

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-glia 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.^{1,5} 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.^{1,6,7} 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 extent *MLLT10/AF10* (10p32).^{6,10} *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

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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).¹⁶ In rearranged *MLL*, breakpoints are clustered within an 8.3kb region spanning exons 8 to 14 which contains a number of recombination-prone sequences.⁸ 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.¹⁵ 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 constitutively the elongation phase of transcription by phosphorylating the RNA polymerase II. This results in an excessive transcription of target genes including the clustered *HOX* loci and the HOX-dimerization partner *MEIS1*, and in a subsequent blockage in the differentiation of hematopoietic precursors.¹⁴ In addition, mice

models of leukemogenesis induced by *MLL-AF4* suggest that the oncogenicity of this fusion protein is not sufficient and that secondary events are required to induce leukemia. Consistently with this hypothesis, the activation of the Fms-like tyrosine kinase 3 (*FLT3*) gene has been proposed as secondary synergistic event since mutations in its tyrosine kinase domain have been detected in approximately 15% of *de novo* acute leukemias with *MLL* translocation.¹⁷

Beside *MLL-R*, other classical chromosome alterations are rarely found in infant leukemia (**Table 1**). These include hyperdiploidy, t(1;19)(q23;p13) in ALL and inv(16)(p13q22) in AML M4 associated with abnormal eosinophils.^{18, 19} Unusual translocations such as t(1;22)(p13;q13) and t(8;16)(p11;p13) have been also identified in AML. The t(7;12)(q36;p13) is a new recurrent translocation that has been recently reported in infant acute leukemia, mainly myeloid. It involves the *ETV6/TEL* gene (12p13) and the neighborhood of the *MNX1/HLXB9* gene. It is associated with an ectopic expression of *MNX1* and an adverse outcome in most cases but its role in leukemogenesis remains unclear. Other chromosomal imbalances considered as high risk alterations (monosomy 7, del(7q) del(5q), +8) are also reported in AML or myelodysplasia with excess of blasts.^{20, 21}

Insights from the unique nature of infant leukemia

Infant leukemia has clinical and biological specificities that raises multiple questions to which genetics brings informative perspectives. Through the recently developed high resolution arrays, gene expression profiling studies have provided complementary information into the biology of infant leukemias with *MLL-R*. Multiple analyses demonstrated that *MLL-R* in infant

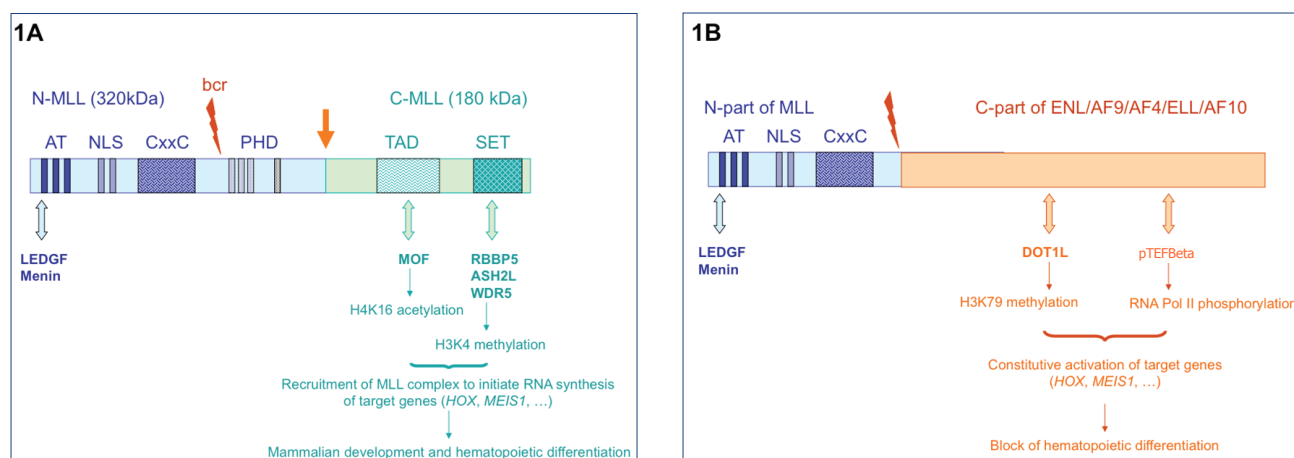


Figure 1 (adapted from (ref. 14, 15)). 1A: Germline MLL complex: AT: DNA binding on minor groove is mediated by the 3 AT-hooks located at the N-terminus of MLL; NLS: 2 nuclear localisation signals; CxxC: this motif recognizing unmethylated CpG dinucleotides is important for target gene selection and 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 H3K79 methyltransferase activity 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

Chromosomal alterations	Involved genes	Incidence in ALL (ref.)	Incidence in AML (ref.)
MLL rearrangements		63-90% (1, 47)	35-66% (1, 47)
t(4;11)(q21;q23)	MLL-AFF1(AF4)	44-70% (1, 47)	<5% (1, 47)
t(11;19)(q23;p13)		12-25% (47)	15% (47)
t(11;19)(q23;p13.3)	MLL-MLLT1(ENL)	12-25% (1, 47)	12% (47)
t(11;19)(q23;p13.1)	MLL-ELL	0	3% (78)
t(9;11)(p22;q23)	MLL-MLLT3(AF9)	4-12% (1, 47)	50% (47)
t(10;11)(p12;q23)	MLL-MLLT10(AF10)	7.5% (47)	15% (47)
Other 11q23		7.5% (47)	15% (47)
inv(11)(p15q23)		+ (79)	0
t(1;11)(p32;q23)		+ (47, 79)	0
t(11;17)(q23;p13)		1 CR (47)	0
t(11;22)(q23;q11)		0	+ (80)
del(11)(q23)		+ (47, 79)	0
dup(11)(q11q23)		1 CR (81)	
Germline MLL		10-37% (1, 47)	34-65% (1, 47)
High hyperdiploidy		5% (47)	0
del(9p)		3% (47)	0
der(X)		2%	5%
t(7;12)(q32;p13)		2% (47)	4% (47)
t(1;22)(p13;q13)		0	5-28%
+8		1 CR (47)	7% (47)
t(8;16)(p11;p13)		0	3%
inv(16)(p13;q22)		0	2% (47)
-7		1 CR (47)	+ (1)
del(5q)		0	1 CR (47) (38)
inv(8)(p11q1)		0	1 CR (47) (38)
t(1;19)(q23;p13)		1 CR (47) (38)	0
dic(9;20)(p11~13;q11)		1 CR (47) (38)	0
t(5;15)(p15;q11-13)		+ (78) (69)	0
Normal karyotype		9%	25%

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.²² 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.¹⁹ 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.^{23, 24} Likewise, infant ALL with t(4;11) has a genetic profile distinct from non infant ALL with t(4;11).²⁵ 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.²³ 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.²⁶

The cellular origin of leukemia, especially when associated to *MLL* rearrangement, is regularly questioned in infant. The common phenotypic features of both ALL and AML are consistent

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 polyploidy 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 bipotent 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 *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 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 later.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, 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 *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, dipyron 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.⁶ 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, **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* ALL 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 folate 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.^{12, 13, 40} 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.⁴⁰ 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.⁴¹ Similarly, the Interfant-99 study did not find a significant difference in the outcome of the infants within the different subgroups.⁴⁰ 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.¹² 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.⁴²

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 (> 50 10⁹ /L) at diagnosis.^{43, 44} The effect of young age and of *MLL*-R is not obvious.⁴³⁻⁴⁶ 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.⁴⁷ Several studies have confirmed that *MLL*-R does not worsen the prognosis of AML in infants and in older children.^{43, 44, 48, 49} 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 (children and adults).^{44, 47, 50} The impact of other cytogenetic factors usually associated with favorable (inversion of chromosome 16, t(8;21), t(15;17)) or unfavorable prognosis (monosomy 7, deletion of the long arm of chromosome 5, highly complex karyotypes) in pediatric and adult AML is not clearly determined in infant because of the small number of cases. The limited results that are available suggest that cytogenetics may be less predictive of outcome in infants than in older children and adults with AML.^{6, 47}

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.⁵¹ 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.⁵² Some studies found that the high sensitivity to cytarabine was related to the presence of 11q23 rearrangement although others did not confirm this association.^{53, 54} 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.⁵⁵ 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.^{56, 57} 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.⁴⁰ 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.⁵⁸ 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.¹ These typically include anthracycline (daunorubicine, doxorubicine, 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.^{59, 60} 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%.⁵⁹ 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.⁶¹ The JILSG MLL98 reported

a posttransplant EFS in the same range (55%).⁶² 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.⁴⁰ 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 JILSG study where 83% of the infants were treated with chemotherapy alone.^{49, 63} 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.⁶⁴

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.^{40, 49} 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 clofarabine and troxacidabine may be interesting candidate drugs for further testing on *MLL*-R cells.^{65, 66} 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.^{24, 67} In particular, the tumor suppressor gene *FHIT* is silenced in these cells by 5'CpG methylation. Stam *et al* observed that the exposition to the demethylating agent decitabine resulted in re-expression of the *FHIT* protein and induced apoptosis.⁶⁸ Similarly, treatment with zebularine reversed aberrant DNA methylation and effectively induces apoptosis in *MLL*-R ALL cells.²⁴

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.⁵² Therefore, the use of synthetic *MCL-1* inhibitors, i.e. Seliciclib

and R-etodolac should be considered in the treatment of *MLL*-R ALL.¹⁰

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 ALL.⁶⁹⁻⁷¹ In addition, *MLL*-R is associated to high expression of *Flt3* in ALL.²² Several small molecule inhibitors such as PKC412 and CEP-701 have been shown to inactivate *FLT3* and to induce apoptosis *in vitro* in AML and *MLL*-R ALL cells.⁷⁰⁻⁷² Furthermore, *Flt3* inhibitors display synergistic cytotoxic effect with chemotherapy in infant *MLL*-R ALL samples.⁷³ 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 lestaurinib (CEP-701) to POG-9407 treatment in *MLL*-R infants (AALL0631).¹

Other targets and pathway have also been considered in the therapeutic approach of infant leukemia. These include the *HOX*, *Menin*⁷⁴ or *Ephrin*/*EphA7*/*ERK* pathways,⁷⁵ the *TRAIL* and *Fas* death receptors,⁷⁶ *CD44* which is highly expressed on *MLL*-R leukemic cells¹⁰ and ultimately the *MLL* fusion gene itself.⁷⁷ Several studies reported promising *in vitro* results but additional investigations are required before their translation into clinical trials.

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.

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