Cytogenetics and outcome of infants with acute lymphoblastic leukemia and absence of *MLL* rearrangements

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Acute lymphoblastic leukemia (ALL) in infants less than 1 year of age is rare and the biological features are different from ALL in older children.[1] Infant ALL is characterized by a high frequency of rearrangements of the *MLL* gene (*MLL*-R) and heterogeneous outcome. However overall, their event-free survival (EFS) is much worse than older children with ALL.[1–5] A large collaborative trial, Interfant-99, demonstrated improved outcome, while characterizing definitively the independent prognostic variables in infant ALL.[6] While cytogenetic data are reported within individual infant ALL clinical trials, the numbers are typically small and many reports are less detailed for those patients without *MLL* gene rearrangements (*MLL*-G). However, it was previously suggested that *MLL*-G had an important predictive influence on outcome.[7,8] These observations were later confirmed in Interfant-99,[6] in which *MLL*-G patients showed a threefold reduced risk of an event compared with *MLL*-R patients, although all *MLL*-G patients were grouped together into a single category. To better understand the association of different chromosomal abnormalities

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and outcome among *MLL*-G infants, here we have carried out detailed cytogenetic investigation of two infant ALL trials: Interfant-99 and Children's Oncology Group (COG)-P9407.

Patients were 365 days old or less with newly diagnosed ALL without a rearrangement of the *MLL* gene enrolled to Interfant-99 (May 1999–December 2005; *n* = 110) and COG-P9407 (June 1996–October 2006; *n* = 52).[6,9] Individual study groups obtained ethical approval, and treating physicians obtained informed consent from parents or guardians. The presence of *MLL* gene rearrangements was excluded using fluorescence *in situ* hybridization (FISH), reverse transcription (RT)-PCR and/or Southern blotting, as previously reported.[6] Each national study group provided patient data, including cytogenetics, FISH and molecular results. EFS and overall survival (OS) were calculated from the date of trial enrolment to the date of the first event (induction failure, relapse, second malignancy or death) or last follow-up. Median follow-up time was 7 years.

Among 162 *MLL*-G patients, no cytogenetic data were available for 34 (21%), resulting in a success rate of 79%. An abnormal karyotype was detected in 90/128 (70%) patients with a successful cytogenetic result (Supplementary Table 1) with the remainder classified as normal based on the presence of at least 10 (but usually 20) normal metaphases. They were categorized according to cytogenetic risk group as previously defined for childhood ALL.[10] Compared with childhood ALL (1–18 years) using data from the UKALL97/99 treatment trial,[10] the frequency of good risk cytogenetic abnormalities among *MLL*-G infants was significantly lower (12 vs 60%, *P*<0.01), whereas the frequency of poor risk abnormalities (excluding *MLL* translocations) was similar (8 vs 10%). Although *ETV6–RUNX1* fusion is present in 25% of childhood ALL, we found no *ETV6–RUNX1* cases among the 75 patients tested by FISH or RT-PCR. High hyperdiploidy (HeH) was the most prevalent abnormality, although the frequency was also much lower than childhood ALL (12 vs 38%, *P*<0.01).[10] It is possible that some cases with normal or failed cytogenetic results harbored a hidden HeH clone. Among patients for whom DNA index was available, a value of 1.13–1.2 correlated with HeH in five patients; one with no cytogenetic result had a DNA index of 1.13, likely indicating the presence of a HeH clone. The chromosome number of HeH karyotypes ranged from 53 to 64 (Supplementary Table 1). The karyotypes of patients 12 and 14 may represent doubling of low hypodiploid clones, although it was not proven. Other established translocations were observed: t(9;22)(q34;q11) (*n* = 2), t(1;19)(q23;p13) (*n* = 3) and t(7;12) (q36;p13) (*n* = 1). Interestingly, the incidence of t(9;22) and t(1;19) among *MLL*-G infants was not markedly different from childhood ALL:[10] 2/128 (1.6%) versus 43/1633 (2.6%) and 3/128 (2.3%) versus 50/1420 (3.5%), respectively. Abnormalities of the short arm of chromosome 9 (9p) were observed in 14 (11%) cases at a similar incidence to childhood ALL; karyotypes included dic(7;9) (*n* = 3) and dic(9;20)(p11 ~ 13;q11) (*n* = 2).

The translocation, t(7;12)/*ETV6–HLXB9* fusion, most frequently occurs in infant acute myeloid leukemia (AML) and rarely in infant ALL.[11] Although associated with a dismal outcome in AML, the outcome in ALL is unknown as numbers of cases are too low. One patient was identified in this series (36). However, as this cryptic abnormality is often linked to deletions of the long arm of chromosome 7 (7q) and gain of chromosome 19, patients 34 and 35 may also harbor this translocation. Unfortunately no material was available for

molecular testing of these or the other six cases with 12p13 breakpoints, suggesting involvement of the *ETV6* gene.

Chromosome 15 abnormalities with variable breakpoints were found in 12 patients. The more frequent occurrence of 15q abnormalities in infant compared with childhood ALL has been previously noted.[12] The translocation, t(5;15)(p15;q11–13), was observed in two patients (21, 25), which has previously been specifically associated with infant ALL.

It is well established that *MLL*-R infants are younger than their *MLL*-G counterparts: in Interfant-99, 91% of infants <6 months were *MLL*-R compared with 66% of those 6–12 months (*P*<0.0001).[6] Classification of *MLL*-G patients by cytogenetic risk group showed further correlation with age. The majority of cytogenetic good risk and all poor risk patients were >9 months old, whereas half of the cytogenetic intermediate risk patients were <9 months (Supplementary Table 2). In addition, there was evidence of outcome heterogeneity according to cytogenetic risk group (Table 1 and Figure 1). Despite the modest numbers of *MLL*-G cases, especially in the cytogenetic good and poor risk groups, the pattern of outcome was very similar to that observed in childhood ALL when the same classification was applied.[10] However, the difference in outcome among the three cytogenetic risk groups among the *MLL*-G patients did not quite reach statistical significance (likely due to low numbers in the good and poor risk categories). It was of interest that two patients in the poor risk group were Philadelphia chromosome (Ph) positive. When these patients were removed from the survival analysis, there was no change to the EFS of the poor risk group, although there was an improvement in OS because one Ph-positive case was among the four patients in the cohort who died. This situation will likely change in future, as Ph-positive infants will be treated with tyrosine kinase inhibitors in the same manner as older children.

Recently, Kang *et al.*[13] reported that gene expression profiles were predictive of age and outcome in 97 infants (including 17 *MLL*-G) with ALL treated in COG-P9407. Their statistical modeling of an outcome predictor revealed three genes (*FLT3*, *IRX2* and *TACC2*) to be highly predictive of EFS beyond age and *MLL* status. In particular, low *FLT3* expression was found to be associated with an excellent outcome, as had been previously indicated.[14] Supplementary Figure 1 shows the *FLT3* intensities for the entire cohort of infants tested in COG-P9407, which was validated in Interfant-99. Interestingly, the majority (10/11) of the cases with low expression were *MLL*-G. However, low *FLT3* expression was not simply a reflection of *MLL*-G status, as seven of the *MLL*-G patients had high *FLT3* expression. However, it is also thought to be linked to the very immature pro B cells, in which the *MLL* rearrangements often occur. These low-expressing *MLL*-G patients emerged from all cytogenetic groups, as shown in Supplementary Table 3. Interestingly, none of the *MLL*-G patients with low expression had an event, whereas all five of the events occurred in the high expressers.

We have confirmed a unique cytogenetic profile among infants with ALL. We demonstrate that the infants without *MLL* translocations (*MLL*-G) share the same cytogenetic abnormalities as older children with ALL. Generally infants with *MLL*-G ALL are older, have low *FLT3* expression and have an improved outcome compared to their *MLL*-R counterparts. Despite small numbers of *MLL*-G infants, when classified into the same good,

intermediate and poor risk cytogenetic subgroups as childhood ALL, their pattern of outcome was very similar to that observed in older children. However, their overall worse outcome likely reflects the differences in distribution of good and poor risk abnormalities: a lower incidence of the good risk abnormality, such as high hyperdiploidy and absence of *ETV6–RUNX1*, and a higher incidence of poor risk abnormalities. Nevertheless, these data suggested that some *MLL*-G infants, especially those with good risk cytogenetics, may benefit from treatment on childhood protocols, which are generally less intensive and less toxic than infant ALL regimens.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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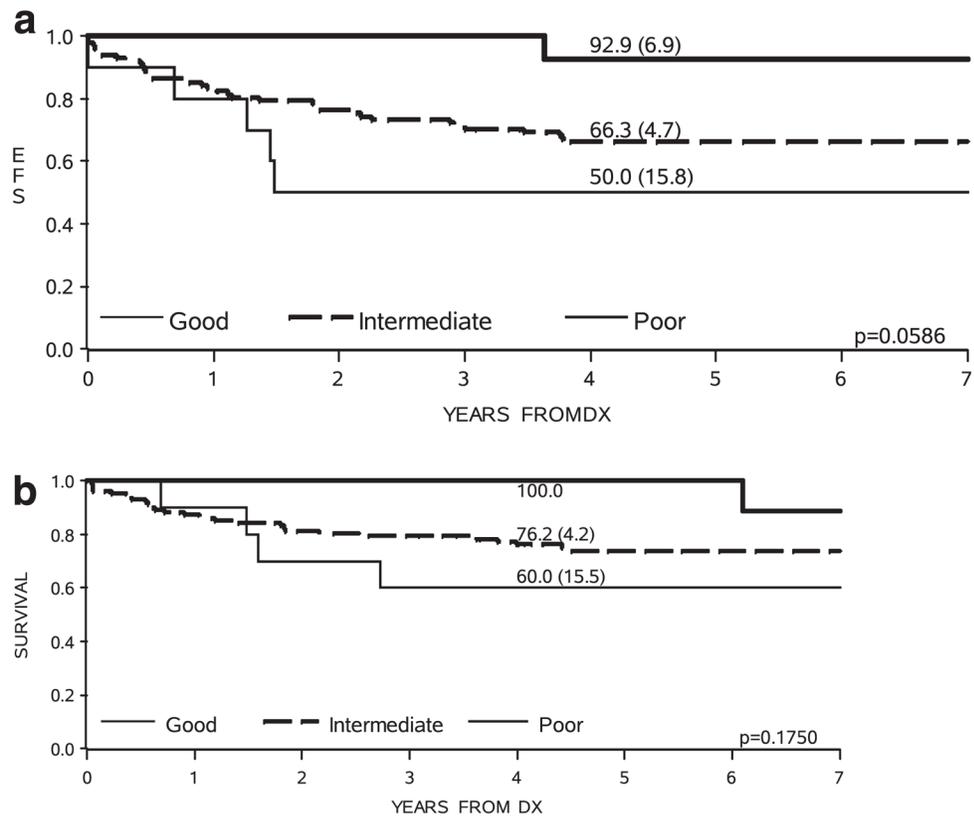
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Figure 1.
Kaplan–Meier curves showing event-free (**a**) and overall (**b**) survival of 128 *MLL*-G infant ALL patients by cytogenetic risk group.

Table 1

Incidence and outcome of 162 *MLL*-G infants treated on INTERFANT-99 and COG-P9407 by cytogenetic risk group

	Total	Cytogenetic risk group[a]		
		Good[b]	Intermediate[c]	Poor[d]
Total	162	15	103	10
Incidence (%)	100	12	80	8
Outcome variables				
Induction failure	10 (6%)	0	6 (6%)	1 (10%)
Relapses	33 (20%)	1 (7%)	24 (23%)	2 (20%)
Death in CCR	9 (6%)	0	4 (4%)	2 (20%)
Events	52 (32%)	1 (7%)	34 (33%)	5 (50%)
Deaths	41 (25%)	1 (7%)	26 (25%)	4 (40%)
EFS at 4 years (s.e.)	67% (3.7)	93% (6.9)	66% (4.7)	50% (15.8)
OS at 4 years (s.e.)	77% (3.4)	100% (—)	76% (4.2)	60% (15.5)

Abbreviations: EFS, event-free survival; OS, overall survival.

[a] Cytogenetic data were only available for 128 patients.

[b] Good risk: all patients with high hyperdiploidy (HeH).

[c] Intermediate—all other cases.

[d] Poor risk: all patients with t(9;22)/*BCR-ABL1*, abnormal 17p and loss of 13q.