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Acute leukemia in early childhood

M. Emerenciano[1], S. Koifman[2] and M.S. Pombo-de-Oliveira[1]

[1]Divisão de Medicina Experimental, Centro de Pesquisa, Instituto Nacional de Câncer, Rio de Janeiro, RJ, Brasil
[2]Escola Nacional de Saúde Pública, FIOCRUZ, Rio de Janeiro, RJ, Brasil

Abstract

Acute leukemia in early childhood is biologically and clinically distinct. The particular characteristics of this malignancy diagnosed during the first months of life have provided remarkable insights into the etiology of the disease. The pro-B, CD10 negative immunophenotype is typically found in infant acute leukemia, and the most common genetic alterations are the rearrangements of the *MLL* gene. In addition, the *TEL/AML1* fusion gene is most frequently found in children older than 24 months. A molecular study on a Brazilian cohort (age range 0-23 months) has detected *TEL/AML1*$^{+ve}$ (N = 9), *E2A/PBX1*$^{+ve}$ (N = 4), *PML/RARA*$^{+ve}$ (N = 4), and *AML1/ETO*$^{+ve}$ (N = 2) cases. Undoubtedly, the great majority of genetic events occurring in these patients arise prenatally. The environmental exposure to damaging agents that give rise to genetic changes prenatally may be accurately determined in infants since the window of exposure is limited and known. Several studies have shown maternal exposures that may give rise to leukemogenic changes. The Brazilian Collaborative Study Group of Infant Acute Leukemia has found that mothers exposed to dipyrone, pesticides and hormones had an increased chance to give birth to babies with infant acute leukemia [OR = 1.48 (95%CI = 1.05-2.07), OR = 2.27 (95%CI = 1.56-3.31) and OR = 9.08 (95%CI = 2.95-27.96)], respectively. This review aims to summarize recent clues that have facilitated the elucidation of the biology of early childhood leukemias, with emphasis on infant acute leukemia in the Brazilian population.

Correspondence
Correspondence
M.S. Pombo-de-Oliveira
Coordenação de Pesquisa, CPq
Instituto Nacional de Câncer
Rua André Cavalcanti, 37
20231-050 Rio de Janeiro, RJ
Brasil
Fax: +55-21-3233-1470
E-mail: mpombo@inca.gov.br

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- Molecular and exploratory epidemiology

Clinical characterization

Acute leukemia in early infancy comprises a group of leukemias characterized by the diagnosis of acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML) during the first years of life. The term infant acute leukemia (IAL) is usually applied when the diagnosis is made within the first twelve months after birth. The medical services of some countries, however, extend this time frame to 18 months of age. Leukemia is the second most common malignancy that occurs in the first year of life, following neuroblastoma (1-3). Although not as frequent as neuroblastoma, leukemia is the leading cause of death from neoplastic disease during the perinatal period. Despite being the second most common malignant disease in the first year of life, leukemia is a very rare disease in younger children, occurring much more frequently in later childhood (3). In some cases, the signs of leukemia appear at birth and the neonates die shortly thereafter, while others show normal development following delivery, with clinical and hematological prob-

lems appearing later (4). Today it is possible to correlate these differences with the variable biological background of each individual case, such as cases with Down syndrome that represent a distinct leukemia with spontaneous clinical remission (5).

Clinically, IAL is characterized by a high leukocyte count, hepatosplenomegaly, and central nervous system involvement. The pro-B immunophenotype, CD10 negative, and sometimes with aberrant expression of monocytoid differentiation is the one most frequently observed. These features indicate that the most common form of infant ALL originates in a stem cell still not fully committed to lymphoid and/or myeloid differentiation. This is corroborated by the observation that the multilineage gene expression profile precedes commitment of stem and progenitor cells to unilineage differentiation in the hematopoietic system, in which unilineage commitment is prefaced by a "promiscuous" phase of multilineage locus activation (6,7). On the other hand, the presence of leukemia-specific translocations in primitive lymphoid-restricted $CD34^+CD19^-$ cells purified from t(4;11)-positive ALL samples has been recently reported (8), indicating a primitive lymphoid-restricted progenitor/stem cell origin. In early infancy cases of myeloid origin (AML), the distribution of subtypes seems to be mostly M4 and M5, followed by the M7 subtype (characteristic of those with Down syndrome) (1). Surprisingly, however, in Brazil an apparent excess of acute promyelocytic leukemia has been reported in children younger than 18 months (9).

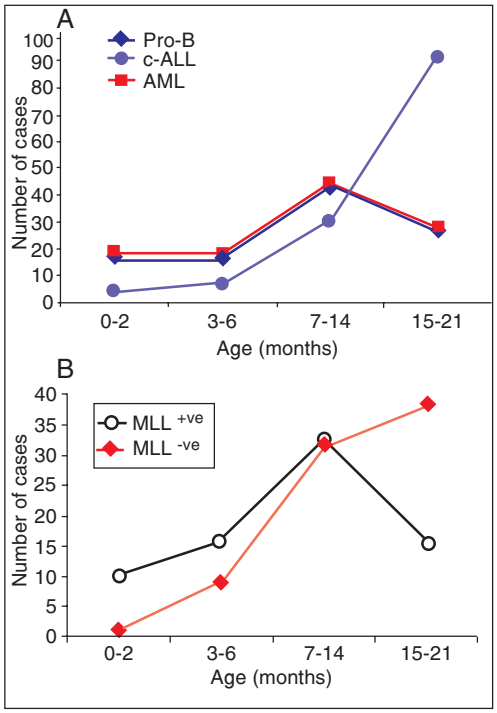

Figure 1. Distribution of infant acute leukemia cases according to immuno-molecular characterization and age. *A*, Immunophenotyping profile. *B*, *MLL* status. Pro-B = $CD19^+/CD10^-$ B precursor acute lymphoblastic leukemia; c-ALL = $CD19^+/CD10^+$ common acute lymphoblastic leukemia; AML = acute myeloid leukemia.

The most common genetic events occurring in children younger than 12 months, both in ALL and AML, are the rearrangements of the *MLL* gene on chromosome 11q23, which may be as high as 85% depending on the techniques applied (10,11). Infants diagnosed with acute leukemia harboring an 11q23 rearrangement have a particularly poor prognosis when compared to other children with acute leukemia (12). These singular characteristics seen in IALs - lack of CD10 expression, presence of myeloid markers, and *MLL* gene rearrangements - are inter-correlated, and their presence is inversely associated with age. For example, *MLL* rearrangement is associated with 90% of $CD10^-$ cases but only with 20% of $CD10^+$ cases. Also, the younger the child the higher is the frequency of *MLL* translocations (10,13). In series of IAL cases from different regions of Brazil, the comparison between age, immunophenotyping, and *MLL* rearrangements demonstrated that up to 14 months of age the pro-B subtype and the *MLL*-positive cases predominated ($P < 0.0001$), and among older cases, most of them were c-ALL and *MLL*-negative cases, as shown in Figure 1. Regarding the *MLL* gene status, it is important to note that even in cases older than 14 months, some were *MLL* positive (Figure 1B).

Regarding outcome, unfortunately, the prognosis in this age group still remains poor. At present, the overall 5-year survival for infant ALL patients remains approxi-

mately 40-50% (12,14). The above features and the short latency periods define IAL as a biologically and clinically distinct disease from childhood acute leukemia in general. Therefore, it seems probable that different genetic and environmental factors may be involved in the mechanisms of pathogenesis of IAL, childhood ALL and AML (1,13), and this knowledge is of great importance when dealing with this aggressive type of leukemia.

Mechanisms of pathogenesis

MLL gene rearrangements and topoisomerase II inhibitors

The mixed-lineage leukemia gene (*MLL/HRX/ALL1*), located at cytogenetic band 11q23, is often altered in IAL, being rearranged in more than 80% of cases (12,13). This gene consists of at least 36 exons, encoding a 3969-amino acid nuclear protein with a molecular weight of nearly 430 kDa that functions as a positive regulator of gene expression in early embryonic development and hematopoiesis. *MLL* translocation breakpoints cluster within an 8.3-kb region spanning exons 5-11 (Figure 2I). In its germ-line form, MLL protein, a human homologue of the transcriptional regulator Trithorax of *Drosophila*, is an upstream transcriptional effector of *HOX* genes, which play a key role in the regulation of hematopoietic development (15). To distinguish whether the *MLL* gene acts as an oncogene or whether the fusion

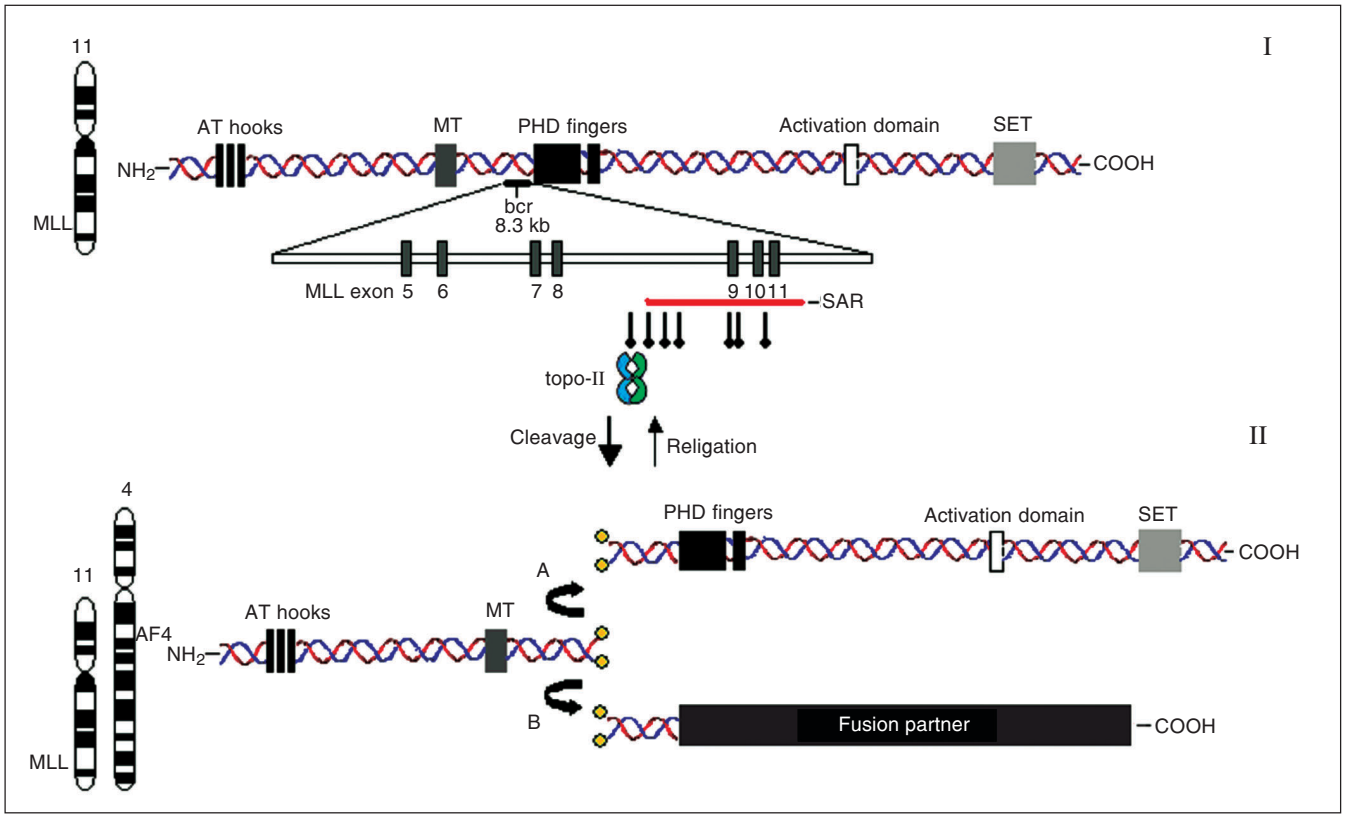

Figure 2. *I*, Structure of the *MLL* gene, with protein motifs indicated in the diagram. Three AT hooks are located near the N-terminal region. MT = DNA methyltransferase homology region; PHD = plant homeodomain zinc fingers; SET = su(var)3-9, enhancer of zeste, trithorax domain. Solid black bar just behind the PHD zinc fingers indicates the location of the breakpoint cluster region (bcr), and its exon/intron structure is highlighted. Solid red bar below exons 9, 10, and 11 indicates the high-affinity scaffold attachment region (SAR). Vertical arrows indicate the topo-II consensus sites. *II*, Consequences of topo-II cleavage. *A*, *MLL* double strand is correctly re-ligated, generating wild-type *MLL*. *B*, *MLL* is fused with frequent partner *AF4*, generating a fusion oncogene. The NCBI website (http://www.ncbi.nlm.nih.gov/) was extensively consulted for elaboration of this figure.

proteins resulting from the translocations have dominant negative effects, *in vitro* experiments using hematopoietic progenitors from embryos of homozygous *MLL* knockout mice were performed. The ability of wild-type AB2.1 embryonic stem (ES) cells and of single- or double-*ALL-1* gene knockout cells derived from them to differentiate along hematopoietic lineages was tested using *in vitro* colony formation assays. The results showed that ALL-1 double-knockout ES cells formed a significantly greater number of colonies with faster kinetics than wild-type and *ALL-1* single-knockout ES cells. Furthermore, parental ES cells formed lineage-restricted colonies, whereas single- and double-knockout ES cells developed, at high frequency, immature and/or biphenotypic colonies, mimicking the aberrant hematopoiesis typical of leukemia patients (16).

To date, over 87 chromosome partners of *MLL* have been described with diverse functions and variable function domains, and 51 of the presumptive gene partners of *MLL* have been cloned and analyzed at the molecular level (11). This marked promiscuity raises the question of whether the diverse partners contribute with common functions or have different effects in leukemogenesis. *MLL* partners can be divided into two groups. First, the nuclear fusion partners, including AF4, AF9, AF10, ENL, ELL, AF17, and others, which are associated with different aspects of transcriptional regulation. The other group is mainly cytoplasmic and frequently associated with cytoskeleton-dependent signal transduction, including *AF6*, *Septin 6*, *ABI-1*, *EEN*, and so on (17). Although the fusion partners display many different features, all of them delete a large 3' portion of the *MLL* gene and connect the remaining part with the corresponding partner and, regardless of whether the fusion partner itself is nuclear or cytoplasmic, the MLL chimeric proteins consistently form punctuated patterns in the nucleus (18), indicating that the localization of the fusion protein and its potential targets is mainly determined by the DNA-binding activity of the MLL portion and not by the fusion partner. Remarkable is the fact that the frequency and diversity of MLL-associated abnormalities vary according to *de novo* acute leukemia and therapy-related leukemias (19). Considering the role of the partner genes, these reports indicate that the transforming capacity of the truncated MLL alone is ineffective, suggesting that a gain of MLL function via a partner gene is crucial.

Although the mechanisms by which these rearrangements result in leukemia remain largely unknown, clues for understanding the mechanism of main translocations come from genomic details of the *MLL* gene. Several DNA motifs implicated in recombination of DNA have been identified and localized within the *MLL* breakpoint cluster region (bcr). These include topoisomerase-II (topo-II)-binding sites (20) and Alu sequences (21). Also, a high-affinity scaffold attachment region (SAR) was identified within the telomeric (3') region of the bcr (Figure 2II). SARs are sites for binding of DNA to nuclear matrix proteins, functioning to maintain the structure of chromosomal loops and to allow regulation of transcription, DNA replication, and recombination (20).

Suggestively, most of the topo-II-consensus sites overlap the SAR, clustering in the telomeric part of the *MLL* bcr. The observation of these recombination-prone sequences in the *MLL* bcr region indicates that the rearrangement might result from DNA breakage and recombination events (20). Several *in vitro* studies support the link between *MLL* cleavage and topo-II inhibitors in human hematopoietic cells (22,23). Nevertheless, these studies still do not prove the occurrence of recombination. The same biased distribution of gene breakpoints within *MLL* bcr seen in treatment-related leukemias has also been shown in IALs (24). On the basis of this finding, it is very likely that

the mechanisms of cleavage of the *MLL* gene in IALs are similar to those of topo-II inhibitor-induced secondary leukemias (20). It was then suggested that the critical leukemogenic event(s) occurring *in uterus* might similarly involve prenatal exposure to topo-II inhibitors as represented by several natural and medicinal substances, further discussed in molecular and exploratory epidemiology.

Other molecular markers

It is clear that chromosome translocations, especially those involving the *MLL* gene, are the genetic alterations most frequently found in early IAL. However, not only *MLL* rearrangements but also other chromosome translocations may be found within this group, especially when the age limit is 18 months of age.

For instance, another hallmark in pediatric acute leukemias is the chromosomal translocation t(12;21)(p13;q22), which fuses the *TEL* and *AML1* genes, resulting in the most common chromosomal alteration in childhood cancer. The *TEL/AML1* fusion gene occurs in approximately 25% of B-cell precursor ALL in children 2-12 years old. There is evidence that *TEL/AML1* usually arises prenatally as an early or initiating mutation. However, the low-twin concordance rate (~10%), the protracted and variable latency of the disease, and the transgenic modeling indicate that secondary postnatal genetic alterations are also required (25,26). While *MLL*-positive cases present a poor prognosis and are often associated with $CD10^-$ immunophenotypes, ALL cases expressing the TEL/AML1 fusion protein are associated with $CD10^+$ immunophenotypes and excellent prognosis (27).

A molecular study conducted on a Brazilian cohort (age range 0 to 23 months) has detected *TEL/AML1*$^{+ve}$ (N = 9), *E2A/PBX1*$^{+ve}$ (N = 4), *PML/RARA*$^{+ve}$ (N = 4), and *AML1/ETO*$^{+ve}$ (N = 2) cases (10). It is unlikely, however, that a single-chromosome translocation itself would be enough to cause overt leukemia. According to Greaves' hypothesis (28), genetic alterations that impair differentiation probably cooperate with a second class of mutations that alter the proliferation and survival of a malignant clone. The short latency and the high-concordance rate between twins with infant leukemia could suggest that the *MLL*-fusion gene should be sufficient to cause leukemia. Perhaps more likely, however, and accepting the same *TEL/AML1* minimal two-step model for childhood leukemia, the disruptive effect of the *MLL*-gene fusion on, for example, DNA repair or cell-cycle regulation could facilitate the rapid acquisition of additional, necessary genetic changes, particularly with continued exposure to a genotoxic chemical *in utero* (29,30).

The gathering of multiple, independent and complementary genetic lesions as a requirement for abnormal development of a hematopoietic progenitor supports studies that aim to investigate other genes in the pathogenesis of early acute leukemia, bearing in mind the cell type and the corresponding chromosomal lesions. Accordingly, secondary chromosomal or genetic alterations are detected at diagnosis in a significant number of *MLL*-positive cases. In children ≤3 years old, among whom it is believed that the first genetic alteration required to fully develop childhood ALL occurs *in utero*, the leukemic cells prevailingly exhibit fetal-type DJ_H junctions of the complementarily determining region 3 of the immunoglobulin H chain that lack the so-called N regions, which are added during DJ_H recombination events later in fetal development (31). Interestingly, N regions were later found to be present in 11 of 12 IAL cases with t(4;11) translocation (32), indicating that t(4;11)-positive IAL is initiated later in fetal development than most B-cell precursor ALL in children younger than 3 years and that they have a shorter latency period already *in utero*. Ultimately,

considering the *MLL* translocation to be the first genetic hit undoubtedly arising *in utero*, but yet displaying N region-positive DJ_H junctions, it is very likely that an exquisitely short latency period for only limited mutagenic events is further required (14,32). Furthermore, it was recently found that the receptor tyrosine kinase FLT3 is highly expressed in *MLL*-rearranged ALL as compared with other leukemias (6). Moreover, it was found that approximately 20% of *MLL*-rearranged ALL cases possess activating mutations in the activation loop region (33). These data provide evidence that leukemogenic fusion proteins such as MLL fusions cooperate with activated kinases to promote leukemogenesis. Thus, the conundrum of whether one or more secondary mutations are necessary for leukemia development in *MLL*-positive acute leukemias is a challenge that still needs to be fully elucidated. The combination of molecular and exploratory epidemiology methods is a good strategic model to test this hypothesis.

Molecular and exploratory epidemiology

What makes these leukemias so unique?

There is no doubt that the risks of developing early acute leukemia are modulated by complex interactions between inherited predispositions, environmental exposure to damaging agents and chance events (28,34). Despite the fact that such leukemias are very rare, their investigation is absolutely necessary for the study of leukemogenesis because they have a short latency period together with the known immune molecular markers. The molecular epidemiological approach to genetic studies has suggested the concept that most, if not all, childhood acute leukemia cases originate *in utero*. The evidence for this is based on the following considerations: first, studies of identical twins with *MLL*-rearranged leukemias, presenting the same fusion gene sequences, indicated that *MLL* rearrangements are acquired *in utero*. Such cases may be plausibly explained by the metastasis of a clonal event originating in one twin to the other twin via placental anastomoses (30); second, the fusion gene sequences have been retrospectively identified in archived neonatal heel blood spot cards of children in whom leukemia had subsequently developed (35). Finally, the same fetal origin has been established in older children through monozygotic twin studies or detection of other specific fusion gene sequences (e.g., *TEL/AML1* and *AML1/ETO*) in neonatal blood spots (25). These findings have led to the interpretation that many childhood leukemias originate prenatally during fetal hematopoiesis. The mechanisms of leukemogenesis in early infancy are related to the fact that the growing fetus and/or child is more sensitive to the effects of potential DNA damage insults during the early stage of pregnancy and/or first semester of life. Because reciprocal rearrangement of the *MLL* gene is the most common genetic feature, it is important to understand how these fusions could possibly result from the transplacental exposure to DNA-damaging substances. It has been well demonstrated that substances that target topo-II, inhibiting the re-sealing of a previously cleaved double-strand end by the same enzyme, trigger the most common mechanism in the formation of *MLL* rearrangement (model for therapy-related secondary AML (s-AML)) (22,36).

In s-AML, older children and adults are exposed to drugs that function as topo-II inhibitors, such as epipodophyllotoxins (e.g., etoposide) and anthracyclines. Therapy-related *MLL*-positive leukemia cases have been increasingly reported (37). It was then postulated that transplacental exposures to DNA-topo-II inhibitors, which form cleavable complexes, may be related to the etiology of IAL with *MLL* rearrangements (38). As discussed before, the same biased distribution of gene breakpoints within *MLL* bcr seen in treat-

ment-related leukemias has also been shown in IALs (24,38). Topo-II inhibitors include chemotherapeutic agents, benzene metabolites (such as benzoquinone), isoflavones, flavonoids, lignans, podophyllin resin, quinolone antibiotics, and some pesticides. Specific fruits and vegetables that contain quercetin, soybeans (genistein), tea, cocoa, wine (catechins), and caffeine have all been related to an increased risk of infant AML (36,38-40). Thus, to better understand the environmental exposures to damaging agents that give rise to these genetic changes *in utero*, infants represent a more accurate group than older children, since the window of exposure is limited and known (shortly before or during pregnancy). Although the epidemiological data are still limited to support correlations, several studies have shown maternal exposures that may give rise to these leukemogenic genetic changes (34,40-44). In a literature review consulting the MEDLINE databank, we retrieved published papers focusing on IAL (1990-2006), summarized them in Table 1, and briefly discussed their valuable results. These studies were searched through an intensive combination of both MeSH terms and part of the text words and titles. The following terms were used: infant acute leukemia, childhood

Table 1. Data concerning leukemias reported in epidemiological literature.

Study group	N	Risk factor, adjusted odds ratio, and 95%CI	Reference
Children's Cancer Group	1753 AL 2081 controls	ALL and AML diagnosed before 2 years of age, previous fetal loss was associated with a 5-fold increased risk (P < 0.001), whereas 2 or more fetal losses were associated with 12-fold increased risk (P < 0.001).	Yeazel et al., 1995 (47)
Children's Cancer Group	IAL: 203 ALL + 88 AML 558 controls	Maternal alcohol consumption during pregnancy (compared with no drinking) was associated with OR = 1.43 (95%CI = 1.00-2.04) for ALL and OR = 2.64 (95%CI = 1.36-5.06) for AML.	Shu et al., 1996 (43)
Children's Cancer Group	303 IAL 468 controls	The relationships between birthweight, prior fetal loss, and risk of infant leukemia appear to be complex, requiring studies that incorporate molecular analysis.	Ross et al., 1997 (48)
International Study Group. UK, 1994-1999	136 IAL 266 controls	DNA-damaging drugs: OR = 2.83 (95%CI = 1.15-6.99) P < 0.03. Mosquitocidals: OR = 5.14 (95%CI = 1.27-20.85) P < 0.003.	Alexander et al., 2001 (40)
Children's Cancer Group. USA, Canada and Australia	64 IAL 81 controls	Maternal exposure to mind-altering drugs: early pre-B ALL with OR = 1.8 (95%CI = 1.0-3.1) P < 0.01.	Wen et al., 2002 (44)
NDSHO. Denmark, Sweden, Norway, Iceland, 1984-1999	3812 AL 10,745 controls	Overall risk of ALL was associated with birth weight (OR = 1.26 per 1 kg increase in birth weight; 95%CI = 1.13-1.41). The association was similar for B- and T-ALL and across all diagnostic ages (0-14 years).	Hjalgrim et al., 2004 (41)
Children's Oncology Group. USA, 1996-2002	240 IAL 255 controls	Maternal consumption of dietary specific DNA topo-II inhibitors associated with AML (*MLL*$^{+ve}$): OR = 1.9 (95%CI = 0.5-7.0); 2.1 (0.6-7.7) and 3.2 (90.9-11.9) comparing 2nd to 4th quartiles.	Spector et al., 2005 (34)
Multicenter Study. France	280 AL 288 controls	Maternal alcohol consumption: LL: OR = 2.0 (95%CI = 1.4-3.0) and AML: OR = 2.6 (95%CI = 1.3-5.8).	Menegaux et al., 2005 (42)
Brazilian Collaborative Study Group of IAL. Brazil, 1999-2005	202 IAL 440 controls	Maternal exposure to estrogens. Dipyrone. Domestic pesticides.	Pombo-de-Oliveira et al., 2006 (45)

These studies were searched through an intensive combination of both MeSH terms and part of the text words and titles. The following terms were used: infant acute leukemia, childhood "leuk(a)emia", risk factor, and leukemia with *MLL* gene rearrangements. AL = acute leukemia; ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; IAL = infant acute leukemia.

"leuk(a)emia", risk factor, and leukemia with *MLL* gene rearrangements.

Ross et al. (43) suggested that maternal alcohol consumption during pregnancy results in an increased risk for infant ALL (OR = 1.43; 95%CI = 1.00-2.04) and for infant AML (OR = 2.64; 95%CI = 1.36-5.06). The mechanism is explained by the ethanol induction of microsomal enzymes, such as cytochrome P450, which subsequently activate pre-carcinogens (43). The same study showed that paternal smoking one month prior to pregnancy was associated with an increased risk (OR = 1.56; 95%CI = 1.03-2.36) of infant ALL. High-birth weights were also shown to be correlated with higher rates of infant ALL and AML. Concordant results were observed within the Brazilian Collaborative Study Group of Infant Acute Leukemia (BCSGIAL), using birth weight information obtained from the questionnaires of 202 IAL cases and 440 controls (45). Since insulin-like growth factor-1 is important in blood formation and regulation and has been shown to stimulate the growth of both myeloid and lymphoid cells in culture, it was postulated that high levels of insulin-like growth factor-1 might produce large babies and contribute to the development of leukemia (46). A maternal history of fetal loss has also been associated with a five- to 12-fold increased risk of developing ALL or AML (47). However, Ross et al. (48) later showed that the relationships between birth weight, prior fetal loss, and risk of infant leukemia appear to be complex, and that only high-birth weight was in fact a significant risk factor of developing childhood leukemia.

An international collaborative study involving patients in Japan, Italy, China and Hong Kong, Greece, Egypt, Chile, and Brazil found the selective link between pregnancy exposures to pesticides, in particular propoxur (a mosquitocidal compound, OR = 9.68; P = 0.003), or consumption during pregnancy of substances such as dipyrone (OR = 5.84; P = 0.001), and IALs with *MLL* gene rearrangements (40). However, in this international study about transplacental chemical exposure and risk of IAL with *MLL* gene fusion, there were a small number of cases molecularly classified and possible confounding factors related to selection of controls in the Brazilian settings. Hence, further analysis was required to confirm these apparent associations. More recently, the BCSGIAL conducted a molecular study combining epidemiological data obtained from interviews with mothers in order to re-evaluate the hypothesis that exposure to pharmaceutical drugs is associated with IAL. The results of that study confirmed reports that mothers exposed to dipyrone and pesticides had an increased chance to give birth to babies with IAL (OR = 1.48; 95%CI = 1.05-2.07 and OR = 2.27; 95%CI = 1.56-3.31, respectively) (45). Furthermore, significant associations with risks for IAL were found, a strong positive association with maternal hormone intake during pregnancy (OR = 9.08; 95%CI = 2.95-27.96). The strong and significant association between IAL and estrogen exposure observed in the BCSGIAL study deserves further investigation. Since a potential role of exogenous estrogens in other cancer was demonstrated in experimental models, and since the metabolite products in estrogen biosynthesis are semiquinones and quinines (49), we speculate that this high association found in the Brazilian series of early infancy acute leukemia could be explained by the same pathway as that of topo-II inhibition caused by metabolite products in estrogen metabolism. On a hopeful note, a recently reported study demonstrated that maternal consumption of fresh vegetables and fruits during pregnancy was associated with a decreased risk of IAL (34).

Gene-environment interaction

Epidemiological and genetic studies support the contention that the *in utero* origin of *MLL* fusion genes in IAL is the result of

transplacental chemical exposures (quinone-based chemicals) (38,40). The probability of the secondary event arising within a short time would be enhanced if the genetic background conferred greater susceptibility of the individual to chemical damage. In this context, constitutive genetic vulnerability may not only act as a predisposing factor for the induction of *MLL* gene fusion but may also increase the risk of the occurrence of further mutations. For most leukemias, and also IALs, multiple genetic polymorphisms of xenobiotic metabolizing enzymes may interact with environmental, dietary, maternal, and other factors to modulate the development of acute leukemia. For example, quinones, which have been shown to cleave both the *MLL* gene and its frequent fusion partner *AF4* at topo-II cleavage sites (50), may be poorly detoxified depending on the activity of NAD(P)H:quinone oxidoreductase 1 (NQO1), an enzyme that detoxifies chemicals with quinone rings including benzene metabolites and flavonoids. The *NQO1* gene is subject to polymorphisms that generate an NQO1 protein with a significantly decreased enzymatic activity. Studies of Caucasian and Japanese patients have shown that the occurrence of alleles conferring low-activity variants of NQO1 was associated with an increased risk of IAL, especially with *MLL/AF4* fusion genes (51,52). Sirma et al. (53) demonstrated that in pediatric ALL without *MLL* rearrangements, the null genotype of the *NQO1* gene is not associated with the etiology of the disease. Recently, in a series of Italian IAL cases, contradictory results were obtained, with this polymorphism appearing to be associated with infant ALL without *MLL* rearrangements, but not with *MLL*-positive infants (54). In the Brazilian series, preliminary results also did not show a significant increased risk (OR = 1.10; 95%CI = 0.62-1.95) of developing leukemia with rearrangements of the *MLL* gene in those individuals with low-activity variants of the NQO1 enzyme (Amorim MR, Silva FA, Emerenciano M, Pombo-de-Oliveira MS, unpublished data).

Polymorphisms of folate-metabolizing enzymes have also been associated with the development of ALL. First, methylenetetrahydrofolate reductase (*MTHFR*) 677C>T gene polymorphism has been linked to a decreased risk of pediatric ALL (55,56). This protective effect may be due to the greater availability of 5,10-methylenetetrahydrofolate and thymidine pools and to an increased fidelity of DNA synthesis. We recently demonstrated interesting effects of MTHFR 677C>T and 1298A>C polymorphisms in a Brazilian childhood series of cases including 62 infants. The results demonstrated a protective role of MTHFR 677C>T polymorphism, linked to a significant 2.18-fold decreased risk of developing ALL, whereas the 1298A>C polymorphism demonstrated a significant 2.01-fold increased risk for ALL in non-white children. It is possible that the opposite roles of 677C>T and 1298A>C polymorphisms in non-white children found in our study result from the different binding sites of MTHFR affected by the polymorphisms (57).

Another example is the inactivating polymorphisms of detoxifying enzymes involved in carcinogen metabolism, such as glutathione S-transferases (GST) that have been associated with the development of ALL (58). A similar study showed the possible role of GST gene polymorphisms in susceptibility to IAL, but the authors analyzed the genotypes of the diseased infants' parents. Surprisingly, they found that the deletion of both the *GSST1* and *GSTM1* genes in either parent might affect the risk of infant leukemia through a pathway independent of the *MLL* gene (59). To investigate whether these polymorphisms represent risk-modifying factors for childhood Brazilian ALL, a study was conducted involving 113 patients and 221 controls with similar ethnic backgrounds. The data revealed that carriers of the rare GSTP1 Val allele were at higher risk of ALL

(OR = 2.7; 95%CI = 1.1-6.8; P = 0.04) (60).

All of these findings need to be extended by larger studies with careful attention to ethnic and geographic diversity in the frequency of polymorphisms, and to socioeconomic status that directly influence the exposures and supplementation features.

Conclusions

Early childhood ALLs and AMLs consistently present *MLL* gene rearrangements, which, besides conferring an adverse outcome, have represented an important molecular diagnosis and monitoring marker, and have been of great importance to identify the unique molecular biological history of this disease. As reviewed here, many molecular features and environmental risk factors have been suggested to cooperate in the development of this malignancy. However, a combination of chances and interactions in the molecular-cellular differentiation pathways is necessary to produce the malignant phenotype. Therefore, continued molecular epidemiological studies are needed to better elucidate the biology of IAL, ultimately leading to an improvement in the present outcome situation.

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Appendix

Members of the *Brazilian Collaborative Study Group of Infant Acute Leukemia* (*BCSGIAL*)

Paulo Ivo C. Araújo[3], Dora Márcia Alencar[4], Silvia R. Brandalise[5], Eni Guimarães Carvalho[6], Virginia M. Coser[7], Imaruí Costa[7], José Carlos Córdoba[8], Mariana Emerenciano[1], Jane Dobbin[1], Maria Célia Moraes Guerra[3], Venâncio Gumes Lopes[4], Isis Q. Magalhães[8], Núbia Mendonça[4], Andrea Gadelha[9], Gilson Guedes[9], Sérgio Koifman[2], Flávia Pimenta[9], Vitória P. Pinheiro[5], Waldir Pereira[7], Maria S. Pombo-de-Oliveira[1], Gilberto Ramos[10], Terezinha J.M. Salles[11], Denise Bousfield da Silva[12], Marcelo P. Land[3], Elaine Sobral[3], Fernando Werneck[13], Carlos Scridelli[14], Luis Gonzaga Tone[14], Lincoln Vermondi[12], Luis Fernando Lopes[15], Wellington Mendes[15].

[1]Centro de Pesquisa, Instituto Nacional de Câncer, Rio de Janeiro, RJ, Brazil
[2]Escola Nacional de Saúde Pública, FIOCRUZ, Rio de Janeiro, RJ, Brazil
[3]Instituto de Pediatria e Puericultura Martagão Gesteira, UFRJ, Rio de Janeiro, RJ, Brazil
[4]Sociedade de Oncologia da Bahia, Salvador, BA, Brazil
[5]Centro Infantil de Investigações Hematológicas Dr. Boldrini, Campinas, SP, Brazil
[6]Hospital Martagão Gesteira, Salvador, BA, Brazil
[7]Departamento de Hematologia, Universidade de Santa Maria, Santa Maria, RS, Brazil
[8]Hospital de Apoio Brasília, Unidade de Onco-Hematologia Pediátrica, Brasília, DF, Brazil
[9]Hospital Napoleão Laureano, João Pessoa, PB, Brazil
[10]Departamento de Pediatria, Faculdade de Medicina, UFMG, Belo Horizonte, MG, Brazil
[11]Hospital Oswaldo Cruz, CEON, Recife, PE, Brazil
[12]Serviço de Oncologia, Hospital Joana de Gusmão, Florianópolis, SC, Brazil
[13]Departamento de Pediatria, Hospital dos Servidores do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil
[14]Departamento de Pediatria, Hospital das Clínicas, Ribeirão Preto, SP, Brazil
[15]Hospital do Câncer A.C. Camargo, São Paulo, SP, Brazil