton, NJ). 2009;471:305–33. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19109787. Accessed 21 Nov 2014.

Joseph T, et al. DNA repair gene XRCC1 polymorphisms in childhood acute lymphoblastic leukemia. Cancer Lett. 2005;217(1):17–24. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15596292. Accessed 21 Nov 2014.

Juárez-Ocaña S, et al. Trends of 6 primary causes of mortality in Mexican children in 1971–2000. Epidemiologic transition in children. Gac Med Mex. 2003;139(4):325–36. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14574753. Accessed 20 Nov 2014.

Kamper-Jørgensen M, et al. Childcare in the first 2 years of life reduces the risk of childhood acute lymphoblastic leukemia. Leukemia. 2008;22(1):189–93. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17690702. Accessed 18 Nov 2014.

Kim AS, Eastmond DA, Preston RJ. Childhood acute lymphocytic leukemia and perspectives on risk assessment of early-life stage exposures. Mutat Res. 2006;613(2–3):138–60. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17049456. Accessed 21 Nov 2014.

Kinlen L. Evidence for an infective cause of childhood leukaemia: comparison of a Scottish new town with nuclear reprocessing sites in Britain. Lancet. 1988;2(8624):1323–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2904050. Accessed 20 Nov 2014.

Kinlen LJ. Epidemiological evidence for an infective basis in childhood leukaemia. Br J Cancer. 1995;71(1):1–5. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2033475&tool=pmcentrez&rendertype=abstract. Accessed 20 Nov 2014.

Kleinerman RA, et al. Are children living near high-voltage power lines at increased risk of acute lymphoblastic leukemia? Am J Epidemiol. 2000;151(5):512–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10707920. Accessed 17 Nov 2014.

Krajinovic M, et al. Susceptibility to childhood acute lymphoblastic leukemia: influence of CYP1A1, CYP2D6, GSTM1, and GSTT1 genetic polymorphisms. Blood. 1999;93(5):1496–501. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10029576. Accessed 21 Nov 2014.

Krajinovic M, et al. Role of NQO1, MPO and CYP2E1 genetic polymorphisms in the susceptibility to childhood acute lymphoblastic leukemia. Int J Cancer. 2002;97(2):230–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11774269. Accessed 21 Nov 2014.

Kulkarni KP, Marwaha RK. Seasonality in diagnosis of childhood acute lymphoblastic leukemia: impact on disease presentation, survival outcome and resources. J Pediatr Hematol Oncol. 2013;35(1):81–2. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23242329. Accessed 17 Nov 2014.

Kwan ML, et al. Breastfeeding patterns and risk of childhood acute lymphoblastic leukaemia. Br J Cancer. 2005;93(3):379–84. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2361562&tool=pmcentrez&rendertype=abstract. Accessed 17 Nov 2014.

Kwan ML, et al. Maternal diet and risk of childhood acute lymphoblastic leukemia. Public Health Rep (Washington, DC: 1974). 2009;124(4):503–14. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2693164&tool=pmcentrez&rendertype=abstract. Accessed 18 Nov 2014.

Lan L, et al. In situ analysis of repair processes for oxidative DNA damage in mammalian cells. Proc Natl Acad Sci U S A. 2004;101(38):13738–43. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=518826&tool=pmcentrez&rendertype=abstract. Accessed 21 Nov 2014.

Lariou M-S, et al. Allergy and risk of acute lymphoblastic leukemia among children: a nationwide case control study in Greece. Cancer Epidemiol. 2013;37(2):146–51. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23182223. Accessed 18 Nov 2014.

Lee Y, et al. The genesis of cerebellar interneurons and the prevention of neural DNA damage require XRCC1. Nat Neurosci. 2009;12(8):973–80. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2831284&tool=pmcentrez&rendertype=abstract. Accessed 21 Nov 2014.

Lehtinen M, et al. Maternal herpesvirus infections and risk of acute lymphoblastic leukemia in the offspring. Am J Epidemiol. 2003;158(3):207–13. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12882942. Accessed 18 Nov 2014.

Linabery AM, Ross JA. Trends in childhood cancer incidence in the U.S. (1992–2004). Cancer. 2008;112(2):416–32. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18074355. Accessed 5 Nov 2014.

Liu R, et al. Paternal smoking and risk of childhood acute lymphoblastic leukemia: systematic review and meta-analysis. J Oncol. 2011;2011:854584. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3132639&tool=pmcentrez&rendertype=abstract. Accessed 17 Nov 2014.

Look AT. Oncogenic transcription factors in the human acute leukemias. Science (New York, NY). 1997;278(5340):1059–64. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9353180. Accessed 21 Nov 2014.

Lowengart RA, et al. Childhood leukemia and parents' occupational and home exposures. J Natl Cancer Inst. 1987;79(1):39–46. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3474448. Accessed 20 Nov 2014.

Ma X, et al. Daycare attendance and risk of childhood acute lymphoblastic leukaemia. Br J Cancer. 2002;86(9):1419–24. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2375371&tool=pmcentrez&rendertype=abstract. Accessed 17 Nov 2014.

Ma X, et al. Ethnic difference in daycare attendance, early infections, and risk of childhood acute lymphoblastic leukemia. Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cospons Am Soc Prev Oncol. 2005;14(8):1928–34. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16103439. Accessed 18 Nov 2014.

Magnani C, et al. Increasing incidence of childhood leukemia in northwest Italy, 1975–98. Int J Cancer. 2003;105(4):552–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12712449 Accessed 21 Nov 2014.

Maule MM, et al. Parental age and risk of acute lymphocytic leukaemia and embryonal tumours in the Piedmont Region, Italy. Int J Epidemiol. 2007;36(3):691–2. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17550942. Accessed 17 Nov 2014.

McNally RJQ, Eden TOB. An infectious aetiology for childhood acute leukaemia: a review of the evidence. Br J Haematol. 2004;127(3):243–63. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15491284. Accessed 20 Nov 2014.

McNally RJ, et al. Examination of temporal trends in the incidence of childhood leukaemias and lymphomas provides aetiological clues. Leukemia. 2001;15(10):1612–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11587220. Accessed 21 Nov 2014.

McNally RJQ, Alexander FE, Birch JM. Space-time clustering analyses of childhood acute lymphoblastic leukaemia by immunophenotype. Br J Cancer. 2002;87(5):513–5. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2376144&tool=pmcentrez&rendertype=abstract. Accessed 4 Nov 2014.

Mehta PA, et al. Perforin polymorphism A91V and susceptibility to B-precursor childhood acute lymphoblastic leukemia: a report from the Children's Oncology Group. Leukemia. 2006;20(9):1539–41. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2922049&tool=pmcentrez&rendertype=abstract. Accessed 21 Nov 2014.

Mejía-Aranguré JM, et al. Incidence of leukemias in children from El Salvador and Mexico City between 1996 and 2000: population-based data. BMC Cancer. 2005;5:33. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1090561&tool=pmcentrez&rendertype=abstract. Accessed 18 Nov 2014.

Metayer C, Colt JS, et al. Exposure to herbicides in house dust and risk of childhood acute lymphoblastic leukemia. J Expo Sci Environ Epidemiol. 2013a;23(4):363–70. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23321862. Accessed 17 Nov 2014.

Metayer C, Zhang L, et al. Tobacco smoke exposure and the risk of childhood acute lymphoblastic and myeloid leukemias by cytogenetic subtype. Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cospons Am Soc Prev Oncol. 2013b;22(9):1600–11. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3769478&tool=pmcentrez&rendertype=abstract. Accessed 17 Nov 2014.

Meza-Espinoza JP, et al. XRCC1 polymorphisms and haplotypes in Mexican patients with acute lymphoblastic leukemia. Genet Mol Res GMR. 2009;8(4):1451–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20013659. Accessed 21 Nov 2014.

Milne E, et al. Fetal growth and risk of childhood acute lymphoblastic leukemia: results from an Australian case-control study. Am J Epidemiol. 2009;170(2):221–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19478236. Accessed 17 Nov 2014.

Milne E, et al. Maternal folate and other vitamin supplementation during pregnancy and risk of acute lymphoblastic leukemia in the offspring. Int J Cancer. 2010;126(11):2690–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19839053. Accessed 18 Nov 2014.

Milne E, et al. Maternal consumption of coffee and tea during pregnancy and risk of childhood ALL: results from an Australian case-control study. Cancer Causes Control CCC. 2011;22(2):207–18. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21113653. Accessed 18 Nov 2014

Milne E, et al. Parental prenatal smoking and risk of childhood acute lymphoblastic leukemia. Am J Epidemiol. 2012;175(1):43–53. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22143821. Accessed 17 Nov 2014.

Milne E, Greenop KR, Metayer C, et al. Fetal growth and childhood acute lymphoblastic leukemia: findings from the childhood leukemia international consortium. Int J Cancer. 2013a;133(12):2968–79. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23754574. Accessed 17 Nov 2014.

Milne E, Greenop KR, Scott RJ, et al. Parental alcohol consumption and risk of childhood acute lymphoblastic leukemia and brain tumors. Cancer Causes Control CCC. 2013b;24(2):391–402. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23247637. Accessed 17 Nov 2014.

Monge P, et al. Childhood leukaemia in Costa Rica, 1981–96. Paediatr Perinat Epidemiol. 2002;16(3):210–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12123433. Accessed 20 Nov 2014.

Morales-Sánchez A, et al. EBV, HCMV, HHV6, and HHV7 screening in bone marrow samples from children with acute lymphoblastic leukemia. Biomed Res Int. 2014;2014:548097. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4189769&tool=pmcentrez&rendertype=abstract. Accessed 18 Nov 2014.

Murray L, et al. Association of early life factors and acute lymphoblastic leukaemia in childhood: historical cohort study. Br J Cancer. 2002;86(3):356–61. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2375228&tool=pmcentrez&rendertype=abstract. Accessed 17 Nov 2014.

Neglia JP, et al. Patterns of infection and day care utilization and risk of childhood acute lymphoblastic leukaemia. Br J Cancer. 2000;82(1):234–40. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2363184&tool=pmcentrez&rendertype=abstract. Accessed 17 Nov 2014.

Nyári TA, et al. Childhood acute lymphoblastic leukaemia in relation to population mixing around the time of birth in south Hungary. Pediatr Blood Cancer. 2006;47(7):944–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16421899. Accessed 17 Nov 2014.

Nyari TA, Kajtár P, Parker L. Seasonality of birth and acute lymphoblastic leukemia. J Perinat Med. 2006;34(6):507–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17140306. Accessed 17 Nov 2014.

Nyári TA, et al. Seasonal variation of childhood acute lymphoblastic leukaemia is different between girls and boys. Pathol Oncol Res POR. 2008;14(4):423–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18409021. Accessed 17 Nov 2014.

Nyari TA, et al. Spatial clustering of childhood acute lymphoblastic leukaemia in Hungary. Pathol Oncol Res POR. 2013;19(2):297–302. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23229439. Accessed 17 Nov 2014.

Op het Veld CW, et al. Methyl methanesulfonate-induced hprt mutation spectra in the Chinese hamster cell line CHO9 and its xrcc1-deficient derivative EM-C11. Mutat Res. 1998;398(1–2):83–92. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9626968. Accessed 21 Nov 2014.

Ou SX, et al. Birth characteristics, maternal reproductive history, hormone use during pregnancy, and risk of childhood acute lymphocytic leukemia by immunophenotype (United States).

Cancer Causes Control CCC. 2002;13(1):15–25. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11899114. Accessed 16 Nov 2014.

Pakakasama S, et al. Genetic polymorphisms and haplotypes of DNA repair genes in childhood acute lymphoblastic leukemia. Pediatr Blood Cancer. 2007;48(1):16–20. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16435384. Accessed 21 Nov 2014.

Papaemmanuil E, et al. Loci on 7p12.2, 10q21.2 and 14q11.2 are associated with risk of childhood acute lymphoblastic leukemia. Nat Genet. 2009;41(9):1006–10. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19684604. Accessed 20 Nov 2014.

Parkin DM, et al. The international incidence of childhood cancer. Int J Cancer. 1988;42(4):511–20. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3170025. Accessed 20 Nov 2014.

Pelissari DM, Barbieri FE, Wünsch Filho V. Magnetic fields and acute lymphoblastic leukemia in children: a systematic review of case-control studies. Cad Saude Publica. 2009;25 Suppl 3:S441–52. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20027391. Accessed 17 Nov 2014.

Pérez-Saldivar ML, et al. Childhood acute leukemias are frequent in Mexico City: descriptive epidemiology. BMC Cancer. 2011;11:355. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3171387&tool=pmcentrez&rendertype=abstract. Accessed 8 Nov 2014.

Petra BG, Janez J, Vita D. Gene-gene interactions in the folate metabolic pathway influence the risk for acute lymphoblastic leukemia in children. Leuk Lymphoma. 2007;48(4):786–92. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17454638. Accessed 21 Nov 2014.

Petridou E, et al. Maternal diet and acute lymphoblastic leukemia in young children. Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cospons Am Soc Prev Oncol. 2005;14(8):1935–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16103440. Accessed 18 Nov 2014.

Petridou ET, et al. Childhood leukaemias and lymphomas in Greece (1996–2006): a nationwide registration study. Arch Dis Child. 2008;93(12):1027–32. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18676433. Accessed 21 Nov 2014.

Pratt JA, et al. Racial differences in acute lymphocytic leukemia mortality and incidence trends. J Clin Epidemiol. 1988;41(4):367–71. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3162512. Accessed 20 Nov 2014.

Pui C-H, Evans WE. A 50-year journey to cure childhood acute lymphoblastic leukemia. Semin Hematol. 2013;50(3):185–96. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3771494&tool=pmcentrez&rendertype=abstract. Accessed 16 Nov 2014.

Pui C-H, et al. Biology, risk stratification, and therapy of pediatric acute leukemias: an update. J Clin Oncol Off J Am Soc Clin Oncol. 2011;29(5):551–65. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3071256&tool=pmcentrez&rendertype=abstract. Accessed 22 Nov 2014.

Ramot B, Magrath I. Hypothesis: the environment is a major determinant of the immunological sub-type of lymphoma and acute lymphoblastic leukaemia in children. Br J Haematol. 1982;50(2):183–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/6977370. Accessed 18 Nov 2014.

Reid A, et al. Risk of childhood acute lymphoblastic leukaemia following parental occupational exposure to extremely low frequency electromagnetic fields. Br J Cancer. 2011;105(9):1409–13. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3241544&tool=pmcentrez&rendertype=abstract. Accessed 17 Nov 2014.

Ribeiro KB, Buffler PA, Metayer C. Socioeconomic status and childhood acute lymphocytic leukemia incidence in São Paulo, Brazil. Int J Cancer. 2008;123(8):1907–12. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18688860. Accessed 17 Nov 2014.

Roberts MR, et al. Single-nucleotide polymorphisms in DNA repair genes and association with breast cancer risk in the web study. Carcinogenesis. 2011;32(8):1223–30. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3149207&tool=pmcentrez&rendertype=abstract. Accessed 21 Nov 2014.

Roman E, et al. Childhood acute lymphoblastic leukemia and infections in the first year of life: a report from the United Kingdom childhood cancer study. Am J Epidemiol. 2007;165(5):496–504. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17182983. Accessed 18 Nov 2014.

Roman E, et al. Childhood acute lymphoblastic leukaemia and birthweight: insights from a pooled analysis of case-control data from Germany, the United Kingdom and the United States. Eur J Cancer (Oxf, Engl: 1990). 2013;49(6):1437–47. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23266048. Accessed 17 Nov 2014.

Rosenbaum PF, Buck GM, Brecher ML. Early child-care and preschool experiences and the risk of childhood acute lymphoblastic leukemia. Am J Epidemiol. 2000;152(12):1136–44. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11130619. Accessed 17 Nov 2014.

Rosenbaum PF, Buck GM, Brecher ML. Allergy and infectious disease histories and the risk of childhood acute lymphoblastic leukaemia. Paediatr Perinat Epidemiol. 2005;19(2):152–64. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15787890. Accessed 18 Nov 2014.

Ruíz Argüelles GJ. Fundamentos de Hematología. 4th ed. México: Editorial Médica Panamericana; 2009.

Rull RP, et al. Residential proximity to agricultural pesticide applications and childhood acute lymphoblastic leukemia. Environ Res. 2009;109(7):891–9. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2748130&tool=pmcentrez&rendertype=abstract. Accessed 17 Nov 2014.

Sherborne AL, et al. Variation in CDKN2A at 9p21.3 influences childhood acute lymphoblastic leukemia risk. Nat Genet. 2010;42(6):492–4. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3434228&tool=pmcentrez&rendertype=abstract. Accessed 25 Nov 2014.

Shiraishi K, et al. Association of DNA repair gene polymorphisms with response to platinum-based doublet chemotherapy in patients with non-small-cell lung cancer. J Clin Oncol Off J Am Soc Clin Oncol. 2010;28(33):4945–52. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20940192. Accessed 18 Nov 2014.

Shu XO, et al. Diagnostic X-rays and ultrasound exposure and risk of childhood acute lymphoblastic leukemia by immunophenotype. Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cospons Am Soc Prev Oncol. 2002;11(2):177–85. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11867505. Accessed 16 Nov 2014.

Sohrabi M-R, et al. Living near overhead high voltage transmission power lines as a risk factor for childhood acute lymphoblastic leukemia: a case-control study. Asian Pac J Cancer Prev. 2010;11(2):423–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20843128. Accessed 17 Nov 2014.

Soldin OP, et al. Pediatric acute lymphoblastic leukemia and exposure to pesticides. Ther Drug Monit. 2009;31(4):495–501. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3622217&tool=pmcentrez&rendertype=abstract. Accessed 17 Nov 2014.

Sørensen HT, et al. Seasonal variation in month of birth and diagnosis of early childhood acute lymphoblastic leukemia. JAMA. 2001;285(2):168–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11176808. Accessed 17 Nov 2014.

Spix C, et al. Temporal trends in the incidence rate of childhood cancer in Germany 1987–2004. Int J Cancer. 2008;122(8):1859–67. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18076067. Accessed 21 Nov 2014.

Stack M, et al. Childhood cancer in Ireland: a population-based study. Arch Dis Child. 2007;92(10):890–7. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2083220&tool=pmcentrez&rendertype=abstract. Accessed 21 Nov 2014.

Stanczyk M, et al. The association of polymorphisms in DNA base excision repair genes XRCC1, OGG1 and MUTYH with the risk of childhood acute lymphoblastic leukemia. Mol Biol Rep. 2011;38(1):445–51. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20364408. Accessed 21 Nov 2014.

Stiller CA. Epidemiology and genetics of childhood cancer. Oncogene. 2004;23(38):6429–44. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15322515. Accessed 18 Nov 2014.

Stiller CA, et al. Population mixing, socioeconomic status and incidence of childhood acute lymphoblastic leukaemia in England and Wales: analysis by census ward. Br J Cancer.

2008;98(5):1006–11. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2266854&tool=pmcentrez&rendertype=abstract. Accessed 17 Nov 2014.

Suk WA, Murray K, Avakian MD. Environmental hazards to children's health in the modern world. Mutation Research. 2003;544(2–3):235–42. ISSN 0027-5107.

Tebbs RS, et al. Requirement for the Xrcc1 DNA base excision repair gene during early mouse development. Dev Biol. 1999;208(2):513–29. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10191063. Accessed 21 Nov 2014.

Thompson JR, et al. Maternal folate supplementation in pregnancy and protection against acute lymphoblastic leukaemia in childhood: a case-control study. Lancet. 2001;358(9297):1935–40. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11747917. Accessed 18 Nov 2014.

Treviño LR, et al. Germline genomic variants associated with childhood acute lymphoblastic leukemia. Nat Genet. 2009;41(9):1001–5. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2762391&tool=pmcentrez&rendertype=abstract. Accessed 21 Nov 2014.

Tumer TB, et al. DNA repair XRCC1 Arg399Gln polymorphism alone, and in combination with CYP2E1 polymorphisms significantly contribute to the risk of development of childhood acute lymphoblastic leukemia. Leuk Res. 2010;34(10):1275–81. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20394984. Accessed 21 Nov 2014.

Urayama KY, et al. A meta-analysis of the association between day-care attendance and childhood acute lymphoblastic leukaemia. Int J Epidemiol. 2010;39(3):718–32. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2878455&tool=pmcentrez&rendertype=abstract. Accessed 18 Nov 2014.

Urayama KY, et al. Early life exposure to infections and risk of childhood acute lymphoblastic leukemia. Int J Cancer. 2011;128(7):1632–43. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3165002&tool=pmcentrez&rendertype=abstract. Accessed 18 Nov 2014.

Vestergaard TR, et al. Hospitalisation for infection prior to diagnosis of acute lymphoblastic leukaemia in children. Pediatr Blood Cancer. 2013;60(3):428–32. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22915267. Accessed 18 Nov 2014.

Vijayakrishnan J, Houlston RS. Candidate gene association studies and risk of childhood acute lymphoblastic leukemia: a systematic review and meta-analysis. Haematologica. 2010;95(8):1405–14. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2913091&tool=pmcentrez&rendertype=abstract. Accessed 21 Nov 2014.

Ward MH, et al. Residential levels of polybrominated diphenyl ethers and risk of childhood acute lymphoblastic leukemia in California. Environ Health Perspect. 2014;122(10):1110–6. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4181922&tool=pmcentrez&rendertype=abstract. Accessed 17 Nov 2014.

Wen W, et al. Allergic disorders and the risk of childhood acute lymphoblastic leukemia (United States). Cancer Causes Control CCC. 2000;11(4):303–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10843442. Accessed 17 Nov 2014.

Wen W, et al. Parental medication use and risk of childhood acute lymphoblastic leukemia. Cancer. 2002;95(8):1786–94. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12365028. Accessed 16 Nov 2014.

Wesolowska A, et al. Cost-effective multiplexing before capture allows screening of 25 000 clinically relevant SNPs in childhood acute lymphoblastic leukemia. Leukemia. 2011;25(6):1001–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21415851. Accessed 21 Nov 2014.

Wiemels J. Perspectives on the causes of childhood leukemia. Chem Biol Interact. 2012;196(3):59–67. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3839796&tool=pmcentrez&rendertype=abstract. Accessed 20 Nov 2014.

Wiemels JL, et al. Protracted and variable latency of acute lymphoblastic leukemia after TEL-AML1 gene fusion in utero. Blood. 1999;94(3):1057–62. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10419898. Accessed 21 Nov 2014.

Wilkinson JD, et al. Lymphoma and lymphoid leukemia incidence in Florida children: ethnic and racial distribution. Cancer. 2001;91(7):1402–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11283943. Accessed 20 Nov 2014.

Woodruff TJ, et al. Trends in environmentally related childhood illnesses. Pediatrics. 2004; 113(4 Suppl):1133–40. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15060210. Accessed 20 Nov 2014.

Wünsch-Filho V, et al. Exposure to magnetic fields and childhood acute lymphocytic leukemia in São Paulo, Brazil. Cancer Epidemiol. 2011;35(6):534–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21840286. Accessed 17 Nov 2014.

Zelent A, Greaves M, Enver T. Role of the TEL-AML1 fusion gene in the molecular pathogenesis of childhood acute lymphoblastic leukaemia. Oncogene. 2004;23(24):4275–83. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15156184. Accessed 15 Nov 2014.

Zhou Y, et al. Maternal benzene exposure during pregnancy and risk of childhood acute lymphoblastic leukemia: a meta-analysis of epidemiologic studies. PLoS One. 2014;9(10):e110466. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4198238&tool=pmcentrez&rendertype=abstract. Accessed 18 Nov 2014.

Zierhut H, et al. Family history of cancer and non-malignant diseases and risk of childhood acute lymphoblastic leukemia: a Children's Oncology Group Study. Cancer Epidemiol. 2012;36(1):45–51. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3962042&tool=pmcentrez&rendertype=abstract. Accessed 17 Nov 2014.

Chapter 9
Early Hematopoietic Differentiation in Acute Lymphoblastic Leukemia: The Interplay Between Leukemia-Initiating Cells and Abnormal Bone Marrow Microenvironment

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Abstract By virtue of their self-renewal and tightly regulated multi-lineage differentiation properties, hematopoietic stem cells (HSCs) generate the whole blood system throughout postnatal life. During malignant hematological disorders, including acute leukemias, a number of intrinsic and extrinsic cues influence the hematopoietic differentiation pathway and cooperate to make aberrant cell fate decisions concomitant with cell transformation. The cellular origin of these disorders is a fundamental matter in question. In keeping with the hierarchical model of tumor evolution, a conspicuous and unique leukemic stem cell (LSC) population is most likely the foundation of acute and chronic myeloid leukemias. In contrast, all B-cell differentiation stages in acute lymphoblastic leukemia (ALL) function as leukemia-initiating cells (LICs), are endowed with primitive stem cell properties and are

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apparently responsible for the long-term maintenance of tumor growth within the bone marrow (BM) and for relapse of the disease following remission. Furthermore, LICs reveal the ability to create irregular BM microenvironments that may result in proinflammatory scenarios with a permissive role by allowing leukemic cell development at the expense of normal hematopoiesis. This chapter outlines the recent findings contributing to the understanding of malignant hematopoiesis through the biology of early stem and progenitor cells in the context of abnormal microenvironments within leukemic BM. By unraveling the role of leukemic precursor cells in the initiation of local inflammatory processes leading to hematopoietic instability, we may learn about additional mechanisms co-participating in the etiology and maintenance of this pathological condition.

Keywords Leukemia-initiating cells • Acute lymphoblastic leukemia • Early hematopoiesis • Bone marrow • Proinflammatory microenvironment

Introduction

Cancer is the leading cause of nonaccidental death among children in high-income countries and in a growing number of middle-income countries and is therefore being considered a global child health priority (Magrath et al. 2013; Gupta et al. 2014). Decreasing overall childhood cancer mortality requires a comprehensive understanding of the origins and pathobiology of the disease, along with more accurate diagnoses and the identification of high-risk groups in order to apply effective treatments. Strikingly, acute leukemias (ALs) are the most frequent childhood malignancies worldwide and remain a leading cause of morbidity and mortality among relapsed patients. Even though more efficient therapeutic agents have been developed over the past 10 years that have increased overall survival rates, leukemic cell infiltration, relapse, and treatment failure change the prognosis and significantly worsen the outcome of the disease, underlining the need for new strategies to better identify the cell root and to predict its dynamics according to the microenvironmental and clinical context.

The hierarchical theory of cancer development sustains the notion that cancer stem cells (CSCs) support the initiation and maintenance of tumors and may also constitute the subpopulation of tumor cells responsible for the invasion and development of metastatic tumors (Kakarala and Wicha 2008; Rajendran and Dalerba 2014). Of note, current information indicates that while in both acute and chronic myeloid leukemias a rare LSC population is responsible for their development and recurrence (Chung and Park 2014; Chavez-Gonzalez et al. 2013), a unique contribution of these primitive cells in ALL is not apparent (Raff and Bruggermann 2014). Furthermore, recent advances suggest that some changes in hematopoietic microenvironment might account for aberrant cell differentiation pathways in this pathological condition.

Biology of ALL

The uncontrolled production of hematopoietic precursor cells of the myeloid or lymphoid series within the BM is the prominent feature of ALs (Dorantes-Acosta and Pelayo 2012). Among them, ALL is the most common cause of childhood cancer worldwide and accounts for 23 % of malignancies and for 85 % of the leukemia cases, whereas acute myeloid leukemia (AML) constitutes 15 % of acute leukemia cases (Xie et al. 2003; Perez-Saldivar et al. 2011). Of note, although important breakthroughs in treatment strategies have influenced the outcome of these disorders, leading to increases in overall survival rates of up to 80 %, the last 25 years have recorded a slight but gradual increase in the incidence of ALL that appears to be highest in Latin America (McNeil et al. 2002; Perez-Saldivar et al. 2011; Xu et al. 2013), where superior rates of high-risk patients are also apparent.

According to international classifications of lineage and maturation stages based on the number and specificity degree of lineage markers that are expressed by leukemic cells (Dorantes-Acosta and Pelayo 2012), 80–85 % of ALL cases have a B-cell immunophenotype, while nearly 15 % show a T-cell immunophenotype. Congenital leukemia, a rare entity distinct from typical ALL, represents only 3 % of ALs, whereas mixed-lineage leukemias (MLLs), endowed with properties of both lymphoid and myeloid lineage cells, constitute about 2 % of ALs. Proper disease management based on the stratification of patients by risk groups and the identification of relapse factors contributes greatly to disease-free survival (Izraeli 2010; Juarez-Velazquez et al. 2014). Currently, the most useful prognostic indicators are age, white blood cell count, immunophenotype, minimal residual disease detection, and therapy responses. The phenotype of leukemic cells is one of the factors that set the risk of relapse, which is suffered by 20–30 % of patients whose response to therapies is frequently of poorer quality and shorter duration. Hence, T-cell lineage and biphenotypic leukemias are associated with unfavorable prognosis (Pui et al. 1998; Rubnitz et al. 2009; Meijerink et al. 2009). Moreover, a lineage conversion/switch is occasionally recorded upon relapse, a phenomenon that has been considered to be an uncommon type of MLL, representing either a relapse of the original clone with plasticity attributes or the emergence of new leukemic clones.

The molecular mechanisms driving relapse in any of the leukemia entities are still poorly understood. In addition, cell infiltration remains an obstacle to curing ALL patients. The central nervous system (CNS) is the most frequently affected extramedullary site (30–40 %), and a number of apparent risk factors related to CNS relapse include T-cell immunophenotype, high-risk cytogenetic abnormalities, hyperleukocytosis, and leukemic cells present in the CNS or in traumatic lumbar puncture at diagnosis (Pui and Evans 2013). Most parameters universally considered remain insufficient for establishing an early stratification, highlighting the complexity of the disease and the need for new biomarkers associated with cell origins to better predict outcomes. Of note, the combination of genomics and clonal studies with xenotransplant approaches has revealed unsuspected genetic diversity

within the various ALL-initiating cells, supporting the multiclonal – possibly stochastic – evolution of leukemogenesis (Notta et al. 2011b; Purizaca et al. 2012).

Recurring chromosome abnormalities are detected in approximately 80 % of ALL patients. Aneuploidy, translocations, inversions, or deletions are some of the numerical and structural changes that are often associated with risk of relapse (Pui et al. 2008). Among them, chromosome translocations are the most frequent and may constitute early or initiating events in leukemogenesis (Greaves and Wiemels 2003). They result in fusion proteins with altered functions and oncogenic properties. *ETV6/RUNX1* and *BCR/ABL1* fusions are universally related to good and bad prognosis, respectively. *ETV6/RUNX1* (*TEL/AML1*) resulting from the t(12;21)(p12;q21) translocation has been considered to be a putative prenatal first lesion which is acquired in utero, but requires additional somatic mutations for overt leukemia (Lilljebjorn et al. 2012; Zuna et al. 2011), whereas the translocation t(9;22)(q34;q11) (Ph chromosome) leads to expression of the *BCR/ABL1* product, found in 5 % of childhood ALL cases and resulting in a constitutive tyrosine kinase activity with alterations in IKZF1. Interestingly, a BCR/ABL1-like ALL has been identified, where *IKZF1*, *CRLF2*, and *JAK* mutations show gene expression profiles similar to Ph+ B-cell ALL (B-ALL), but lack the *BCR/ABL1* rearrangement (Mullighan and Willman 2011; Woo et al. 2014). The *MLL* gene is involved in more than 50 fusions mostly connected to cell transformation and adverse outcome, largely due to cellular drug resistance (Meijerink et al. 2009). Translocation t(4;11)(q21;q23) producing the fusion of the *MLL* and *AF4* genes has been documented in nearly 80 % of infant ALL. On the other hand, aneuploidy (hyperdiploidy and hypodiploidy) occurs in high frequency of ALL cases (Mullighan 2012). Hyperdiploidy is characterized by a nonrandom gain of chromosomes X, 4, 6, 10, 14, 17, 18, and 21 and clinically by a favorable prognosis. In contrast, the majority of the hypodiploidy cases show 45 chromosomes and adverse evolution of the disease.

Novel high-resolution genomic technologies and next-generation sequencing have been decisive in the identification of a growing list of DNA lesions affecting genes involved in key cellular pathways, including normal hematopoiesis, tumor suppression, apoptosis, and cell cycle regulation (Mantovani et al. 2008; Meijerink et al. 2009), targeting new genes of potential interest, including *CDKN2A*, *COL6A2*, *PTPRO*, *CSMD1*, *HMGA1*, *CASP8AP2*, and *H2AFZ*. Additionally, a possible convergence of the WNT, JAK, and MAP kinase pathways has been related to leukemogenesis (Hogan et al. 2011; Bhatla et al. 2014; Juarez-Velazquez et al. 2014).

As will be further discussed, hematopoiesis is driven to a large extent by lineage-specific transcription factors. Remarkably, genetic alterations of master regulators of the early hematopoietic differentiation, including Ikaros (*IKZF1*), and the downstream lymphoid development pathway *PAX5*, *EBF1*, *E2A*, *CRLF2*, and *LEF1* are hallmarks of ALL (Fig. 9.1) (Mullighan and Willman 2011). Of special interest, by disrupting the transcriptional program of normal B-cell differentiation, the contribution of these factors to neoplastic growth regulation has been demonstrated (McManus et al. 2011; Kawamata et al. 2012). Moreover, crucial growth factor receptors which are primarily expressed in stem cells and early multipotent (myeloid and lymphoid) progenitors, playing important roles in their proliferation and differentiation, are compromised in ALL. The best example is the FMS-like tyrosine kinase-3 (*FLT3*), a group of class III receptor tyrosine kinases that also includes

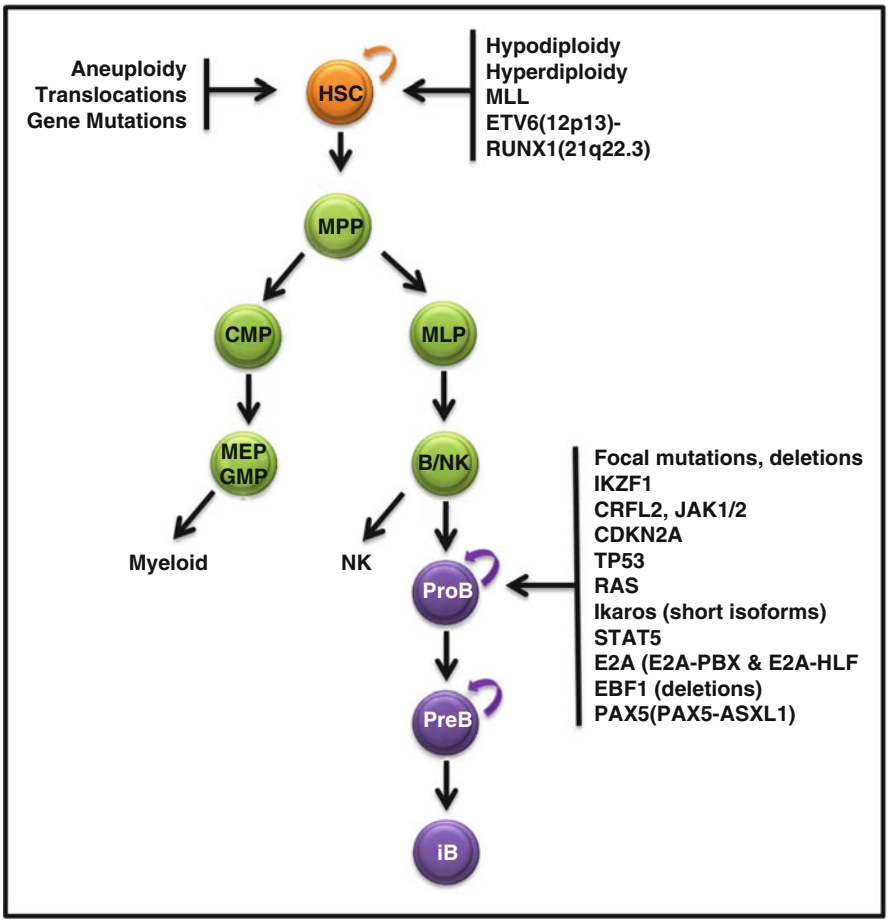


Fig. 9.1 Genetic abnormalities in B-ALL development. B-cell development starts within the BM from a primitive hematopoietic stem cell endowed with self-renewal and multipotent differentiation properties. HSC gives rise to multipotent progenitors that have the ability to differentiate into MPPs, where commitment to the early lymphoid lineage program starts and ends with the formation of immature B-cells. Numerous recurrent abnormalities have been identified along the B-cell pathway in leukemogenesis, including aneuploidy, translocations, and gene mutations that may be associated with the earliest steps of malignant hematopoiesis, as well as aberrant receptors, signaling proteins, and transcription factors involved in commitment and regulation of pro- and pre-B-cell precursors. *B-ALL* B-cell acute lymphoblastic leukemia, *HSC* hematopoietic stem cell, *MPP* multipotent progenitor, *CMP* common myeloid progenitor, *MEP* megakaryocyte and erythrocyte progenitor, *GMP* granulocyte and monocyte progenitor, *MLP* multi-lymphoid progenitor, *B/NK* progenitor for B and natural killer cells, *iB* immature B-cell

C-KIT, C-FMS, and platelet-derived growth factor receptor (Takahashi 2011; Gu et al. 2011). The FLT3 internal tandem duplications (Flt3-ITDs) occurring in 25–30 % of B-ALL patients suggest that a constitutive activation of FLT3 is apparent from the earliest stages of the hematopoietic differentiation program, underlining the relevance of lineage commitment and primitive cell differentiation in the pathobiology of ALL.

Normal vs Malignant Hematopoiesis: The Beginning of Leukemic Processes

Development and Function of Normal Hematopoietic Cells

Stem cells are the origin of all cell types within the organism. The characterization and definition of their transcriptional activity patterns controlling lineage fate decisions have allowed the construction of cell differentiation maps that are, today, the best models for understanding normal and aberrant core processes. The term stem cell describes a functional primitive cell capable of producing multiple cell types by differentiation and self-renewal activity. According to their differentiation potentials, these rare cells can be categorized into totipotent stem cells, pluripotent stem cells, and multipotent stem cells. The latter are the source of various specialized tissues, including the hematopoietic, which is hierarchically organized and maintains homeostasis via a complex and continuous biological process called hematopoiesis (Purizaca et al. 2012; Pelayo et al. 2012; Vadillo et al. 2013).

The hematopoietic system supplies and replenishes erythrocytes, thrombocytes, and all cell categories of the immune system throughout life. The tightly regulated developmental steps take place within BM starting from a unique population of HSCs that reside in endosteal, perivascular, or reticular niches (Vadillo et al. 2013). While intrinsic early differentiation programs – including genetic and epigenetic networks – strictly control the self-renewing and most multi-lineage potential properties in this infrequent population, specialized BM niches and a number of crucial microenvironmental cues function by promoting maintenance of the HSC pool and contributing lymphoid or myeloid cell fate decisions along the pathway (Purizaca et al. 2012; Vadillo et al. 2013).

The current understanding of the biology of HSC and hematopoietic system development is largely the result of research in genetically modified mouse models that offer the possibility of carrying out in vivo experiments to demonstrate precursor cell activity. Additionally, powerful technologies as flow cytometry and xenotransplantation approaches have enabled the identification of five cell compartments within BM: stem cells, multipotent progenitors (MPPs), oligopotent progenitors, precursor cells, and mature cells. Maintenance of the hematopoietic hierarchy and the precise balance between proliferation and differentiation of the various cell fractions are critical to the proper operation of the system.

Murine HSCs were first isolated as Lin^{-} $Sca\text{-}1^{+}$ $Thy\text{-}1^{lo}$ and were shown to constitute about 0.05 % of BM cells (Spangrude et al. 1988). Further characterization based on the expression of signaling lymphocyte activation molecule (SLAM) family members has distinguished $CD150^{+}CD244^{-}CD48^{-}$ HSC from $CD150^{-}CD244^{+}CD48^{+}$ multipotent and committed progenitors concomitant with gradual reconstitution capability loss (Kiel et al. 2005). Of interest, it has been recently proposed that HSCs have "intrinsic tendencies" to produce lymphoid, myeloid, or a mixture of both lineages that are sensitive to microenviromental factors promoting lineage-specific blood cell production (Benz et al. 2012). Together with SLAM family markers, CD229 allows

the phenotypic distinction of a lymphoid-biased from a myeloid-biased HSC (Oguro et al. 2013). Moreover, HSCs are thought to possess exquisite affinity for specialized niches within BM, providing a putative advantage for the production of lineages to which these cells are committed (Benz et al. 2012).

On the other hand, human $Lin^-CD34^+CD38^-CD45RA^-CD49f^+CD90^+$ HSC represent less than 0.04 % of BM mononuclear cells and are capable of long-term reconstitution when testing in xenotransplantation models. Additional useful detection markers are Flt3 and null CD7 or CD10 expression (Doulatov et al. 2010; Notta et al. 2011a). HSCs give rise to MPPs, which no longer possess self-renewal potential and have therefore lost long-term reconstitution capabilities (Dorantes-Acosta and Pelayo 2012). MPPs embed lymphoid-primed multipotent progenitors (LMPPs), a heterogeneous population where L-selectin$^+$ progenitors (LSPs), early lymphoid progenitors (ELPs), and dendritic cell (DC)-biased LMPPs (DC-LMPPs) (Iwasaki and Akashi 2007; Naik et al. 2013) are contained, each generating specific cell lineages. LSPs are the origin of interferon killer dendritic cells, while ELPs produce plasmacytoid dendritic cells. Human L-selectin$^+$ progenitors have also been described (Kohn et al. 2012) that may precede the earliest steps of the lymphoid program concomitant with multi-lymphoid progenitor (MLP) differentiation. Both LSPs and MLPs are efficient in producing all lymphoid lineage cells, monocytes, and DCs but lack granulocyte potential (Doulatov et al. 2010). Whereas in mice $Lin^-ckit^{lo}Sca1^+IL\text{-}7R\alpha^+CD27^+$ common lymphoid progenitors (CLPs) arising from LMPPs/ELPs constitute 0.02 % of total BM cells and the main B and natural killer (NK) cell producer (Pelayo et al. 2006; Kondo et al. 1997; Dorantes-Acosta and Pelayo 2012), its human counterpart endowed with B/NK lymphoid potential is characterized as $Lin^-CD34^+CD38^+CD90^-CD45RA^+Flt3^+CD10^+$ (Vadillo and Pelayo 2011). Downstream the pathway, sequential differentiation of fully committed progenitors gives rise to $CD34^+CD10^+CD19^+$ pro-B-cells, $CD34^-CD10^+CD19^+$ Pre-B-cells, and $CD34^-CD10^-CD19^+$ immature B-cells that are eventually exported to the periphery (Fig. 9.1). Early B-cell development stages are crucially mediated by interleukin 7 (IL-7) signals that activate the major signaling pathway, JAK-STAT5 (Pelayo et al. 2012).

T-cell production depends on the thymic colonization by BM-exported early progenitors expressing chemokine receptors like CCR7, CCR9, CXCR4, and PSLG1 (Sultana et al. 2012; Bell and Bhandoola 2008; Zlotoff et al. 2010; Shah and Zuniga-Pflucker 2014). The thymic microenvironment induces the loss of multipotency as cell progenitors advance on their developmental program. Thymocyte specification starts in a $CD4^-CD8^-$ double negative population and progresses to double-positive (DP) $CD4^+CD8^+$ stages concomitant with their migration from the cortex to the subcapsular regions of the thymus. Then, T-cell production is fully achieved upon positive selection of DP clones (Bhandoola and Sambandam 2006). At the time of lymphopoiesis, common myeloid progenitors (CMPs) are constantly formed by MPPs and in turn give rise to granulocyte–monocyte progenitors (GMPs) and megakaryocytic–erythroid progenitors (Fig. 9.1) (Manz et al. 2002; Dorantes-Acosta and Pelayo 2012).

In the aforementioned, normal hematopoiesis is largely governed by a growing number of cell–cell interactions and growth/differentiation factors that control the expression of lineage-specific transcription factors (TFs) (Vadillo et al. 2013).

Accordingly, the B-cell differentiation pathway depends on the expression of PU.1, E2A, EBF, and Pax5, while the T-cell program is controlled by GATA3 and Notch1. Strikingly, deficiency in E2A and EBF1 blocks B-cell differentiation and the loss of Pax5 redirects B-cells into other lineages. Pax5 acting together with EBF conducts the display of key molecules such as FOXO1, LEF1, CD19, RAG, and CD79a. This master regulator, Pax5, also functions as a repressor of M-CSFR, NOTCH1, and FLT3, inhibiting the possibility of commitment to myeloid, T-cell, or DC lineages respectively, and therefore promoting B-cell specification for further differentiation (Pelayo et al. 2012). On the other hand, lymphoid-derived DC formation requires the activity of TFs like FLT3, STAT3, E2.2, IRF8, and Spi-B (Shortman et al. 2013). Interestingly, recent research on lymphoid development has generated a new classification, where innate lymphoid cells (ILCs) are now integrated (Vosshenrich and Di Santo 2013; Spits and Cupedo 2012). Innate lymphoid cells emerge from $Id2^+$ CLPs (Boos et al. 2007) and include NK cells and other immune-dedicated populations (Spits and Cupedo 2012). Though the whole path is still poorly understood, the transcriptional control of NK cell production is known to be governed by Ikaros, Aiolos, and PU.1 in the very primitive stages and become dependent on E4bp4 for the induction of Id2 and Eomes expression that culminates in NK cell commitment in dependency on IL15 (Blom and Spits 2006; Male et al. 2014). Factors like Notch, RORα, RORγt, and GATA3 are essential for other subsets of ILC (Wong et al. 2012; Mjosberg et al. 2012; Serafini et al. 2014; Vosshenrich and Di Santo 2013). Within the myeloid differentiation pathway, TFs such as PU.1, RUNX1, SCL, Ikaros, and Gfi1 play a substantial role in the early commitment of CMP (Dorantes-Acosta and Pelayo 2012), whereas GATA1 is required for megakaryocytic–erythroid development, and downstream the path c/EBPα is involved in the acquisition of myeloid function (Iwasaki and Akashi 2007). Of note, any deregulation of this transcriptional balance may lead to a new pathological lineage.

A number of hematopoietic cells and molecules that compose the innate or adaptive immune systems function as key components of defense against cancer, where their ability to unequivocally identify and destroy tumor cells contributes to a dynamic process termed cancer immunosurveillance (Vesely et al. 2011), highlighting the relevance of a continuous homeostatic control of hematopoietic cell proliferation and differentiation to produce functional extrinsic tumor suppressor elements. However, a recent investigation has suggested that in addition to playing an effective protection role against tumorigenesis, the immune system can further participate in the promotion of the outgrowth of malignant cells by editing of tumor immunogenicity. Such cancer immunoediting process includes three distinct phases: elimination, equilibrium, and escape (Vesely et al. 2011). During the elimination phase, normal hematopoietic cells and molecules terminate potential cancer cells. Entering the equilibrium phase due to tumor dormancy is an unsafe situation because some components of the immune system are exposed to loss of function and ultimately permit the adaptation of transformed cells (escape phase). The cancer immunoediting mechanisms that may operate once transformed leukemic cells are differentiating are still unclear.

Because hematopoiesis – particularly lymphopoiesis – can undergo adjustments in cell fate decisions under inflammatory conditions and during malignant development, the presumptive implication of a "permissive or inductive normal hematopoiesis" in the etiology of ALL should be supported by future investigations using discrimination approaches to follow normal vs malignant hematopoiesis within leukemic BM. In fact, hematopoiesis in both childhood ALL and AML is crucially defective when tested in controlled culture systems (Purizaca et al. 2013; Dorantes-Acosta et al. 2008). Surprisingly, the content and ability of early lymphoid and myeloid progenitors to proliferate and undergo multi-lineage differentiation are reduced, revealing major deficiencies in the presumed normal hematopoietic compartment of leukemia BM.

Cancer Stem Cells and Leukemia-Initiating Cells in Malignant Hematopoietic Development

Despite the important advances in unraveling genetic, molecular, and cell abnormalities associated with leukemic hematopoiesis, a complete understanding of the mechanisms damaging the earliest developmental program and controlling the emergence of leukemia-initiating cells that drive lineage instability is still lacking. Moreover, a discrepancy in the cellular origins of myeloid and lymphoid leukemias has increased the complexity of these neoplastic entities. As discussed below, very primitive CSCs in myeloid acute and chronic leukemias are apparently responsible for tumor initiation and maintenance. However, the identification of a unique population of MSCs sustaining ALL has not been confirmed. Besides, there is tremendous intersubject heterogeneity that makes it impossible to firmly apply the CSC model to lymphoblastic leukemias. Instead, various precursor differentiation stages may function as LICs (Fig. 9.2).

Cancer cell of origin constitutes the normal clonal population target of the first oncogenic hit and in which the earliest tumoral process takes place (Abollo-Jiménez et al. 2011; Abraham et al. 2014). This premalignant clone would lead to increased survival and may function as a precursor of CSCs, which are defined as a rare "reprogrammed" population capable of indefinitely generating tumors as well as all cellular types that compose the malignant mass. CSCs control the maintenance, propagation, metastasis, and relapse of transformed cells (Ailles and Weissman 2007). In contrast, cancer-initiating cells can be either progenitor or differentiating cells where the normal developmental program is damaged in such a way that they display some stemness and plasticity properties. Leukemia-initiating cells are believed to be part of this group.

Clonal phenotyping and genotyping have allowed the functional evaluation of CSCs; thus, their biological behavior could be classified within the hierarchical or the stochastic model. According to the hierarchical model, tumors are "pathological organs" where CSCs are located at the top of a cellular hierarchy, implying that they self-renew and consequently produce more differentiated clones with limited or no self-renewal potential that are not capable of giving rise to primitive CSCs (Rajendran and Dalerba 2014). Additionally, CSCs must be responsive to

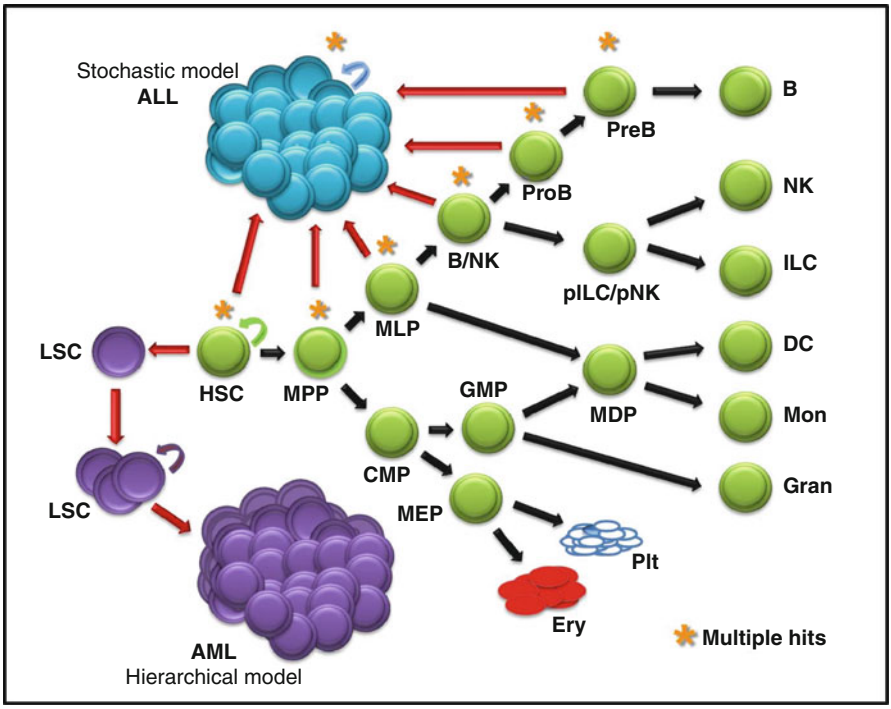


Fig. 9.2 Cancer stem cells and leukemia-initiating cells in the etiology of acute leukemias. Normal hematopoietic process (*central panel*) implicates the differentiation of HSC toward myeloid and lymphoid progenitors producing committed precursor and mature cells of physiological proportions. According to the hierarchical model of cancer evolution, *HSC* transformation upon multiple genetic or epigenetic hits (*yellow stars*) give rise to self-renewing leukemia stem cells (*LSCs*) which are capable of generating acute myeloid leukemia (*AML*; *left panel*, *purple cells*). In contrast, acute lymphoblastic leukemia (*ALL*) fits the stochastic model as it can be initiated from all B-cell differentiation stages susceptible to leukemic transformation and endowed with self-renewal ability (*upper panel*). In both hematological malignancies, residual normal hematopoiesis is carried out but at very low extent as leukemic blasts are preferentially produced. *Black arrows* indicate diminished normal hematopoietic differentiation

environmental cues and show interdependence on specialized niches where they can exert their aberrant function. In contrast, the random or stochastic model suggests that the cellular heterogeneity within a tumor is the result of progressive and divergent formation of genetic subclones. In this model, all tumor cells possess CSC potential (Fig. 9.2), and environmental factors may not definitively influence tumoral growth (Nguyen et al. 2012).

Thus, the natural attribute of normal stem cells to self-renew and reconstitute in the long term has suggested that in the earliest stage of neoplastic transformation, a number of genetic aberrations accumulate in this precise compartment that in turn become reprogrammed (Rajendran and Dalerba 2014; Abollo-Jiménez et al. 2011). This scenario does not exclude any of the proposals explaining leukemogenesis,

that is, the "population mixing" hypothesis or the "multiple hits" hypothesis (Pelayo et al. 2012; Kinlen 1995).

In recent years, our understanding of leukemogenesis has deepened due to the combination of xenotransplantation models, genome-wide methods, and bioinformatic analysis of transcriptional programs applied to CSC research (Eppert et al. 2011).

Primitive CSCs were first documented in AML, where only a minor fraction of leukemic cells were capable of in vitro proliferation and differentiation and could recapitulate leukemia in transplanted mice, suggesting that leukemogenic activity might reside in a cell fraction (Krause and Van Etten 2007) endowed with some stem cell properties and indicating a hierarchical organization of the disease (Fig. 9.2). Based on their slow-cycling, self-renewal, differentiation potential, gene expression program, and surface phenotype, LSCs in AML have shown remarkable similarities to normal HSCs (Deng and Zhang 2010; Eppert et al. 2011; Greve et al. 2012). They can reversibly enter quiescence, alternating this cell cycle status with active proliferative stages. The resulting low cycling rates, together with ATP-binding cassette multidrug transporter pumps, antiapoptotic proteins, and disrupted DNA repair mechanisms, confer LSCs in AML resistance to conventional chemotherapy (Baccelli and Trumpp 2012). By using multiparametric flow cytometry, LSCs were identified as $Lin^{-}CD34^{+}CD38^{-}$, whereas $CD34^{+}CD38^{+}$ and $CD34^{-}$ fractions were shown to lack functional features of LICs (Tan et al. 2006; Lapidot et al. 1994; Bonnet and Dick 1997) (Table 9.1). However, in contrast to what is observed in normal HSCs, they lack expression of CD90 and CD117 and "aberrantly" display CD123 (Jordan et al. 2000; Chavez-Gonzalez et al. 2014). Furthermore, LSC frequencies within the stem cell compartment in AML BM presumably have a strong prognostic impact and emphasize the need for discrimination between LSCs and normal HSCs (Terwijn et al. 2014). Of high relevance, a conspicuous population of pre-LSCs has been identified in AML patients harboring DNMT3A and IDH2 mutations (Shlush et al. 2014). These peculiar cells survive induction chemotherapy and can persist at remission (Table 9.1).

LSCs also constitute the cellular root in chronic myeloid leukemia (CML), a clonal hematological disease of adult individuals, characterized by a t(9;22)(q34;q11) reciprocal translocation rendering the Philadelphia (Ph) chromosome and the fusion protein Bcr-Abl (Holyoake et al. 1999; Chavez-Gonzalez et al. 2013; Chung and Park 2014). As in AML, CML LSCs have a similar phenotype to normal HSCs but show strong activation of signaling pathways that depend on the constitutively active tyrosine kinase Bcr-Abl. Only recently, Lemoli and colleagues discovered a $CD34^{-}$ population among CML patients corresponding to treatment-resistant LSCs (Table 9.1) (Lemoli et al. 2009).

In contrast, the cellular origin of ALL is less clear. Both LICs with immature phenotypes and the various B-cell differentiation stages are able to recapitulate the disease, challenging the hierarchical stem cell model and suggesting that the self-renewal property is maintained in B-committed cells (Fig. 9.2) (Purizaca et al. 2012). Moreover, the unsuspected genetic diversity within LICs and an increasingly complex pattern of acquisition of mutations in B precursor cells support the multiclonal evolution of leukemogenesis (Dick 2008). In concordance, at diagnosis, several leukemic clones bear common mutations, but an apparent predominant

Table 9.1 Primitive hematopoietic cells in myeloid and lymphoid leukemias

Disease	Normal hematopoietic potential	Pre-LSC	LSC	LIC	Phenotype	Genetic aberrations	Biological properties	Correlation with clinical outcome	References
AML		Yes			Lin^- $CD34^+$ $CD38^-$ $CD33^-$ $CD45^+$	$DNMT3A^{mut}$	Chemoresistant Persistence at remission		Shlush et al. (2014)
	↓↓								Dorantes-Acosta et al. (2008)
									Mayani et al. (2009)
								Yes	Terwijn et al. (2014)
			Yes		Lin^- $CD34^+$ $CD38^{-/+}$		Cell hierarchy		Lapidot et al. (1994)
					$CD90^-$ $Sca\text{-}1^-$ $c\text{-}kit^+$ $CD16^+$ $CD45RA^+$ $TIM3^+$ $ALDH^+$	$DNMT3a^-$	1:1,000 A stem cell disease		Reviewed in Chung (2014)
					$CD123^+$				Jordan et al. (2000)
					$CD47^+$				Majeti et al. (2009)
							Chemoresistant Quiescence		Ishikawa et al. (2007)
						NPMI, DHI, Flt3-ITD, SMC1A, TET2			Jan et al. (2012)
CML	↓→ ↑		Yes		Lin^- $CD34^+$ $CD38^{\pm}$ $CD123^+CD45RA^-CD71^-$	Bcr-abl t(9:22) Wnt	Cell hierarchy Quiescence Constitutive activation of JAK/STAT and Ras/Raf/MEK/ERK		Chavez-Gonzalez et al. (2013)
									Holyoake et al. (1999)
									Reviewed in Chung (2014)

				Yes	$CD34^-$		Resistant to imatinib		Chavez-Gonzalez et al. (2013)
									Lemoli et al. (2009)
B-ALL	↓↓								Purizaca et al. (2012)
			Yes		$CD34^+$ $CD38^-$ $CD10^-$ $CD19^*$ $CD133^+$				Cobaleda et al. (2000)
									Cox et al. (2009)
									Diamanti et al. (2013)
				Yes	$CD34^+$ $CD19^-$ $CD34^+$ $CD19^+$ $CD34^-$ $CD19^+$	EBF1 PAX5 IZKF1	Reconstitution of the original phenotype Activation of the JAK/STAT and NF-κB pathways		Le Viseur et al. (2008)
									Mullighan et al. (2009)
									Maude et al. (2012)
									Vilchis et al. (2015)
									Diamanti et al. (2013)
						ETV6-RunX1	No obvious stem cell hierarchical structure Plasticity		Bomken et al. (2010)
									Rehe et al. (2013)
									Raff (2014)
T-ALL		Yes			$CD34^+$ $CD7^-$ $CD4^-$		Activation of Notch pathway		Cox et al. (2007)
									Zhang et al. (2013)
									Armstrong et al. (2009)

LSC leukemic stem cell, *LIC* leukemia-initiating cell, *AML* acute myeloid leukemia, *CML* chronic myeloid leukemia, *B-ALL* B-cell acute lymphoblastic leukemia, *T-ALL* T-cell acute lymphoblastic leukemia

clone shows additional genetic lesions that may confer them selective advantages over the common ones. During treatment, if the prominent clones are successfully abated, additional clones which may have acquired resistance to chemotherapy can emerge and lead to the relapse of patients (Raff and Bruggermann 2014).

The participation of primitive cells in the etiology of ALL has been suggested by three lines of evidence: first, the identification of leukemic clones harboring unrelated DJ sequences and cytogenetic abnormalities in lineage-negative cells; second, the engraftment capability and leukemia reconstitution in immunodeficient xenotransplantation mouse models driven by a primitive $CD34^+$ $CD10^-$ $CD19^-$ fraction within ALL BM; and third, the resistance of $CD133^+$ cells and $CD34^+$ $CD19^-$ cells to treatment with conventional or small-molecule chemotherapy, respectively (Diamanti et al. 2013; Cox et al. 2004, 2009). Similar results were shown when T-cell ALL was evaluated and only primitive fractions ($CD34^+$ $CD4^-$ and $CD34^+$ $CD7^-$) were capable of engrafting NOD/SCID mice and reconstituting the disease (Table 9.1) (Cox et al. 2007). On the other hand, this is still under debate as the work by Vormoor et al. strongly suggests not a hierarchical, but a stochastic, origin of ALL. The leukemic phenotype can be completely reestablished in vivo by early progenitors or by any of the B-cell precursor subsets (Fig. 9.2) (Rehe et al. 2013; le Viseur et al. 2008). Interestingly, by transplanting irradiated newborn NOD/SCID/IL2rγ^{null} mice, Ishikawa and colleagues found that committed B-cell precursors ($CD34^+$ $CD38^{\pm}$ $CD19^+$) are the origin of ALL and have the potential to infiltrate extramedullary tissues including the liver, spleen, and kidney. In contrast, more primitive progenitors ($CD34^+$ $CD38^-$ $CD10^-$ $CD19^-$) apparently direct normal hematopoietic reconstitution (Kong et al. 2008).

A Role of Leukemic Stem-Initiating Cells in Tumor Infiltration?

The proposal that metastases originate from a conspicuous population of primitive surviving cells has been experimentally proven in a number of cancer models. Liquid tumors, including leukemias, also display site-specific homing to microdomains within BM and infiltration/metastasis into extramedullary compartments that may result in residual disease and consequent relapse episodes.

Under homeostatic conditions, HSCs are constantly migrating within BM and between BM and peripheral tissues via blood–lymph–thoracic duct–blood (Massberg et al. 2007; Mazo et al. 2011; Merchand-Reyes et al. 2014). HSC mobilization out of the marrow implies the active participation of proteolytic enzymes, chemokines, and adhesion molecules. It is uncertain whether primitive cells govern the clinical infiltration setting in ALs. A better understanding of the molecular mechanisms that control the trafficking of LSCs/LICs and mediate their recruitment is crucial.

When leukemic cells acquire the capacity to invade extramedullary tissues, they form tumor masses referred to as chloromas (Dias et al. 2000). Interestingly, some myeloid leukemias are capable of disseminating into many organs, but they do not infiltrate the CNS, whereas ALL often infiltrates the CNS and testis. Organ infiltration is always prognostic for poor outcomes, and increased frequencies of LSCs in AML have been suggested to correlate with relapse after treatment.

While leukemic cells create abnormal inhibitory niches for normal hematopoietic cells in BM (Colmone et al. 2008; Raaijmakers et al. 2010), LSC maintenance and trafficking may depend on microenvironmental cues that usually support self-renewal and development of healthy HSCs (Sipkins et al. 2005; Purizaca et al. 2012). Moreover, their migration into the BM seems to be promoted by CXCL12 and CXCR4 or specific integrins (Sipkins et al. 2005), supporting the postulate that metastasis of LSCs and the trafficking of normal HSCs may involve similar mechanisms (Kucia et al. 2005). Clearly, the importance of the SDF/CXCR4 axis in the motility regulation of LSCs in AML has been defined (Tavor et al. 2004), leading to speculate that a close connection between CXCR4, LSCs, and infiltration is likely. Furthermore, to investigate the dynamics of liver invasion by $CD45^{+}$ leukemic cells, a model of myeloproliferative disease-like myeloid leukemia was recently used by Drize and colleagues. In this model, CCR1, CCR2, and CCR5 were shown to be highly expressed by the liver cells of leukemic animals, suggesting that they might play an important role in the retention of leukemic cells in the liver (Bigildeev et al. 2011). At least 4 % of the cells that invaded the liver were LSCs that have activated the NFkB pathway. Of particular interest, IL1a, which is one of the most potent activators of NFkB, and a number of genes targeted by NFkB, such as Vcam1, Icam1, CyclinD1, and Myc, were all upregulated (Guzman et al. 2001; Bigildeev et al. 2011).

How leukemic cells break down the blood–brain barrier (BBB) and enter the CNS is less understood. Transmembrane molecules like claudin-5 and occludin have been identified as key components of BBB integrity through the formation of tight junctions between adjacent brain microvascular endothelial cells. Their disruption can lead to the loss of BBB integrity. Using an in vitro model of BBB and a powerful in vivo model of CNS AML, Feng and colleagues reported MMP-2- and MMP-9-dependent invasion mediated by the disruption of claudin-5, occludin, and accessory molecules (Feng et al. 2011). Whether the same mechanism is used by conspicuous populations of LSC/LIC to increase the permeability of the BBB during clinical settings of infiltration is yet to be determined (Merchand-Reyes et al. 2014).

Phenotype Plasticity in Acute Leukemias

Physiological plasticity is an essential feature of developing tissues and refers to the capacity of fate-changing processes, where a well-defined cell adopts the biological properties of other cell types (Abollo-Jiménez et al. 2011). In this phenomenon, the original plastic cells might be endowed with primitive or differentiated phenotypes, and the output cells may belong to the same or different lineages. Extrinsic environmental cues and epigenetic modifications might cooperate with intrinsic programs to regulate cell fate conversion (Dorantes-Acosta and Pelayo 2012). Multi-lineage potential, biological dedifferentiation, transdifferentiation, and reprogramming are all manifestations of plasticity, and their study is currently of great interest, because of its implications in regenerative medicine and for understanding neoplastic processes, which are considered to be a result of erroneous reprogramming (Abollo-Jiménez et al. 2011).

Cell reprogramming can be caused in experimental conditions when inducing pluripotency or programmed dedifferentiation, verifying the participation of key

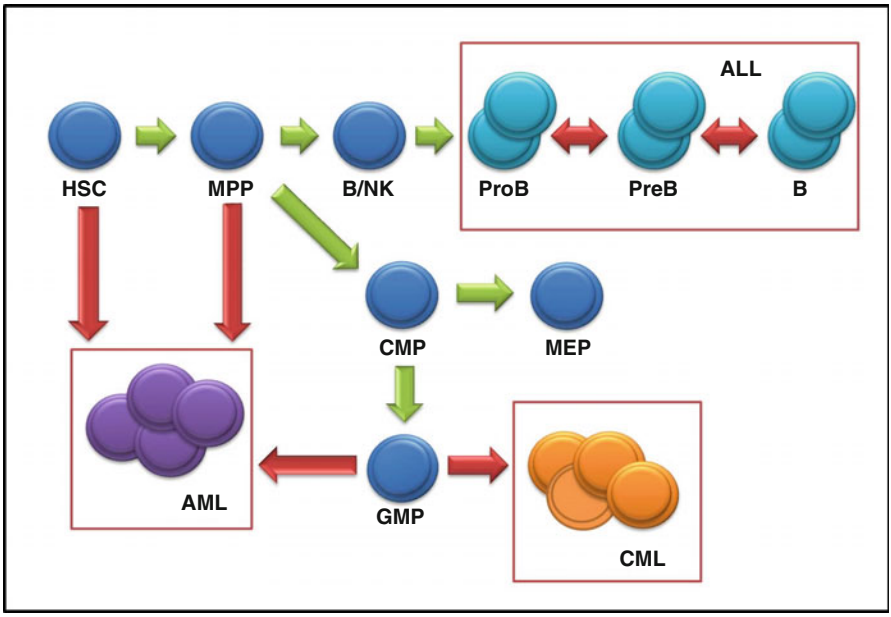


Fig. 9.3 Plasticity of the leukemia phenotype. Leukemia cells display phenotype plasticity. In *ALL*, pro-B, pre-B, and B-cells are able to upregulate and downregulate key lineage markers and gain the capacity for self-renewal at all differentiation stages, leading to dedifferentiation. *ALL* precursors are marked in *light blue*, and *red arrows* represent phenotype plasticity. Although AML origin has been demonstrated to take place in the most primitive progenitor compartment, it has also been suggested that cells with *GMP* and *MPP* phenotypes can function as LSC. In *CML*, an LSC with a *GMP*-like phenotype has been proposed. *Orange* and *purple* cells represent *CML* and *AML* cells, respectively. *Dark blue* cells and *green arrows* represent normal differentiation pathways

transcription factors in lineage decisions. The absence of EBF allows early lymphoid progenitors to differentiate into myeloid lineage, and downstream the path, the conditional deletion of PAX5 in mature B-cells can induce conversion to different fates, including macrophages and T-cells (Nutt et al. 1999; Dorantes-Acosta and Pelayo 2012). Of note, the forced expression of c/EBP in committed B-cell precursors leads to expedited reprogramming to macrophages (Xie et al. 2003; Orkin and Pera 2007). Similar mechanisms might be used by leukemic cells, such as during lineage switching; conversions from lymphoblastic leukemia to myeloid leukemia, or vice versa, are recorded (Dorantes-Acosta and Pelayo 2012). Recent studies suggest that the lineage commitment of plastic leukemic progenitors may be reversible upon specific signals provided by environmental inducers that include microbial components (Dorantes-Acosta et al. 2013). This may also occur under "instruction" from oncogenic lesions, as lessons from the clinic suggest that committed B-cells might be targets of dedifferentiation to earlier progenitor stages. In addition, the phenomenon can work in reverse, with progenitor cells differentiating to stages of B-cell commitment (Fig. 9.3) (le Viseur et al. 2008; Campos-Sanchez et al. 2011).

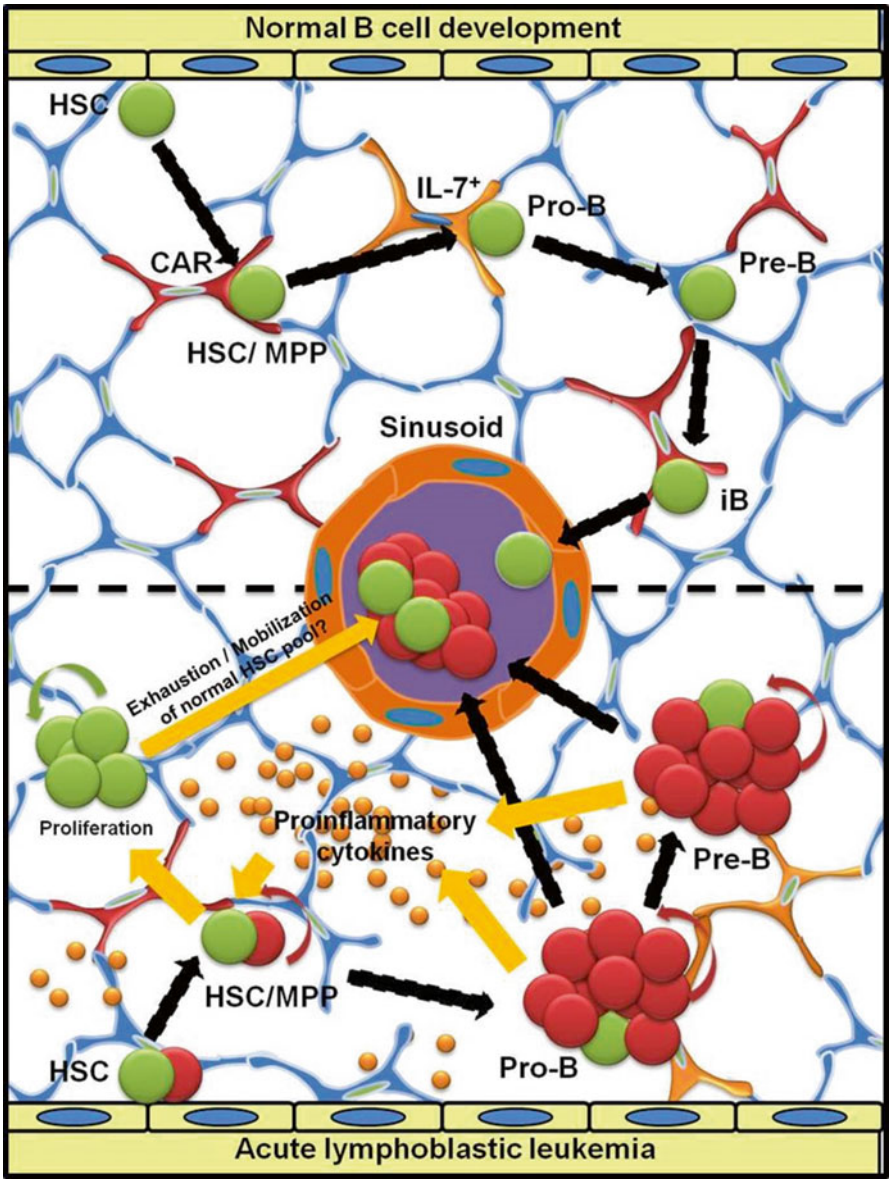
Recent findings of phenotype heterogeneity within the LSC compartment in AML have questioned the unidirectional feature of the hierarchical AML structure (Chung and Park 2014). Nearly 80 % of AML patients develop leukemia with apparent correlation with cell populations that resemble GMPs and LMPPs, supporting the provocative hypothesis that more differentiated LSCs might acquire abnormal self-renewal potential and produce transplantable AML (Goardon et al. 2011). The same is true for CML (Fig. 9.3).

Bone Marrow Microenvironment in the Regulation of Normal and Malignant Hematopoiesis

It has become clear that the ordered series of lineage fate decisions result from the continuing dialogue between developing stem/progenitor cells and the surrounding hematopoietic microenvironment (Dick 2008; Purizaca et al. 2012; Dorantes-Acosta and Pelayo 2012). Such a specialized microenvironment comprises a complex network of mesenchymal cells, osteoblasts, endothelial cells, fibroblasts, adipocytes, innate and adaptive immune cells, and their products, including extracellular matrix, cytokines, chemokines, and growth factors. At least three hematopoietic niches within the BM architecture are dedicated to supporting HSC differentiation throughout life: the endosteal niche, mainly composed of osteoblasts lining the bone surface, the vascular niche, formed of endothelial cells, and the reticular niche, where the chemokine/chemokine receptor CXCL12/CXCR4 axis plays a pivotal role in the regulation of early lymphopoiesis (Fig. 9.4) (Purizaca et al. 2012). A comprehensive model for stem/progenitor cell population dynamics in the context of a regulating (inductive, repressive or permissive) microenvironment is not yet available, but recent advances have suggested that changes in the composition and function of the hematopoietic microenvironment – including soluble and cellular components – might cooperate with the initiation or maintenance of aberrant processes and lead to disease. Of significance, the development of tridimensional culture systems as a novel tool for advancing normal and LSC research, including the identification of therapeutic targets and drug candidates by high-throughput screening applications, is highly encouraged. Moreover, systems biology approaches provide useful complementary instruments for integration of the available information and further prediction of clinical scenarios.

Based on experimental data, the following mechanisms have been proposed to function as "cooperating" microenvironment-related factors in leukemogenesis: a competition of tumor cells for normal HSC niches, the manipulation of normal environments led by tumor cells, and disruption of HSC-niche communication. Any of these scenarios would favor tumor progression at the expense of homeostatic, hematopoietic differentiation (Raaijmakers 2011). Increasing evidence indicates the prevalence of functional defects in both soluble and cellular microenvironmental elements that may accompany oncogenesis. In support of this hypothesis, osteoblastic differentiation impairment by deletion of Dicer1 gene induces the development

of myeloid leukemia upon myelodysplasia (Corre et al. 2007). Additional strong signals, including that provided by the activated Wnt- β catenin pathway, allows the initiation or maintenance of leukemia (Lane et al. 2011). However, it is still unclear whether ALL microenvironmental abnormalities appear as a consequence of leukemic activity or whether they constitute intrinsic pre-leukemic lesions. In ALL, seminal work by Sipkins Lab using a mouse xenograft model of pre-B-cell ALL, has



demonstrated that tumor cells create inhibitory microenvironments for normal HSCs by overproducing CXCL12 and disrupting their niches (Colmone et al. 2008). Recently, the findings of a significant decrease in some biological properties (i.e., the proliferation and hematopoietic support) of mesenchymal stromal cells with no association with genetic abnormalities of ALL underline the active role of leukemic cells and their products in the modulation of the BM microenvironment (Conforti et al. 2013; Vicente Lopez et al. 2014).

Inflammatory Cues Influencing Cell Fate Decisions in All Bone Marrow

Although stability of the hematopoietic system is indubitable, the plasticity of stem and progenitor cells leads the process to undergo adjustments when interacting with extrinsic factors provided by inflammatory conditions (Vadillo et al. 2013). Interestingly, these primitive cells proliferate in response to stress mediated by proinflammatory factors. Furthermore, they are capable of recognizing microbial components via toll-like receptors (TLRs), redirecting cell fate decisions and facilitating their differentiation toward innate immune cells (Welner et al. 2008; Vadillo et al. 2014; Vadillo and Pelayo 2012). While mouse models have been useful in mapping the selective cell production of myeloid and DCs at the expense of B-lymphoid production, controlled culture systems and xenotransplantation approaches have suggested that human multi-lymphoid progenitors over-produce diverse categories of DCs and NK cells – both functionally involved in cancer surveillance – when viral components trigger the TLR signaling pathway. Similar stimulation of more primitive cells promotes myeloid differentiation. The released

Fig. 9.4 The proinflammatory microenvironment of ALL bone marrow. Normal B-cell development (*upper panel*) starts from rare *HSCs* that reside near osteoblasts or CXCL12$^+$ stromal cells (*light yellow* and *red* cells, respectively). Upon a sequential and tightly regulated process, pro-B-cells are formed and stay in close interaction with IL-7$^+$ stromal cells (*orange* cells). As these cells advance in their differentiation, they produce pre-B-cells that no longer need interaction with IL-7. Immature B-cells (*iB*) are ultimately produced, which interact with CXCL12$^+$ stromal cells prior to peripheral blood release and further maturation in secondary lymphoid organs. In ALL (*lower panel*), leukemic precursors (pro-B and pre-B) can be exported to peripheral circulation (normal progenitors are shown in *green* while leukemic clones are in *red*). Patients bearing lineage infidelity (CD13$^+$ leukemic B-cell precursors) may induce a proinflammatory microenvironment, characterized by abnormally increased production of proinflammatory cytokines that in turn activates the proliferation and differentiation of normal hematopoietic clones. Scenarios like nonmalignant hematopoietic exhaustion and mobilization may favor disease progression. *Yellow arrows* depict proinflammatory cytokine production. The central structure represents a sinusoid within BM whereas the upper and lower *yellow* structures the endosteum. The model is based on in vitro experimental findings

cytokines, including IL-1β, TNFα, and IL-6, drive the activation and mobilization of primitive cells to the blood circulatory system (Vadillo et al. 2013).

Compared with hematologically normal individuals, B-ALL patients have shown detrimental potential in stromal cell development, which is remarkably related to levels of IL-6 and TNFα, suggesting that an inflammatory setting might prevail as a pathobiology factor (Espinoza-Hernandez et al. 2001). The implication of these signals in the hematopoietic niches and on normal and malignant hematopoiesis remains to be learned. Along with the abnormalities discussed above, a proinflammatory tumor microenvironment may induce leukemic progenitors to redirect original fates. Accordingly, TLR stimulation of cell precursors in childhood leukemias does not play an apparent critical role in disease progression, but strengthens the production of innate cells (Dorantes-Acosta et al. 2013).

Chronic inflammation and carcinogenesis have lately been closely connected through two potential pathways: the extrinsic pathway, which results from external factors promoting latent inflammatory responses, and the intrinsic pathway, conducted by oncogenes that activate the expression of inflammation-related programs. Crucial molecular regulators include cytokines, chemokines, and components of signaling pathways, such as MyD88, NFkB, and STAT3 (Mantovani et al. 2008; Krawczyc et al. 2014; Lippitz 2013. In addition, innate immune cells, including tumor-associated macrophages, make significant contributions to the regulation of LSCs.

Our recent investigation of possible BM inflammatory scenarios in ALL suggests the modification of the microenvironment by a special type of leukemic cells that may interfere with regular hematopoietic differentiation (Vilchis et al. 2015). Apparently distinct B-ALL cells co-expressing the myeloid-related markers CD13 and CD33, have the capability to produce Th1-type cytokines such as TNFα, IL-1β, IL-12, and GM-CSF, among others, that drive normal stem and progenitor cells into the cell cycle and differentiation (Fig. 9.4). We need to discern whether the entailed NFkB and STAT3 signaling disturbs effective long-term blood cell formation or exposes the activated cells to oncogenic hits and further correlate the resulted information to evidence from the clinics.

Concluding Remarks

Hematopoiesis is a fundamental homeostatic process responsible for the lifelong replenishment of blood cell lineages. Its proper genetic, epigenetic, and microenvironmental regulation is critical to maintain the functional and homeostatic activity of the very primitive stem and differentiating cells. Outstanding work has recently been conducted regarding the role of these seminal cells in the etiology and recurrence of aberrant malignant processes, including childhood myeloid and lymphoid leukemias. It is now clear that a conspicuous and unique LSC population is most likely the origin of AML and CML. The molecular pathways involved in the induction of pre-leukemic lesions within the normal, primitive HSC pool are under

intense investigation. In contrast, the contribution of LSCs in ALL is not that obvious. Moreover, clonal diversity and the tumor-initiating abilities of all lymphoid differentiation stages retaining some stemness and plasticity property highlight the complexity of this childhood disease and increase the uncertainty of its cell origins. Furthermore, changes in the composition and function of the hematopoietic microenvironment – including soluble and mesenchymal stromal cell components – might govern tumor progression and infiltration. Accordingly, constitutively activated inflammation pathways within LIC populations may function as a cooperating element – rather than as a driving factor – which creates abnormal proinflammatory niches, exposing cells in prolonged proliferation to oncogenic mutations and accelerating the multistep process of leukemogenesis.

By unraveling fundamental biological differences between normal and leukemic primitive cells and developing novel strategies to purify them, a better understanding of the hematopoietic–microenvironment interplay that controls the malignant setting is prompted.

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Glossary

Cancer stem cell A primitive cell capable of generating tumor indefinitely and all the cellular subsets that constitute the malignant mass. Cancer stem cells may have the ability to control the maintenance, propagation, metastasis, and relapse of transformed cells.

Hematopoiesis A highly ordered, multi-step differentiation process that starts in a unique population of self-renewing HSCs, which gradually commit to lymphoid or myeloid lineage fates until the formation of mature blood cells.

Hematopoietic microenviroment A specialized structure consisting of a complex network of mesenchymal cells, osteoblasts, endothelial cells, fibroblasts, adipocytes, innate and adaptive immune cells, and their products, including extracellular matrix, cytokines, chemokines, and growth factors. It is essential for supporting HSC differentiation throughout life.

Leukemia-initiating cell Cancer-initiating cells with either progenitor or differentiating cell phenotypes, capable of recapitulating leukemia in serial transplantation mouse models. LICs are endowed with some stemness and plasticity properties.

Plasticity An essential feature of developing tissues that refers to the capacity of cells to adopt biological properties of other cell types. Multi-lineage potential, dedifferentiation, transdifferentiation, and reprogramming are all manifestations of plasticity.

References

Abollo-Jiménez F, Campos-Sanches E, Sagrera A, Muñoz ME, Galan AI, Jimenez R, Cobaleda C. The dark side of cellular plasticity: stem cells in development and cancer. In: Shostak S. editor. Cancer Stem Cells Theories and Practice. Rijeka, Croatia: InTech; 2011. ISBN: 978-953-307-225-8.

Abraham S, Hopcroft L, Bhatia R, Koschmieder S, Whetton A, Holyoake T. Models to study chronic myeloid leukemia cancer stem cells. In*:* Rajasekhar V. editor. Cancer stem cells. Hoboken, New Jersey: John Wiley & Sons, Inc; 2014.

Ailles LE, Weissman IL. Cancer stem cells in solid tumors. Curr Opin Biotechnol. 2007; 18:460–6.

Armstrong F, Brunet de la Grange P, Gerby B, Rouyez MC, Calvo J, Fontenay M, Boissel N, Dombret H, Baruchel A, Landman-Parker J, Roméo PH, Ballerini P, Pflumio F. NOTCH is a key regulator of human T-cell acute leukemia initiating cell activity. Blood. 2009;113(8):1730–40.

Baccelli I, Trumpp A. The evolving concept of cancer and metastasis stem cells. J Cell Biol. 2012;198:281–93.

Bell JJ, Bhandoola A. The earliest thymic progenitors for T cells possess myeloid lineage potential. Nature. 2008;452:764–7.

Benz C, Copley MR, Kent DG, Wohrer S, Cortes A, Aghaeepour N, Ma E, Mader H, Rowe K, Day C, Treloar D, Brinkman RR, Eaves CJ. Hematopoietic stem cell subtypes expand differentially during development and display distinct lymphopoietic programs. Cell Stem Cell. 2012; 10:273–83.

Bhandoola A, Sambandam A. From stem cell to T cell: one route or many? Nat Rev Immunol. 2006;6:117–26.

Bhatla T, Jones CL, Meyer JA, Vitanza NA, Raetz EA, Carroll WL. The biology of relapsed acute lymphoblastic leukemia: opportunities for therapeutic interventions. J Pediatr Hematol Oncol. 2014;36:413–8.

Bigildeev AE, Shipounova IN, Svinareva DA, Drize NJ. Leukemia cells invading the liver express liver chemokine receptors and possess characteristics of leukemia stem cells in mice with MPD-like myeloid leukemia. Exp Hematol. 2011;39:187–94.

Blom B, Spits H. Development of human lymphoid cells. Annu Rev Immunol. 2006;24:287–320.

Bomken S, Fiser K, Heidenreich O, Vormoor J. Understanding the cancer stem cell. Br J Cancer. 2010;103(4):439–45.

Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med. 1997;3:730–7.

Boos MD, Yokota Y, Eberl G, Kee BL. Mature natural killer cell and lymphoid tissue-inducing cell development requires Id2-mediated suppression of E protein activity. J Exp Med. 2007;204: 1119–30.

Campos-Sanchez E, Toboso-Navasa A, Romero-Camarero I, Barajas-Diego M, Sanchez-Garcia I, Cobaleda C. Acute lymphoblastic leukemia and developmental biology: a crucial interrelationship. Cell Cycle. 2011;10:3473–86.

Chavez-Gonzalez A, Aviles-Vazquez S, Moreno-Lorenzana D, Mayani H. Hematopoietic stem cells in chronic myeloid leukemia. In*:* Alimoghaddam K editor. Stem cell biology in normal life and diseases. Rijeka, Croatia: InTech; 2013.

Chavez-Gonzalez A, Dorantes-Acosta E, Moreno-Lorenzana D, Alvarado-Moreno A, Arriaga-Pizano L, Mayani H. Expression of CD90, CD96, CD117, and CD123 on different hematopoietic cell populations from pediatric patients with acute myeloid leukemia. Arch Med Res. 2014;45:343–50.

Chung SS, Park CY. Acute myeloid leukemia cells-updates and controversies. In: Rajasekhar V. editor. Cancer stem cells. Hoboken, New Jersey: John Wiley & Sons, Inc, Wiley-Blackwell; 2014.

Cobaleda C, Gutiérrez-Cianca N, Pérez-Losada J, Flores T, García-Sanz R, González M, Sánchez-García I. A primitive hematopoietic cell is the target for the leukemic transformation in human philadelphia-positive acute lymphoblastic leukemia. Blood. 2000;95(3):1007–13.

Colmone A, Amorim M, Pontier AL, Wang S, Jablonski E, Sipkins DA. Leukemic cells create bone marrow niches that disrupt the behavior of normal hematopoietic progenitor cells. Science. 2008;322:1861–5.

Conforti A, Biagini S, DEL Bufalo F, Sirleto P, Angioni A, Starc N, Li Pira G, Moretta F, Proia A, Contoli B, Genovese S, Ciardi C, Avanzini MA, Rosti V, Lo-Coco F, Locatelli F, Bernardo ME. Biological, functional and genetic characterization of bone marrow-derived mesenchymal stromal cells from pediatric patients affected by acute lymphoblastic leukemia. PLoS ONE. 2013;8:e76989.

Corre J, Mahtouk K, Attal M, Gadelorge M, Huynh A, Fleury-Cappellesso S, Danho C, Laharrague P, Klein B, Reme T, Bourin P. Bone marrow mesenchymal stem cells are abnormal in multiple myeloma. Leukemia. 2007;21:1079–88.

Cox CV, Evely RS, Oakhill A, Pamphilon DH, Goulden NJ, Blair A. Characterization of acute lymphoblastic leukemia progenitor cells. Blood. 2004;104:2919–25.

Cox CV, Martin HM, Kearns PR, Virgo P, Evely RS, Blair A. Characterization of a progenitor cell population in childhood T-cell acute lymphoblastic leukemia. Blood. 2007;109: 674–82.

Cox CV, Diamanti P, Evely RS, Kearns PR, Blair A. Expression of CD133 on leukemia-initiating cells in childhood ALL. Blood. 2009;113:3287–96.

Deng CH, Zhang QP. Leukemia stem cells in drug resistance and metastasis. Chin Med J (Engl). 2010;123:954–60.

Diamanti P, Cox CV, Moppett JP, Blair A. Parthenolide eliminates leukemia-initiating cell populations and improves survival in xenografts of childhood acute lymphoblastic leukemia. Blood. 2013;121:1384–93.

Dias S, Hattori K, Zhu Z, Heissig B, Choy M, Lane W, Wu Y, Chadburn A, Hyjek E, Gill M, Hicklin DJ, Witte L, Moore MA, Rafii S. Autocrine stimulation of VEGFR-2 activates human leukemic cell growth and migration. J Clin Invest. 2000;106:511–21.

Dick JE. Stem cell concepts renew cancer research. Blood. 2008;112:4793–807.

Dorantes-Acosta E, Pelayo R. Lineage switching in acute leukemias: a consequence of stem cell plasticity? Bone Marrow Res. 2012;2012:406796.

Dorantes-Acosta E, Chavez-Gonzalez A, Santos JI, Medina-Sanson A, Mayani H. Defective in vitro growth of primitive hematopoietic cells from pediatric patients with acute myeloid leukemia. Pediatr Blood Cancer. 2008;51:741–6.

Dorantes-Acosta E, Vadillo E, Contreras-Quiroz A, Balandran JC, Arriaga-Pizano L, Purizaca J, Huerta-Yepez S, Jimenez E, Aguilera W, Medina-Sanson A, Mayani H, Pelayo R. TLR stimulation of bone marrow lymphoid precursors from childhood acute leukemia modifies their differentiation potentials. Biomed Res Int. 2013;2013:846724.

Doulatov S, Notta F, Eppert K, Nguyen LT, Ohashi PS, Dick JE. Revised map of the human progenitor hierarchy shows the origin of macrophages and dendritic cells in early lymphoid development. Nat Immunol. 2010;11:585–93.

Eppert K, Takenaka K, Lechman ER, Waldron L, Nilsson B, VAN Galen P, Metzeler KH, Poeppl A, Ling V, Beyene J, Canty AJ, Danska JS, Bohlander SK, Buske C, Minden MD, Golub TR, Jurisica I, Ebert BL, Dick JE. Stem cell gene expression programs influence clinical outcome in human leukemia. Nat Med. 2011;17:1086–93.

Espinoza-Hernandez L, Cruz-Rico J, Benitez-Aranda H, Martinez-Jaramillo G, Rodriguez-Zepeda MC, Velez-Ruelas MA, Mayani H. In vitro characterization of the hematopoietic system in pediatric patients with acute lymphoblastic leukemia. Leuk Res. 2001;25:295–303.

Feng S, Cen J, Huang Y, Shen H, Yao L, Wang Y, Chen Z. Matrix metalloproteinase-2 and -9 secreted by leukemic cells increase the permeability of blood-brain barrier by disrupting tight junction proteins. PLoS ONE. 2011;6:e20599.

Goardon N, Marchi E, Atzberger A, Quek L, Schuh A, Soneji S, Woll P, Mead A, Alford KA, Rout R, Chaudhury S, Gilkes A, Knapper S, Beldjord K, Begum S, Rose S, Geddes N, Griffiths M, Standen G, Sternberg A, Cavenagh J, Hunter H, Bowen D, Killick S, Robinson L, Price A, Macintyre E, Virgo P, Burnett A, Craddock C, Enver T, Jacobsen SE, Porcher C, Vyas P. Coexistence of LMPP-like and GMP-like leukemia stem cells in acute myeloid leukemia. Cancer Cell. 2011;19:138–52.

Greaves MF, Wiemels J. Origins of chromosome translocations in childhood leukaemia. Nat Rev Cancer. 2003;3:639–49.

Greve B, Kelsch R, Spaniol K, Eich HT, Gotte M. Flow cytometry in cancer stem cell analysis and separation. Cytom A. 2012;81:284–93.

Gu TL, Nardone J, Wang Y, Loriaux M, Villen J, Beausoleil S, Tucker M, Kornhauser J, Ren J, Macneill J, Gygi SP, Druker BJ, Heinrich MC, Rush J, Polakiewicz RD. Survey of activated FLT3 signaling in leukemia. PLoS ONE. 2011;6:e19169.

Gupta S, Rivera-Luna R, Ribeiro RC, Howard SC. Pediatric oncology as the next global child health priority: the need for national childhood cancer strategies in low- and middle-income countries. PLoS Med. 2014;11:e1001656.

Guzman ML, Neering SJ, Upchurch D, Grimes B, Howard DS, Rizzieri DA, Luger SM, Jordan CT. Nuclear factor-kappaB is constitutively activated in primitive human acute myelogenous leukemia cells. Blood. 2001;98:2301–7.

Hogan LE, Meyer JA, Yang J, Wang J, Wong N, Yang W, Condos G, Hunger SP, Raetz E, Saffery R, Relling MV, Bhojwani D, Morrison DJ, Carroll WL. Integrated genomic analysis of relapsed childhood acute lymphoblastic leukemia reveals therapeutic strategies. Blood. 2011;118:5218–26.

Holyoake T, Jiang X, Eaves C, Eaves A. Isolation of a highly quiescent subpopulation of primitive leukemic cells in chronic myeloid leukemia. Blood. 1999;94:2056–64.

Ishikawa F, Yoshida S, Saito Y, Hijikata A, Kitamura H, Tanaka S, Nakamura R, Tanaka T, Tomiyama H, Saito N, Fukata M, Miyamoto T, Lyons B, Ohshima K, Uchida N, Taniguchi S, Ohara O, Akashi K, Harada M, Shultz LD. Chemotherapy-resistant human AML stem cells home to and engraft within the bone-marrow endosteal region. Nat Biotechnol. 2007;25(11): 1315–21.

Iwasaki H, Akashi K. Myeloid lineage commitment from the hematopoietic stem cell. Immunity. 2007;26:726–40.

Izraeli S. Application of genomics for risk stratification of childhood acute lymphoblastic leukaemia: from bench to bedside? Br J Haematol. 2010;151:119–31.

Jan M, Snyder TM, Corces-Zimmerman MR, Vyas P, Weissman IL, Quake SR, Majeti R. Clonal evolution of preleukemic hematopoietic stem cells precedes human acute myeloid leukemia. Sci Transl Med. 2012;4(149):149ra118.

Jordan CT, Upchurch D, Szilvassy SJ, Guzman ML, Howard DS, Pettigrew AL, Meyerrose T, Rossi R, Grimes B, Rizzieri DA, Luger SM, Phillips GL. The interleukin-3 receptor alpha chain is a unique marker for human acute myelogenous leukemia stem cells. Leukemia. 2000;14:1777–84.

Juarez-Velazquez R, Reyes-Leon A, Salas-Labadia C, Rivera-Luna R, Velasco-Hidalgo L, Lopez-Hernandez G, Lopez-Santiago N, Paredes-Aguilera R, Dominguez-Lopez A, Bernaldez R, Perez-Vera P. Significance of CASP8AP2 and H2AFZ expression in survival and risk of relapse in children with acute lymphoblastic leukemia. Leuk Lymphoma. 2014;55:2305–11.

Kakarala M, Wicha MS. Implications of the cancer stem-cell hypothesis for breast cancer prevention and therapy. J Clin Oncol. 2008;26:2813–20.

Kawamata N, Pennella MA, Woo JL, Berk AJ, Koeffler HP. Dominant-negative mechanism of leukemogenic PAX5 fusions. Oncogene. 2012;31:966–77.

Kiel MJ, Yilmaz OH, Iwashita T, Terhorst C, Morrison SJ. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. Cell. 2005;121:1109–21.

Kinlen LJ. Epidemiological evidence for an infective basis in childhood leukaemia. Br J Cancer. 1995;71:1–5.

Kohn LA, Hao QL, Sasidharan R, Parekh C, Ge S, Zhu Y, Mikkola HK, Crooks GM. Lymphoid priming in human bone marrow begins before expression of CD10 with upregulation of L-selectin. Nat Immunol. 2012;13:963–71.

Kondo M, Weissman IL, Akashi K. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. Cell. 1997;91:661–72.

Kong Y, Yoshida S, Saito Y, Doi T, Nagatoshi Y, Fukata M, Saito N, Yang SM, Iwamoto C, Okamura J, Liu KY, Huang XJ, Lu DP, Shultz LD, Harada M, Ishikawa F. CD34+CD38+CD19+ as well as CD34+CD38-CD19+ cells are leukemia-initiating cells with self-renewal capacity in human B-precursor ALL. Leukemia. 2008;22:1207–13.

Krause DS, VAN Etten RA. Right on target: eradicating leukemic stem cells. Trends Mol Med. 2007;13:470–81.

Krawczyc J, O'Dwyer M, Swords R, Freeman C, Giles FJ. The role of inflammation in leukemia. In: Agarwal BB et al. editors. Inflammation and Cancer, Advances in Experimental Medicine and biology. Basel: Springer; 2014.

Kucia M, Reca R, Miekus K, Wanzeck J, Wojakowski W, Janowska-Wieczorek A, Ratajczak J, Ratajczak MZ. Trafficking of normal stem cells and metastasis of cancer stem cells involve similar mechanisms: pivotal role of the SDF-1-CXCR4 axis. Stem Cells. 2005;23:879–94.

Lane SW, Wang YJ, Lo Celso C, Ragu C, Bullinger L, Sykes SM, Ferraro F, Shterental S, Lin CP, Gilliland DG, Scadden DT, Armstrong SA, Williams DA. Differential niche and Wnt requirements during acute myeloid leukemia progression. Blood. 2011;118:2849–56.

Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, Minden M, Paterson B, Caligiuri MA, Dick JE. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature. 1994;367:645–8.

Le Viseur C, Hotfilder M, Bomken S, Wilson K, Rottgers S, Schrauder A, Rosemann A, Irving J, Stam RW, Shultz LD, Harbott J, Jurgens H, Schrappe M, Pieters R, Vormoor J. In childhood acute lymphoblastic leukemia, blasts at different stages of immunophenotypic maturation have stem cell properties. Cancer Cell. 2008;14:47–58.

Lemoli RM, Salvestrini V, Bianchi E, Bertolini F, Fogli M, Amabile M, Tafuri A, Salati S, Zini R, Testoni N, Rabascio C, Rossi L, Martin-Padura I, Castagnetti F, Marighetti P, Martinelli G, Baccarani M, Ferrari S, Manfredini R. Molecular and functional analysis of the stem cell compartment of chronic myelogenous leukemia reveals the presence of a CD34- cell population with intrinsic resistance to imatinib. Blood. 2009;114:5191–200.

Lilljebjorn H, Rissler M, Lassen C, Heldrup J, Behrendtz M, Mitelman F, Johansson B, Fioretos T. Whole-exome sequencing of pediatric acute lymphoblastic leukemia. Leukemia. 2012; 26:1602–7.

Lippitz BE. Cytokine patterns in patients with cancer: a systematic review. Lancet Oncol. 2013;14:e218–28.

Magrath I, Steliarova-Foucher E, Epelman S, Ribeiro RC, Harif M, Li CK, Kebudi R, Macfarlane SD, Howard SC. Paediatric cancer in low-income and middle-income countries. Lancet Oncol. 2013;14:e104–16.

Majeti R, Chao MP, Alizadeh AA, Pang WW, Jaiswal S, Gibbs KD Jr, van Rooijen N, Weissman IL. CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. Cell. 2009;138(2):286–99.

Male V, Nisoli I, Kostrzewski T, Allan DS, Carlyle JR, Lord GM, Wack A, Brady HJ. The transcription factor E4bp4/Nfil3 controls commitment to the NK lineage and directly regulates Eomes and Id2 expression. J Exp Med. 2014;211:635–42.

Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008; 454:436–44.

Manz MG, Miyamoto T, Akashi K, Weissman IL. Prospective isolation of human clonogenic common myeloid progenitors. Proc Natl Acad Sci U S A. 2002;99:11872–7.

Massberg S, Schaerli P, Knezevic-Maramica I, Kollnberger M, Tubo N, Moseman EA, Huff IV, Junt T, Wagers AJ, Mazo IB, VON Andrian UH. Immunosurveillance by hematopoietic progenitor cells trafficking through blood, lymph, and peripheral tissues. Cell. 2007; 131:994–1008.

Maude SL, Tasian SK, Vincent T, Hall JW, Sheen C, Roberts KG, Seif AE, Barrett DM, Chen IM, Collins JR, Mullighan CG, Hunger SP, Harvey RC, Willman CL, Fridman JS, Loh ML, Grupp SA, Teachey DT. Targeting JAK1/2 and mTOR in murine xenograft models of Ph-like acute lymphoblastic leukemia. Blood. 2012;120(17):3510–8.

Mayani H, Flores-Figueroa E, Chávez-González A. In vitro biology of human myeloid leukemia. Leuk Res. 2009;33(5):624–37.

Mazo IB, Massberg S, Von Andrian UH. Hematopoietic stem and progenitor cell trafficking. Trends Immunol. 2011;32:493–503.

McManus S, Ebert A, Salvagiotto G, Medvedovic J, Sun Q, Tamir I, Jaritz M, Tagoh H, Busslinger M. The transcription factor Pax5 regulates its target genes by recruiting chromatin-modifying proteins in committed B cells. EMBO J. 2011;30:2388–404.

Mcneil DE, Cote TR, Clegg L, Mauer A. SEER update of incidence and trends in pediatric malignancies: acute lymphoblastic leukemia. Med Pediatr Oncol. 2002;39:554–7; discussion 552–3.

Meijerink JP, DEN Boer ML, Pieters R. New genetic abnormalities and treatment response in acute lymphoblastic leukemia. Semin Hematol. 2009;46:16–23.

Merchand-Reyes G, Pelayo R, Pavon L, Pestell R, Velasco-Velázquez M. Role of cancer stem cells in metastasis. In*:* Rajasekhar V. editor. Cancer stem cells. Hoboken, New Jersey: John Wiley & Sons, Inc; 2014.

Mjosberg J, Bernink J, Golebski K, Karrich JJ, Peters CP, Blom B, Te Velde AA, Fokkens WJ, Van Drunen CM, Spits H. The transcription factor GATA3 is essential for the function of human type 2 innate lymphoid cells. Immunity. 2012;37:649–59.

Mullighan CG, Zhang J, Harvey RC, Collins-Underwood JR, Schulman BA, Phillips LA, Tasian SK, Loh ML, Su X, Liu W, Devidas M, Atlas SR, Chen IM, Clifford RJ, Gerhard DS, Carroll WL, Reaman GH, Smith M, Downing JR, Hunger SP, Willman CL. JAK mutations in high-risk childhood acute lymphoblastic leukemia. Proc Natl Acad Sci U S A. 2009;106(23):9414–8.

Mullighan CG. The molecular genetic makeup of acute lymphoblastic leukemia. Hematol Am Soc Hematol Educ Prog. 2012;2012:389–96.

Mullighan CG, Willman CL. Advances in the biology of acute lymphoblastic leukemia-from genomics to the clinic. J Adolesc Young Adult Oncol. 2011;1:77–86.

Naik SH, Perie L, Swart E, Gerlach C, Van Rooij N, De Boer RJ, Schumacher TN. Diverse and heritable lineage imprinting of early haematopoietic progenitors. Nature. 2013;496:229–32.

Nguyen LV, Vanner R, Dirks P, Eaves CJ. Cancer stem cells: an evolving concept. Nat Rev Cancer. 2012;12:133–43.

Notta F, Doulatov S, Laurenti E, Poeppl A, Jurisica I, Dick JE. Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. Science. 2011a;333:218–21.

Notta F, Mullighan CG, Wang JC, Poeppl A, Doulatov S, Phillips LA, Ma J, Minden MD, Downing JR, Dick JE. Evolution of human BCR-ABL1 lymphoblastic leukaemia-initiating cells. Nature. 2011b;469:362–7.

Nutt SL, Heavey B, Rolink AG, Busslinger M. Commitment to the B-lymphoid lineage depends on the transcription factor Pax5. Nature. 1999;401:556–62.

Oguro H, Ding L, Morrison SJ. SLAM family markers resolve functionally distinct subpopulations of hematopoietic stem cells and multipotent progenitors. Cell Stem Cell. 2013;13:102–16.

Orkin SH, Pera M. Stem cells down under-ISSCR 2007. Cell Stem Cell. 2007;1:271–6.

Pelayo R, Miyazaki K, Huang J, Garrett KP, Osmond DG, Kincade PW. Cell cycle quiescence of early lymphoid progenitors in adult bone marrow. Stem Cells. 2006;24:2703–13.

Pelayo R, Dorantes-Acosta E, Vadillo E, Fuentes-Panana EM. From HSC to B-lymphoid cells in normal and malignant hematopoiesis. In*:* Pelayo R. editor. Advances in hematopoietic stem cell research. Rijeka, Croatia: InTech; 2012.

Perez-Saldivar ML, Fajardo-Gutierrez A, Bernaldez-Rios R, Martinez-Avalos A, Medina-Sanson A, Espinosa-Hernandez L, Flores-Chapa JDED, Amador-Sanchez R, Penaloza-Gonzalez JG, Alvarez-Rodriguez FJ, Bolea-Murga V, Flores-Lujano J, Rodriguez-Zepeda MDELC, Rivera-Luna R, Dorantes-Acosta EM, Jimenez-Hernandez E, Alvarado-Ibarra M, Velazquez-Avina MM, Torres-Nava JR, Duarte-Rodriguez DA, Paredes-Aguilera R, Del Campo-Martinez MDEL, Cardenas-Cardos R, Alamilla-Galicia PH, Bekker-Mendez VC, Ortega-Alvarez MC, Mejia-Arangure JM. Childhood acute leukemias are frequent in Mexico City: descriptive epidemiology. BMC Cancer. 2011;11:355.

Pui CH, Evans WE. A 50-year journey to cure childhood acute lymphoblastic leukemia. Semin Hematol. 2013;50:185–96.

Pui CH, Rubnitz JE, Hancock ML, Downing JR, Raimondi SC, Rivera GK, Sandlund JT, Ribeiro RC, Head DR, Relling MV, Evans WE, Behm FG. Reappraisal of the clinical and biologic significance of myeloid-associated antigen expression in childhood acute lymphoblastic leukemia. J Clin Oncol. 1998;16:3768–73.

Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. Lancet. 2008;371:1030–43.

Purizaca J, Meza I, Pelayo R. Early lymphoid development and microenvironmental cues in B-cell acute lymphoblastic leukemia. Arch Med Res. 2012;43:89–101.

Purizaca J, Contreras-Quiroz A, Dorantes-Acosta E, Vadillo E, Arriaga-Pizano L, Fuentes-Figueroa S, Villagomez-Barragan H, Flores-Guzman P, Alvarado-Moreno A, Mayani H, Meza I, Hernandez R, Huerta-Yepez S, Pelayo R. Lymphoid progenitor cells from childhood acute lymphoblastic leukemia are functionally deficient and express high levels of the transcriptional repressor Gfi-1. Clin Dev Immunol. 2013;2013:349067.

Raaijmakers MH. Niche contributions to oncogenesis: emerging concepts and implications for the hematopoietic system. Haematologica. 2011;96:1041–8.

Raaijmakers MH, Mukherjee S, Guo S, Zhang S, Kobayashi T, Schoonmaker JA, Ebert BL, Al-Shahrour F, Hasserjian RP, Scadden EO, Aung Z, Matza M, Merkenschlager M, Lin C, Rommens JM, Scadden DT. Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia. Nature. 2010;464:852–7.

Raff T, Bruggermann M. Leukemia-initiating cells in acute lymphoblastic leukemia. In: Rajasekhar V. editor. Cancer stem cells. Hoboken, New Jersey: John Wiley & Sons, Inc, Wiley-Blackwell; 2014.

Rajendran P, Dalerba P. Theoretical and experimental foundations of the "Cancer stem cell" model. In*:* Rajashekhar V, editor. Cancer stem cells*.* Hoboken, New Jersey: John Wiley & Sons, Inc; 2014.

Rehe K, Wilson K, Bomken S, Williamson D, Irving J, DEN Boer ML, Stanulla M, Schrappe M, Hall AG, Heidenreich O, Vormoor J. Acute B lymphoblastic leukaemia-propagating cells are present at high frequency in diverse lymphoblast populations. EMBO Mol Med. 2013;5:38–51.

Rubnitz JE, Onciu M, Pounds S, Shurtleff S, Cao X, Raimondi SC, Behm FG, Campana D, Razzouk BI, Ribeiro RC, Downing JR, Pui CH. Acute mixed lineage leukemia in children: the experience of St Jude Children's Research Hospital. Blood. 2009;113:5083–9.

Serafini N, Klein Wolterink RG, Satoh-Takayama N, Xu W, Vosshenrich CA, Hendriks RW, Di Santo JP. Gata3 drives development of RORgammat+ group 3 innate lymphoid cells. J Exp Med. 2014;211:199–208.

Shah DK, Zuniga-Pflucker JC. An overview of the intrathymic intricacies of T cell development. J Immunol. 2014;192:4017–23.

Shlush LI, Zandi S, Mitchell A, Chen WC, Brandwein JM, Gupta V, Kennedy JA, Schimmer AD, Schuh AC, Yee KW, McLeod JL, Doedens M, Medeiros JJ, Marke R, Kim HJ, Lee K, Mcpherson JD, Hudson TJ, Consortium HP-LGP, Brown AM, Yousif F, Trinh QM, Stein LD, Minden MD, Wang JC, Dick JE. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. Nature. 2014;506:328–33.

Shortman K, Sathe P, Vremec D, Naik S, O'Keeffe M. Plasmacytoid dendritic cell development. Adv Immunol. 2013;120:105–26.

Sipkins DA, Wei X, Wu JW, Runnels JM, Cote D, Means TK, Luster AD, Scadden DT, Lin CP. In vivo imaging of specialized bone marrow endothelial microdomains for tumour engraftment. Nature. 2005;435:969–73.

Spangrude GJ, Heimfeld S, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. Science. 1988;241:58–62.

Spits H, Cupedo T. Innate lymphoid cells: emerging insights in development, lineage relationships, and function. Annu Rev Immunol. 2012;30:647–75.

Sultana DA, Zhang SL, Todd SP, Bhandoola A. Expression of functional P-selectin glycoprotein ligand 1 on hematopoietic progenitors is developmentally regulated. J Immunol. 2012;188: 4385–93.

Takahashi S. Downstream molecular pathways of FLT3 in the pathogenesis of acute myeloid leukemia: biology and therapeutic implications. J Hematol Oncol. 2011;4:13.

Tan BT, Park CY, Ailles LE, Weissman IL. The cancer stem cell hypothesis: a work in progress. Lab Invest. 2006;86:1203–7.

Tavor S, Petit I, Porozov S, Avigdor A, Dar A, Leider-Trejo L, Shemtov N, Deutsch V, Naparstek E, Nagler A, Lapidot T. CXCR4 regulates migration and development of human acute myelogenous leukemia stem cells in transplanted NOD/SCID mice. Cancer Res. 2004; 64:2817–24.

Terwijn M, Zeijlemaker W, Kelder A, Rutten AP, Snel AN, Scholten WJ, Pabst T, Verhoef G, Lowenberg B, Zweegman S, Ossenkoppele GJ, Schuurhuis GJ. Leukemic stem cell frequency: a strong biomarker for clinical outcome in acute myeloid leukemia. PLoS ONE. 2014;9:e107587.

Vadillo E, Pelayo R. El sistema hematopoyético a partir de células troncales. In: Pelayo R, editor. Células Troncales y Medicina Regenerativa. Mexico: PUIS; 2011.

Vadillo E, Pelayo R. Toll-like receptors in development and function of the hematopoietic system. Rev Invest Clin. 2012;64:461–76.

Vadillo E, Dorantes-Acosta E, Pelayo R. Regulation of hematopoietic stem/progenitor cells by inflammation cues. In: Perez-Martínez et al (eds). Molecular Aspects of Inflammation Kerala, India: Research Signpost; 2013.

Vadillo E, Dorantes-Acosta E, Arriaga-Pizano L, Chavez-Gonzalez A, Reyes-Maldonado E, Garrett KP, Mayani H, Kincade PW, Pelayo R. Adult, but not neonatal, human lymphoid progenitors respond to TLR9 ligation by producing functional NK-like cells. Exp Hematol. 2014;42:562–73, e3.

Vesely MD, Kershaw MH, Schreiber RD, Smyth MJ. Natural innate and adaptive immunity to cancer. Annu Rev Immunol. 2011;29:235–71.

Vicente Lopez A, Vazquez Garcia MN, Melen GJ, Entrena Martinez A, Cubillo Moreno I, Garcia-Castro J, Orellana MR, Gonzalez AG. Mesenchymal stromal cells derived from the bone marrow of acute lymphoblastic leukemia patients show altered BMP4 production: correlations with the course of disease. PLoS ONE. 2014;9:e84496.

Vilchis A, Contreras-Quiroz A, Vadillo E, Dorantes-Acosta E, Reyes-Lopez A, Quintela-Nuñez Del Prado H, Venegas-Vazquez J, Mayani H, Ortiz-Navarrete V, Lopez-Martinez B, Pelayo R. Boen marrow cells in acute lymphoblastic leukemia create a pro-inflammatory microenviroment influencing normal hematopoietic differentiation fates. Biomed Res Int. 2015;2015:386165. doi: 10.1155/2015/386165.

Vosshenrich CA, Di Santo JP. Developmental programming of natural killer and innate lymphoid cells. Curr Opin Immunol. 2013;25:130–8.

Welner RS, Pelayo R, Kincade PW. Evolving views on the genealogy of B cells. Nat Rev Immunol. 2008;8:95–106.

Wong SH, Walker JA, Jolin HE, Drynan LF, Hams E, Camelo A, Barlow JL, Neill DR, Panova V, Koch U, Radtke F, Hardman CS, Hwang YY, Fallon PG, McKenzie AN. Transcription factor RORalpha is critical for nuocyte development. Nat Immunol. 2012;13:229–36.

Woo JS, Alberti MO, Tirado CA. Childhood B-acute lymphoblastic leukemia: a genetic update. Exp Hematol Oncol. 2014;3:16.

Xie Y, Davies SM, Xiang Y, Robison LL, Ross JA. Trends in leukemia incidence and survival in the United States (1973–1998). Cancer. 2003;97:2229–35.

Xu H, Yang W, Perez-Andreu V, Devidas M, Fan Y, Cheng C, Pei D, Scheet P, Burchard EG, Eng C, Huntsman S, Torgerson DG, Dean M, Winick NJ, Martin PL, Camitta BM, Bowman WP, Willman CL, Carroll WL, Mullighan CG, Bhojwani D, Hunger SP, Pui CH, Evans WE, Relling MV, Loh ML, Yang JJ. Novel susceptibility variants at 10p12.31-12.2 for childhood acute lymphoblastic leukemia in ethnically diverse populations. J Natl Cancer Inst. 2013;105: 733–42.

Zhang M, Lu J, Wang X, Cen J, Fan G, Hu S. Early precursor T-lymphoblastic leukaemia/lymphoma arising from paediatric chronic myeloid leukaemia - unusual lymph node blast crisis. Br J Haematol. 2013;161(1):136-9.

Zlotoff DA, Sambandam A, Logan TD, Bell JJ, Schwarz BA, Bhandoola A. CCR7 and CCR9 together recruit hematopoietic progenitors to the adult thymus. Blood. 2010;115:1897–905.

Zuna J, Madzo J, Krejci O, Zemanova Z, Kalinova M, Muzikova K, Zapotocky M, Starkova J, Hrusak O, Horak J, Trka J. ETV6/RUNX1 (TEL/AML1) is a frequent prenatal first hit in childhood leukemia. Blood. 2011;117:368–9; author reply 370–1.

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