"The genetic signature of acute leukemia in infacy"

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ABSTRACT

Infant leukemia is a rare malignant disease with clinical and biological features distinct from older children. It is characterized by a high incidence of mixed lineage leukemia (MLL) gene rearrangement and a poor outcome despite intensive chemotherapy. Recent genetic studies argue in favor of a unique biology of infant acute leukemia. This review describes the specific genetic signature of infant leukemia. It discusses the important insights it provides into the understanding of leukemogenesis and reviews the influence of genetics on the prognosis and the treatment of leukemia during infancy.

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The genetic signature of acute leukemia in infancy

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Infant leukemia is a rare malignant disease with clinical and biological features distinct from older children. It is characterized by a high incidence of mixed lineage leukemia (MLL) gene rearrangement and a poor outcome despite intensive chemotherapy. Recent genetic studies argue in favor of a unique biology of infant acute leukemia. This review describes the specific genetic signature of infant leukemia. It discusses the important insights it provides into the understanding of leukemogenesis and reviews the influence of genetics on the prognosis and the treatment of leukemia during infancy.

Leukemia is the most frequent neoplastic disease in childhood. It makes up one third of the pediatric cancers. Its incidence peaks between 2 and 4 years corresponding to the high incidence of acute lymphoblastic leukemia (ALL) at these ages.1 But it occurs also in children younger than 1 year and has been described in neonates and in fetus.2-4 Infant leukemia represents 5 to 10% of the overall pediatric leukemias. In this group of patients, leukemia has clinical and biological features distinct from older children. First, infants with acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) have high white blood cell (WBC) counts and frequent extramedullary manifestations such as hepatosplenomegaly, leukemia cutis and CNS infiltration. Second, infant ALL and AML share phenotypic characteristics suggesting a common origin in a progenitor not fully committed to lymphoid or myeloid differentiation. At this age, ALL typically have an early B-cell precursor immunophenotype lacking the common CD10 antigen but co-expressing myeloid and monocytic associated antigens such as CD15, CD65, CD4 and myeloperoxidase. The chondroitin sulfate proteo-glycan neural-glial antigen-2 (NG2) is also relatively, though not absolutely, expressed1. Infant AML includes most frequently the French-American-British (FAB) M4/M5 (myelomonocytic/monoblastic) and M7 (megakaryoblastic) types and is slightly more frequent than ALL. During the first year of life, the incidence of ALL and AML are 19.7 and 20.5 cases per million infants, respectively.5,6 Third, infant leukemia, including ALL and AML, has a typical genetic signature characterized by a high incidence of mixed lineage leukemia (MLL) gene rearrangement. In this article, we focus on the new developments and discoveries concerning this particular signature, we discuss the important insights it provides into the understanding of leukemogenesis and we review its influence on the prognosis and the treatment of this disease in infancy.

The genetic alterations in infant leukemia

The major genetic abnormalities observed in both infant ALL and AML are summarized in Table 1. Rearrangement of MLL gene is the most common genetic finding in infant leukemia. This gene is located at cytogenetic band 11q23 and is rearranged in 80% of infants with ALL and 50 to 60% with AML compared to 2-8% and 10-20% respectively in older children.3-8 More than 100 different MLL rearrangements (MLL-R) have been reported but in infant leukemia they consist mostly in reciprocal translocation. No internal partial duplication has been reported as described in adult AML.8,9 Nearly all the MLL translocations lead to the fusion of the N-terminal part of MLL in frame to the C terminal region of a wide variety of partner genes. At least 64 partner genes have been characterized in both pediatric and adult leukemia. In infant, the most frequent are AFF1/AF4 (4q21), MLLT1/ENL (19p13.3), MLLT3/AF9 (9p22) and to a lesser extend MLLT10/AF10 (10p32).5,10 MLL translocations are usually detected by conventional cytogenetics. However, since this technique failed to detect 16 to 40% of MLL-R and since the spectrum of potential partner gene is broad, fluorescent in situ hybridization (FISH) is considered to be the best method for MLL translocation screening whatever the partner gene.11 An alternative strategy consists in the screening by RT-PCR for the most frequent fusion transcripts (MLL-AF4 in ALL, MLL-AF9, MLL-ENL and MLL-AF10 in AML) although this may fail to identify infrequent chimeric transcript. Once the partner is identified either by cytogenetics or by FISH with specific probes, quantitative RT-PCR is useful for the follow-up of the minimal residual disease.12,13

The MLL gene encodes a large nuclear protein that is widely expressed and plays a critical role for normal mammalian development and hematopoietic differentiation. MLL protein acts as a transcription regulator of genes including members of the HOX family. Recent studies have demonstrated that MLL has other regulatory functions. It is incorporated into a large macromolecular complex involved in chromatin modification which is supposed to be recruited by transcription factors in order to initiate RNA synthesis.14,15 The structure and the function of...
MLL protein is illustrated in Figure 1. The highly conserved C-terminal SET domain acts as a histone methyltransferase that methylate histone H3 at lysine 4 (H3K4) around the transcription start site of most of the transcribed genes (Figure 1A). The transactivating domain (TAD) may exhibit a MOF-mediated histone acetyltransferase activity specific acetylation of histone 4 at lysine 16 (H4K16). In rearranged MLL, breakpoints are clustered within an 8.3kb region spanning exons 8 to 14 which contains a number of recombination-prone sequences. In the chimeric fusion proteins, the methyltransferase activity of the SET domain of MLL is lost but the fusion partner provides novel biological properties that confer a new role in oncogenesis. The characterized partner genes encode a large variety of proteins whose contribution in the leukemogenic process is not elucidated yet. Several mechanisms including histone methylation catalyzed by DOT1L and associated with RNA polymerase II phosphorylation, histone acetylation, histone methylation mediated by SAM68 and MLL dimerisation have been suggested to explain the transcriptional activation induced by MLL rearrangement. In infant acute leukemia, the most frequent partner genes (AF4, AF9, ENL, ELL, AF10) encode nuclear proteins that belong to the same network involved in chromatin remodeling through histone methylation (Figure 1B). In these cases, MLL-fusion proteins recruit another histone methyltransferase (DOT1L) within a complex of proteins to induce the accessibility of chromatin at inappropriate loci and to stimulate constitutive the elongation phase of transcription by phosphorylating RNA polymerase II. This results in an excessive transcription of target genes including the clustered breakpoints in MLL between CxxC and PHD motifs; orange arrow: the large MLL protein is cut by a protease into a N-terminal 320 kDa fragment and a C-terminal 180 kDa moiety that are both core components of MLL complex; AT: DNA binding on minor groove is mediated by the 3 AT-hooks located at the N-terminal of MLL; NLS: 2 nuclear localisation signals; CxxC: this motif recognizing unmethylated CpG dinucleotides is important for target gene selection and recruit repressive factors such as histone deacetylase; PHD: the 4 plant homeodomain fingers are involved in chromatin-mediated transcriptional regulation; TAD: the transactivation domain is involved in acetylating H4 histone at lysine 16 (H4K16); SET: this domain located at the C terminal part of MLL is a histone methyltransferase active site methylating H3 histone at lysine 4 (H3K4); NLS: 2 nuclear localisation signals; CxxC: this motif recognizing unmethylated CpG dinucleotides is important for target gene selection and recruit repressive factors such as histone deacetylase; PHD: the 4 plant homeodomain fingers are involved in chromatin-mediated transcriptional regulation; TAD: the transactivation domain is involved in acetylating H4 histone at lysine 16 (H4K16); SET: this domain located at the C terminal part of MLL is a histone methyltransferase active site methylating H3 histone at lysine 4 (H3K4); bcr: clustered breakpoints in MLL between CxxC and PHD motifs; orange arrow: the large MLL protein is cut by a protease into a N-terminal 320 kDa fragment and a C-terminal 180 kDa moiety that are both core components of the MLL complex. The recruitment of the MLL complex by transcription factors allows the initiation of RNA synthesis of genes, such as HOX, which play a major role in mammalian development and hematopoietic differentiation. MLL-fusion complex with the most frequent nuclear partners involved in infant acute leukemias: The ENL, AF9, AF4, ELL, AF10 are members of a complex that combines 2 activities: histone methyltransferase catalyzed by DOT1L; transcription elongation stimulation by pTEFb which induces phosphorylation of RNA polymerase II. The resulting constitutive activation of target genes favors inhibition of hematopoietic differentiation.

Figure 1 (adapted from ref. 14,15). 1A: Germline MLL complex: AT: DNA binding on minor groove is mediated by the 3 AT-hooks located at the N-terminus of MLL; NLS: 2 nuclear localisation signals; CxxC: this motif recognizing unmethylated CpG dinucleotides is important for target gene selection and recruit repressive factors such as histone deacetylase; PHD: the 4 plant homeodomain fingers are involved in chromatin-mediated transcriptional regulation; TAD: the transactivation domain is involved in acetylating H4 histone at lysine 16 (H4K16); SET: this domain located at the C terminal part of MLL is a histone methyltransferase active site methylating H3 histone at lysine 4 (H3K4); bcr: clustered breakpoints in MLL between CxxC and PHD motifs; orange arrow: the large MLL protein is cut by a protease into a N-terminal 320 kDa fragment and a C-terminal 180 kDa moiety that are both core components of the MLL complex. The recruitment of the MLL complex by transcription factors allows the initiation of RNA synthesis of genes, such as HOX, which play a major role in mammalian development and hematopoietic differentiation. 1B: MLL-fusion complex with the most frequent nuclear partners involved in infant acute leukemias: The ENL, AF9, AF4, ELL, AF10 are members of a complex that combines 2 activities: histone methyltransferase catalyzed by DOT1L; transcription elongation stimulation by pTEFb which induces phosphorylation of RNA polymerase II. The resulting constitutive activation of target genes favors inhibition of hematopoietic differentiation.
Table 1. Genetic findings in infant ALL and AML: review of the literature

<table>
<thead>
<tr>
<th>Chromosomal alterations</th>
<th>Involved genes</th>
<th>Incidence in ALL (ref.)</th>
<th>Incidence in AML (ref.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MLL rearrangements</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(4;11)(q21;q23)</td>
<td>MLL-AFF1(AF4)</td>
<td>44-70% (1, 47)</td>
<td>&lt;5% (1, 47)</td>
</tr>
<tr>
<td>t(11;19)(q23;p13)</td>
<td>MLL-MLLT1(ENL)</td>
<td>12-25% (1, 47)</td>
<td>12% (47)</td>
</tr>
<tr>
<td>t(11;19)(q23;p13.1)</td>
<td>MLL-ELL</td>
<td>0</td>
<td>3% (78)</td>
</tr>
<tr>
<td>t(9;11)(p22;q23)</td>
<td>MLL-MLLT3(AF9)</td>
<td>4-12% (1, 47)</td>
<td>50% (47)</td>
</tr>
<tr>
<td>t(10;11)(p12;q23)</td>
<td>MLL-MLLT10(AF10)</td>
<td>7.5% (47)</td>
<td>15% (47)</td>
</tr>
<tr>
<td>Other 11q23</td>
<td></td>
<td>7.5% (47)</td>
<td>15% (47)</td>
</tr>
<tr>
<td>inv(11)(p15q23)</td>
<td></td>
<td>+ (79)</td>
<td>0</td>
</tr>
<tr>
<td>t(1;11)(p32;q23)</td>
<td></td>
<td>+ (47, 79)</td>
<td>0</td>
</tr>
<tr>
<td>t(11;17)(q23;p13)</td>
<td></td>
<td>1 CR (47)</td>
<td>0</td>
</tr>
<tr>
<td>t(11;22)(q23;q11)</td>
<td></td>
<td>0</td>
<td>+ (80)</td>
</tr>
<tr>
<td>del(11)(q23)</td>
<td></td>
<td>+ (47, 79)</td>
<td>0</td>
</tr>
<tr>
<td>dup(11)(q11q23)</td>
<td></td>
<td>1 CR (81)</td>
<td></td>
</tr>
<tr>
<td><strong>Germline MLL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High hyperdiploidy</td>
<td></td>
<td>5% (47)</td>
<td>0</td>
</tr>
<tr>
<td>del(9p)</td>
<td></td>
<td>3% (47)</td>
<td>0</td>
</tr>
<tr>
<td>der(11q23)</td>
<td></td>
<td>2% (47)</td>
<td>4% (47)</td>
</tr>
<tr>
<td>t(7;12)(q32;p13)</td>
<td></td>
<td>2% (47)</td>
<td>5-28%</td>
</tr>
<tr>
<td>t(1;22)(p13;q13)</td>
<td></td>
<td>0</td>
<td>2% (47)</td>
</tr>
<tr>
<td>+8</td>
<td></td>
<td>1 CR (47)</td>
<td>7% (47)</td>
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<tr>
<td>t(8;16)(p11;p13)</td>
<td></td>
<td>0</td>
<td>3%</td>
</tr>
<tr>
<td>inv(16)(p13;q22)</td>
<td></td>
<td>0</td>
<td>2% (47)</td>
</tr>
<tr>
<td>-7</td>
<td></td>
<td>1 CR (47)</td>
<td>+ (1)</td>
</tr>
<tr>
<td>del(5q)</td>
<td></td>
<td>0</td>
<td>1 CR (47) (38)</td>
</tr>
<tr>
<td>inv(8)(p11q11)</td>
<td></td>
<td>0</td>
<td>1 CR (47) (38)</td>
</tr>
<tr>
<td>t(1;19)(q23;p13)</td>
<td></td>
<td>1 CR (47) (38)</td>
<td>0</td>
</tr>
<tr>
<td>dic(9;20)(p11→13q11)</td>
<td></td>
<td>1 CR (47) (38)</td>
<td>0</td>
</tr>
<tr>
<td>t(5;15)(p15q11-13)</td>
<td></td>
<td>+ (78) (69)</td>
<td>0</td>
</tr>
<tr>
<td>Normal karyotype</td>
<td></td>
<td>9%</td>
<td>25%</td>
</tr>
</tbody>
</table>

1 CR: one case report; +: more than one case report without indication of the incidence.

ALL induces an expression pattern that is clearly distinguishable from other ALL subtypes as well as from AML. Recently, in samples of MLL-R leukemias, Zangrando et al. identified a specific gene expression signature, characterized notably by MEIS1 overexpression, that is independent of the lineage of origin and reinforces the hypothesis of a common genetic deregulation in MLL-R infant AML and ALL. Besides a core signature shared by all MLL-R infant ALL, each of the 3 main subtypes of MLL translocation (with AF4, ENL, AF9) harbors specific biologic modifications: they are associated to particular gene expression profiles and distinct genome-wide promoter methylation patterns. Likewise, infant ALL with t(4;11) has a genetic profile distinct from non infant ALL with t(4;11). Independently to the MLL signature, several studies argue in favor of an unique biology of infant leukemia compared to pediatric leukemia of the same phenotype, reflecting the influence of very young age. Despite significant differences, the gene expression pattern of infant ALL with germline MLL (MLL-G) is closer to MLL-R infant ALL than to the MLL-G precursor B ALL of older children. Similarly, most of the infant AML display a distinct gene expression in comparison to older children with AML which may explain why the presence of MLL gene rearrangement failed to correlate with treatment response in infant AML.
with the microarray analyses and suggest that leukemia originates in a stem cell still not fully committed to lymphoid or myeloid differentiation. The favorable response of infant ALL to hybrid treatment protocols that include AML regimens has reinforced this assumption. A recent genetic study has opened interesting perspective about this issue. This analysis detected MLL-AF4 fusion gene in bone marrow mesenchymal stem cells of all tested samples from infant with MLL-AF4 ALL.\(^ {27}\) The absence of polyplody and immunoglobulin monoclonal rearrangement in these MLL-AF4 positive mesenchymal cells excludes cell fusion plasticity or de-differentiation of ALL leukemic cells. This observation was consistent with the previous identification of BCR-ABL fusion gene in endothelial cells derived from chronic myelogenous lymphoma. It suggests that the first leukemogenic hit might arise very early in the stem cell hierarchy targeting a bipotential precursor for endothelial and hematopoietic cells and it sustains a potential role for bone marrow microenvironment in infant leukemia.

The development of leukemia as early as in fetus, neonates and infant is intriguing. It has been clearly demonstrated that early step of neoplastic transformation such as chromosome translocation, and in particular MLL-R, occurs \textit{in utero} in most infant ALL. This was initially suspected because of the very high concordance rate of leukemia observed between identical twins. In older children, this rate was estimated to 10% but in infant monozygotic twins it was, until recently, thought to be close to 100%.\(^ {28, 29}\) This observation suggested that leukemia originates \textit{in utero} in one twin and is subsequently transmitted to the second twin through conjoined circulation. The identification of MLL translocation in cord blood samples of aborted fetuses and neonates with congenital leukemia as well as in archived neonatal blood spots of infants who subsequently developed leukemia confirmed the prenatal origin of infant leukemia.\(^ {28}\) However, animal models of leukemogenesis induced by MLL have demonstrated that MLL-R is not, in itself, sufficient.\(^ {17}\) This multistep process was first admitted in older children. Preleukemic clone with chromosomal translocation such as \(t(12;21)\) is often isolated in the cord blood of these patient but additional genetic events are necessary to induce leukemia that arise several years latter.\(^ {28-30}\) The fact that these secondary abnormalities are distinct within twin pairs confirms that they occur mostly after birth.\(^ {30}\) Their role in infant leukemia has been recently confirmed by the report of the first discordance in monozygotic twins where one twin developed a MLL-R ALL at 9 months of age and the second showed a spontaneous clearance of preleukemic clone with MLL-R.\(^ {31}\) Nevertheless, \textit{infant leukemia contrasts with other pediatric leukemia} by its short latency. This rapid transformation of preleukemic cells with MLL rearrangement suggests an increased susceptibility to mutagenic influences that favor accumulation of secondary genetic events even \textit{in utero}. This latter is supported by some experiments demonstrating an inhibition of p53-mediated responses to DNA damage.\(^ {32}\)

Infant leukemia raises also the question of causative factors and in particular the contribution of environmental factors that are attributed to parental exposure prior or after conception as well as genetic constitutional factors in leukemogenesis. The role of inherited mutations is relatively small. Infant leukemia, mainly AML, has an increased incidence in several constitutional syndromes such as Down, Noonan, neurofibromatosis-1, and to a lesser extend, Turner syndrome and trisomy 9. However, most of the time, it occurs in patients with no obvious predisposing condition. In epidemiologic studies, it has been associated with more or less evidence to prior miscarriage, higher birth order, high birth weight, exposure to radiation, maternal (consumption of alcohol, marijuana, mind-altering drug, antihistamine, metronidazole, estrogen, dipyrone analgesic, insecticide, herbal medicine) and paternal exposures (smoking, pesticide exposure, mind-altering drug use) (for review see (1)). Important clues about potential environmental factors and the multi-step pathogenesis of infant leukemia have been provided by the comparison between infant leukemia and therapy-related AML associated with MLL-R rearrangement (MLL-R t-AML). Both share similarities: short latency, expression of myeloid markers and similar clustering of MLL genomic breakpoints in the telomeric region. MLL-R t-AML usually follows treatment with cytotoxic agents that inhibit the DNA topoisomerase II and are, therefore, considered as mutagens in the induction of MLL rearrangement.\(^ {9}\) Other agents such as quinolon antibiotics, caffeine, flavonoids, quercetin (contained in fruits and vegetables) catechins (tea, cocoa and wine), podophylline resin, insecticides and benzene metabolites and estrogens are able to inhibit topoisomerase II. Although neither the infants nor their parents are likely exposed to chemotherapy, \textit{an increased maternal consumption of foods containing those environmental DNA topoisomerase II inhibitors} has been correlated to an increased risk of MLL-R AML but not of MLL-G AML and both MLL-R and MLL-G AML in infant.\(^ {33}\) In addition, some constitutional pharmacogenetic factors might modulate the sensitivity to these compounds and other potential environmental mutagens for developing acute leukemia. MLL-R leukemia has been associated to genetic polymorphisms that confer low activity of NAD(P)H quinone oxidoreductase (NQO1), an enzyme involved in the detoxification and metabolism of many topoisomerase II-inhibitors.\(^ {34, 35}\) Similarly prenatal folic supplementation and genetic polymorphism conferring low activity of methyltetrahydrofolate reductase (MTHFR) seems to reduce the risk of MLL-R leukemia.\(^ {36}\) Moreover, a high incidence of deletion in the glutathione-S-transferase (GST) genes that encode enzymes involved in the detoxification has been detected in either parents of infant with MLL-G leukemia. This suggests that the effect of prenatal exposure to carcinogens may also be modulated by parental pharmacogenetic factors and that these factors may affect the risk of infant leukemia through a pathway independent of the MLL gene.\(^ {37}\)

**Impact on prognosis**

The influence of genetics on the prognosis of infant leukemia depends mainly on the presence of MLL-R and is different in ALL and AML. The 5-year event free survival (EFS) for infants with ALL is the worst of all children with ALL apart from those with \(t(9;22)\) and near-haploidy and is in the order of 35-50%, 6, 38, 39 The major predictive factors for poor outcome include age younger than 6 months, very high WBC, absence of CD10 expression,
poor response to initial corticosteroid therapy and the presence of MLL-R. It is noteworthy that these characteristics are closely interrelated and their presence is inversely related to the age of the infant. The recent Interfant-99 study that enrolled 482 infants with ALL of which 79% had a MLL-R, showed a 4-year event-free survival (EFS) for patients with MLL-R of 36.9% compared to 74.1% for those with MLL-G. The effect of specific fusion genes on prognosis remains unclear. A large retrospective analysis in ALL with various 11q23 abnormalities, showed no significant difference in outcome among cytogenetic subgroups including the t(4;11), t(11;19), t(9;11) and other 11q23 rearrangements in infants whereas in older children, t(4;11) and t(9;11) were associated with a worse outcome than were other rare 11q23 changes. Similarly, the Interfant-99 study did not find a significant difference in the outcome of the infants within the different subgroups. However, the CCG-1953 study revealed a better outcome in infants with other 11q23 rearrangements compared to those with t(4;11), t(11;19) and t(9;11) although no significant difference was found in the 5-year EFS among these latter subgroups. Recently, in the P9407, a pilot study of the Children Oncology Group (COG) testing the utility of intensified induction therapy, 202 infants with ALL were analyzed. The 5-year EFS varied significantly depending on the partner gene and was 29 +/- 10% for ENL, 34.2 +/- 7.4% for AF4, 45 +/- 14.9% for other partner genes, 67.7 +/- 17.2% for AF9 and 66.2 +/- 9.3% for MLL-G. This negative effect of ENL and AF4 was even larger in the infants less than 90 days of age.

The outcome of AML is poorer than ALL in pediatrics. In infant as in older children, it is characterized by a much lower relapse rate and a 4-year EFS of approximately 60%. The prognostic factors of infant AML are not clearly defined: the only reproducible factor associated with a poor prognosis is the high WBC (> 50 10^9/L) at diagnosis. The effect of young age and of MLL-R is not obvious. In a series of 57 infants with AML, the 4-year EFS was 56%, 55% and 64% in patients with normal cytogenetics, 11q23 abnormalities and other abnormalities, respectively. Several studies have confirmed that MLL-R does not worsen the prognosis of AML in infants and in older children. Interestingly, some but not all studies have found that the presence of t(9;11) was associated with a better outcome in infant AML as it has been claimed for older patients.

Impact on treatment

Genetics may guide the treatment of infant leukemia at three levels: (i) it may be an indicator of drug sensitivity, (ii) as factor of prognosis it may suggest an adaptation of treatment intensity, (iii) and it may lead to the identification of novel therapeutic targets.

Drug resistance and sensitivity

Drug sensitivity is a major problem in the treatment of infant leukemia and in particular in infant ALL. In vitro, infant lymphoblasts are significantly more resistant than cells from older children to prednisolone and L-asparaginase but are more sensitive to cytarabine. Leukemic cells with MLL-R are characterized by an overexpression of MCL-1, an anti-apoptotic member of the BCL-2 family which has been associated to prednisolone resistance. Some studies found that the high sensitivity to cytarabine was related to the presence of 11q23 rearrangement although others did not confirm this association. A potential explanation is the increased expression of the equilibrative nucleoside transporter 1 (hENT1) which transports cytarabine across cell membrane in infant ALL cells and in particular in MLL-R cells. Lack of expression of CD10 in preB ALL cells also correlates with this sensitivity pattern and, in vivo, intensification of post-remission therapy with high dose cytarabine has improved outcome in small numbers of adults and infants. These results associated to the frequent coexpression of myeloid markers in infant ALL, have led to the development of hybrid protocols for the treatment of infants with ALL. The interfant-99 protocol consisted of a standard ALL induction with the addition of low-dose cytarabine, a consolidation with high-dose cytarabine and high-dose methotrexate, a hybrid reinduction including elements of both ALL and AML treatment and a maintenance therapy. The overall 4-year EFS and survival for this study were respectively 47% and 55.3% and were better than those achieved with most previous protocols. This improvement in outcome was especially remarkable for the high-risk patients who responded poorly to a prednisone prophase. Their EFS was 30% compared with 15% for those treated with protocols based on Berlin-Frankfurt-Münster (BFM) regimens. The current interfant-06 study stratifies risk according to previously described factors including the presence of MLL-R. Since it has no clear effect on prognosis, the presence of MLL-R does not influence the treatment of infant AML. Infants with AML are currently treated on standard children protocols as they generally have prognosis similar to that of older children with AML. These typically include anthracycline (daunorubicin, doxorubicin, mitoxantrone), high-dose cytarabine, etoposide and 6-thioguanine.

Intensification and hematopoietic stem cell transplant

Whether MLL rearrangement should lead to intensified therapy with hematopoietic stem cell transplant (HSCT) or not remains controversial. In infant ALL, small uncontrolled studies have suggested a benefit for HSCT in patients with MLL-R. In one of the most promising series, Sander et al. reported 11 of 14 infants with ALL and MLL-R treated by HSCT in first complete remission with a 3-year disease-free survival (DFS) of 73%. However, the combined analysis of a larger prospective study, the POG-9407/CCG-1953, failed to demonstrate a survival benefit for transplant with a 5-year EFS of 51 and 49% for infants with and without transplant, respectively. The JILSG MLL98 reported
a posttransplant EFS in the same range (55%). In high-risk patients, the Interfant-99 found a 4-year-DFS of 50% in infants with HSCT compared with 37% DFS in those with chemotherapy alone. But the number of patients was too limited to reach a statistical significance. The major concern in the analysis of these studies is the variability of the conditioning regimens and the donor source. This makes difficult to evaluate the true benefit of a particular transplant approach. For these reasons, the Interfant-06 is currently testing the interest of HSCT in selected high-risk patients. Studies assessing the contribution of HSCT on large series of infants with AML are lacking. In 15 infants with AML, Marco et al. have reported a 5-year EFS of 71% after HSCT which was similar to the 72% 5-year EFS of the JIIaSG study where 83% of the infants were treated with chemotherapy alone. These results suggest that MLL-R should not be taken into consideration in the decision of undertake a HSCT in AML. So far, no general recommendation has been admitted and the role of HSCT for AML in infants as in older children has to be evaluated in each study group according to the outcome levels of chemotherapy.

**Novel therapies**

The intensification of chemotherapy and the use of hybrid regimens combining ALL and AML active drugs have slightly increased the survival of infants with leukemia. However, further significant improvements in the outcome of infants with leukemia will require novel therapies targeting leukemic cells with MLL-R. Given the apparent sensitivity of infant ALL cells to nucleoside analogues, newly developed agents such as cefotarabine and troxacidabine may be interesting candidate drugs for further testing on MLL-R cells. In addition, some nucleoside analogues such as 5-aza-2'-deoxycytidine (decitabine) and zebularine that also inhibit DNA methylation, are promising in the treatment of MLL-R ALL. MLL-R leukemic cells have an abnormally high index of DNA methylation that leads to gene silencing and seems to be correlated to poor outcome. In particular, the tumor suppressor gene FHIT is silenced in these cells by 5’CpG methylation. Stamm et al observed that the exposition to the demethylating agent decitabine resulted in re-expression of the FHIT protein and induced apoptosis. Similarly, treatment with zebularine reversed aberrant DNA methylation and effectively induces apoptosis in MLL-R ALL cells.

As the response to prednisone largely determines the clinical outcome of pediatric ALL patients, overcoming resistance to this drug may be an important step towards improving prognosis. Although it is not the sole mechanism, MCL-1 overexpression in infant ALL cells with MLL-R plays a role in this resistance. Recently, it has been shown that down-regulation of these gene by RNA interference led to prednisolone sensitization. Therefore, the use of synthetic MCL-1 inhibitors, i.e. Seliciclib and R-etodolac should be considered in the treatment of MLL-R ALL.

Flt3 is another relevant target in the treatment of AML and MLL-R ALL. Although they are rare in pediatric ALL, activating mutations of the FLT3 gene are detected in 20-30% of pediatric and adult AML and 15-20% of MLL-R ALL. Several small molecule inhibitors such as PKC412 and CEP-701 have been shown to inactivate FLT3 and to induce apoptosis in vitro and MLL-R ALL cells. Furthermore, Flt3 inhibitors display synergistic cytotoxic effect with chemotherapy in infant MLL-R ALL samples. Altogether, these results strongly support the design of clinical trials with available FLT3 inhibitors. The next COG Infant ALL trial tests the benefits of adding lestaurtinib (CEP-701) to POG-9407 treatment in MLL-R infants (AALL0631).

Other targets and pathway have also been considered in the therapeutic approach of infant leukemia. These include the HOX, Menin or Ephrin/EphA7/ERK pathways, the TRAIL and Fas death receptors, and adult AML and 15-20% of MLL-R ALL with HSCT which was similar to the 72% 5-year EFS of the JIIaSG. Other targets and pathway have also been considered in the therapeutic approach of infant leukemia. These include the HOX, Menin or Ephrin/EphA7/ERK pathways, the TRAIL and Fas death receptors, and the TRAIL and Fas death receptors.

**Conclusion**

Despite dramatic improvement in older children, the outcome of leukemia remains poor in infant. The clinical, biological and genetic features of infant leukemia suggest that it has an unique biology where MLL rearrangement is a key element. A deeper understanding of leukemogenesis at this age has already allowed substantial progress and will provide new perspectives in the prevention and in the therapeutic approach of infant leukemia. By analogy to secondary leukemia with MLL-R, it has been shown that environmental DNA topoisomerase II inhibitors present in particular fruits, vegetables, drinks and chemical might promote the development of leukemia. There is growing evidence that pharmacogenetic factors in the child or in his parents might also modulate the risk of infant leukemia through pathways depending or not on the MLL gene. The positive effect of hybrid chemotherapy regimens and the co-expression of myeloid and lymphoid markers often associated to MLL-R question about therapeutic stratification of infant leukemias based on their genetic profile rather than on the usual ALL/AML distinction. Ultimately, this should lead to innovative treatments targeting MLL-R and/or associated genetic abnormalities such as FLT3 activation, MCL-1 overexpression and excess of DNA methylation.

References


8 Hem Onc Volume 1 Issue 1


