Clinical and molecular genetic characterization of wild-type MLL infant acute lymphoblastic leukemia identifies few recurrent abnormalities

Wild-type *MLL* infant acute lymphoblastic leukemia (ALL) patients account for approximately 20% of all infant ALL cases. Although this group of patients generally fares better than *MLL*-rearranged infant ALL patients, their prognosis is still worse than that of non-infant pediatric B-ALL patients. Extensive characterization of this specific patient group largely remains unacknowledged. In this study, we aim to obtain a clinical and molecular profile of this patient group in order to find new opportunities to optimize treatment. We report on a large cohort of 78 wild-type *MLL* infant ALL samples using clinical parameters, array-comparative genomic hybridization analysis, and gene expression profiling. The frequency of DNA copy number variations and molecular genetic lesions in genes involved in B-cell development are lower in wild-type *MLL* infant ALL than older children with ALL. Wild-type *MLL* infant ALL presented with higher white blood cell (WBC) counts and a more immature immunophenotype than pediatric (non-infant) B-ALL patients. The strongest predictor of outcome in wild-type MLL infant ALL was the level of *MEIS1* expression, which may indicate new opportunities for novel strategies in treating wild-type *MLL* infant ALL.

Acute lymphoblastic leukemia (ALL) in infants (<1 year of age) is a rare but highly aggressive type of leukemia, typically characterized by the presence of *MLL*-rearrangements occurring in approximately 80% of these patients.[1] The prognosis for *MLL*-rearranged infant ALL patients is highly unfavorable.[2] In contrast, infant ALL patients carrying wild-type (or germline) *MLL* genes fare significantly better, with reported event-free survival (EFS) of 60%-74%.[1,2] However, these rates are much lower than the 5-year survival rates in older children with ALL (approx. 90%).[3] We recently showed that wild-type *MLL* infant ALL specifies a gene expression pattern that is different from both *MLL*-rearranged infant ALL and from pediatric non-infant precursor B-ALL.[4] In an unsupervised clustering analysis, wild-type *MLL* infant ALL samples even appeared more closely related to MLL-rearranged infant ALL than to pediatric precursor B-ALL cases. Also, infant ALL patients who do not carry *MLL* translocations share the same cytogenetic abnormalities as older children with ALL, albeit with a different distribution: a lower incidence of the favorable abnormalities *ETV6-RUNX1* and high hyperdiploidy, and a higher incidence of unfavorable abnormalities, including *BCR-ABL1*.[5]

In the present study, we aimed to obtain a clinical and genetic profile of a relatively large cohort of wild-type *MLL* infant ALL patients all treated according to INTERFANT treatment protocols (i.e. Interfant-99 or Interfant-06), in order to find a common denominator in this group that can ultimately be used to optimize treatment for these patients. The results are compared to data obtained in MLL-rearranged infant ALL patients (enrolled in INTERFANT studies) and pediatric (non-infant) precursor ALL patients uniformly treated according to the Dutch Childhood Oncology Group (DCOG) ALL-10 protocol. Patients enrolled in this study from the INTERFANT-99 study (n=61) have been presented elsewhere.[5]

Table 1. Clinical characteristics and prognostic factors of wild-type MLL infant ALL patients.

	Wild-type MLL infant ALL (n=78)	MLL-rearranged infant ALL (n=70)	*P*	Pediatric (non-infant) ALL (n=484)	*P*
Sex			0.62		0.09
Male	36 (46%)	29 (41%)		274 (57%)	
Female	42 (54%)	41 (59%)		210 (43%)	
Age at diagnosis			<0.0001		NA
< 6 months	18 (23%)	41 (59%)		NA	
> 6 months	60 (77%)	29 (41%)		NA	
WBC count (cells/L)			<0.0001		<0.0001
< 100 x 10^9	52 (69%)	16 (24%)		389 (89%)	
100-300 x 10^9	19 (25%)	24 (36%)		38 (9%)	
> 300 x 10^9	6 (8%)	27 (40%)		11 (3%)	
not known	1	3		46	
Immunophenotype			<0.0001		<0.0001
Pro-B cell	8 (11%)	52 (78%)		9 (2%)	
Common B-cell	25 (33%)	3 (4%)		257 (54%)	
Pre-B cell	29 (39%)	10 (15%)		132 (28%)	
T-lineage	9 (12%)	0		80 (17%)	
Other*	4 (5%)	2 (3%)		1 (0%)	
Not known	3	3		5	
Prednisone response			0.009		0.21
good response	62 (85%)	39 (65%)		385 (90%)	
poor response	11 (15%)	21 (35%)		41 (10%)	
not known	5	9		58	

*Other immunophenotypes included both acute undifferentiated and biphenotypic leukemias. WBC: white blood cell; NA: not applicable.

Clinical parameters known to predict outcome in *MLL*-rearranged infant ALL were compared between infant ALL patients carrying wild-type MLL genes (n=78) and *MLL*-rearranged infant ALL cases (n=70), as well as between wild-type MLL infant ALL patients and pediatric (non-infant) ALL patients (n=484). The adverse prognostic factors analyzed included: age under six months, WBC counts more than $300x10^9$ leukemic cells/L, a pro-B (CD10-) immunophenotype, and a poor *in vivo* prednisone window response (Table 1). Compared with *MLL*-rearranged infant ALL cases, infant ALL patients carrying wild-type *MLL* genes were significantly more often diagnosed at over six months of age, presented with more favorable WBC counts, more mature (pre-B or common) immunophenotypes, and generally responded well to a 7-day window of prednisone monotherapy (Table 1).

Next we assessed the prognostic relevance of these predictive parameters in terms of disease-free survival (DFS), overall survival (OS), and cumulative incidence of relapse (CIR) five years after diagnosis in wild-type *MLL* infant ALL patients (n=76) for whom clinical follow-up data were available (Table 2). Overall, 5-year DFS (standard error, SE) was 71.3 (5.3), 5-year OS 82.2 (4.5), and 5-year CIR 21.9 (4.9). Neither age under six months at diagnosis, nor WBC counts more than $300x10^9$ leukemic cells/L were predictive of clinical outcome within this group. In contrast, a poor prednisone response was marginally associated with an inferior outcome (although not significantly so), whereas an immature pro-B immunophenotype was highly predictive of a poor clinical outcome ($P<0.001$). The 5-year OS in the wild-type MLL infant ALL patients diagnosed with pro-B ALL was 14.3 (18.7), whereas this was 92.0 (5.4) and 92.7 (5.0) in wild-type *MLL* infants diagnosed with common B-ALL and pre-B ALL, respectively ($P<0.001$).

In order to identify additional prognostic factors for wild-type *MLL* infant ALL, we applied significance analysis of microarrays (SAM) to screen our gene expression profiles (Affymetrix HU133plus2.0 GeneChips) for genes predictive for clinical outcome. Gene expression profiles were available for 36 wild-type *MLL* infant ALL patients and clinical follow-up data were available for 30. Of these 30 patients, 8 experienced an event. Interestingly, two probe sets appeared highly predictive of clinical outcome: i) the level of *MEIS1* expression (Affymetrix probe set 242172_at); and ii) PENK (Affymetrix probe set 213791_at). Patients expressing low levels of *MEIS1* (i.e. below the median MEIS1 level of the entire patient group, n=16) had a superior outcome over patients expressing high levels (i.e. above the median, n=14): 5-year DFS (SE) was 87.5 (8.3) *versus* 50.0 (13.4; $P=0.01$), while 5-year OS was 100.0 *versus* 71.4 (12.1; $P=0.02$) for low and high *MEIS1*-expression, respectively. Remarkably, differential gene expression analysis between patients with high *MEIS1*-expression (n=18) and patients with low *MEIS1*-expression (n=18) could not identify differentially expressed genes other than MEIS1 itself. Analysis of prognostic factors showed a significant difference in terms of immunophenotype, with more immature phenotype more frequent in the wild-type infant ALL patients with high *MEIS1* expression ($P=0.009$) (*Online Supplementary Table S1*). Strikingly, we could not detect a prognostic value of high-level *MEIS1* expression in the pediatric (non-infant) ALL patients.

Interestingly, high-level expression of *MEIS1* is also closely associated with prognostically unfavorable MLL-rearranged leukemias.[6,7] Hence, the prognostic relevance of *MEIS1* expression in wild-type MLL infant ALL patients may imply transformation events that, to some extent, resemble that of MLL-rearranged infant ALL cases. Furthermore, the strong influence on clinical outcome of *MEIS1* expression suggests that infant ALL expressing high levels of *MEIS1* represent a highly aggressive leukemia that require very few co-operative genetic lesions during leukemogenesis and/or leukemia maintenance.

Kang *et al.* have shown that *FLT3, IRX2* and *TACC2* expression is highly predictive of EFS in infant ALL.[8] That we did not find a significant result from *FLT3*, *IRX* and *TACC2* as predictors of outcome could have been due to the fact that we only used wild-type *MLL* patients or that patient numbers were too low to provide a significant result.

In order to detect submicroscopic deletions and amplifications in the DNA, we performed array-comparative

Table 2. Univariate analysis of prognostic factors in wild-type MLL infant ALL patients.

	N	5-year DFS	SE	*P*	5-year OS	SE	*P*	5-year CIR	SE	*P*
Age at diagnosis				0.75			0.49			0.32
< 6 months	17	76.5	10.3		76.5	10.3		11.8	8.1	
> 6 months	58	70.0	6.1		84.0	4.9		24.8	5.8	
WBC count (cells/L)				0.83			0.30			0.74
< $100x10^9$	49	72.6	6.5		87.2	4.9		25.4	6.4	
100-$300x10^9$	19	68.4	10.7		73.7	10.1		15.8	8.6	
> $300x10^9$	6	66.7	19.3		66.7	19.3		16.7	16.7	
Prednisone response				0.22			0.23			0.33
Good response	61	75.1	5.6		85.1	4.6		18.3	5.1	
Poor response	10	56.3	16.5		67.5	15.5		32.5	16.7	
Immunophenotype				0.003			<0.001			0.03
Pro-B cell	7	14.3	18.7	<0.001*	14.3	18.7	<0.001*	57.1	22.7	0.02*
Common B cell	25	66.3	9.8		92.0	5.4		33.7	10.1	
Pre-B cell	28	85.3	6.8		92.7	5.0		7.3	5.1	
T-lineage	9	66.7	15.7		64.8	16.5		22.2	14.8	
Other	4	–	–		–	–		–	–	

*DFS: disease event-free survival; SE: standard error; OS: overall survival; CIR: cumulative incidence of relapse; WBC: white blood cell. *p-value for comparison of pro-B immunophenotype versus all other phenotypes.*

genomics hybridization (array-CGH) and multiplex ligation-dependent probe amplification (MLPA) on a cohort of wild-type *MLL* infant ALL patients (n=31 and n=32, respectively) for whom genomic DNA was available (*Online Supplementary Table S2*).

The results from array-CGH were compared with data from a group of pediatric (non-infant) B-ALL patients (n=115) (Table 3) selected with a relatively high frequency of B-others and low number of high hyperdiploid patients, and this should be taken into account when interpreting the results. The frequency of structural aberrations found by array-CGH (i.e. partial deletions/amplifications, and translocations) was much lower in wild-type *MLL* infants than in non-infant ALL patients (45% *vs.* 98%, respectively; $P<0.001$) (Table 3). The frequency of patients with numerical aberrations (i.e. gain or loss of complete chromosomes) among wild-type *MLL* infant ALL patients (23%) was comparable to the frequency of numerical aberrations in non-infant pediatric ALL patients (29%).

MLPA analysis was carried out using specific probes for single gene alterations including *CDKN2A, CDKN2B, IKZF1, PAX5* and *ETV6* (*Online Supplementary Table S3*). No alterations of *IKZF1* were detected in any of the wild-type *MLL* infant ALL samples (n=32) tested, whereas 17% of the pediatric non-infant B-ALL patients are known to carry an *IKZF1* deletion.[9] A deletion of *CDKN2A* and *CDKN2B* on 9p21.3 was found in 6 (19%) of the infants. In contrast, deletions of *CDKN2A* and *CDKN2B* were found in 35% and 34% of the pediatric non-infant ALL samples, respectively. Deletions of PAX5 were present together with *CDKN2A* and *CDKN2B* in 4 wild-type MLL infant ALL patients, whereas the PAX5 deletion was the only observed abnormality in 2 infants as determined by MLPA. The incidence of ETV6 deletions was markedly lower in infants: 3% *versus* 26% of the pediatric precursor B-ALL patients ($P=0.002$); however, when *ETV6-RUNX1* cases were excluded, only 11% of precursor B-ALL patients had *ETV6* deletions (11%; $P=0.2$).

In conclusion, wild-type *MLL* infant ALL has a different clinical and molecular profile to pediatric (non-infant) precursor B-ALL and is characterized by a higher incidence of poor prognostic factors and fewer genetic alterations. High *MEIS1* expression is highly predictive of poor outcome in wild-type *MLL* infant ALL.

Table 3. Distribution of DNA copy-number variations in wild-type MLL infant acute lymphoblastic leukemia (ALL) and pediatric non-infant precursor B-ALL patients.

	Wild-type MLL infant ALL		Pediatric non-infant B-ALL		#Fisher *P*-value^	
	all	without HD	all	without HD	all	without HD
Type of aberration*	(n=31)	(n=27)	(n=115)	(n=103)		
No aberrations	10 (32%)	10 (37%)	2 (2%)	2 (2%)	<0.001	<0.001
Numerical aberrations	7 (23%)	3 (11%)	33 (29%)	21 (20%)	0.65	0.49
Structural aberrations	14 (45%)	14 (52%)	113 (98%)	101 (98%)	<0.001	<0.001
Structural aberrations by oncogene/tumor suppressor gene and immunophenotype						
	B-ALL patients (n=28)†					
CDKN2A (9p21.3)						
Deletion	6 (21%)		6 (21%)		33 (29%)	26 (25%)
PAX5 (9p13.2)						
Deletion	6 (21%)		6 (21%)		19 (17%)	19 (18%)
RUNX3 (1p36.11)						
Deletion	1 (4%)		1 (4%)		11 (10%)	10 (10%)
GAS7 (17p13.1)						
Amplification	4 (14%)		1 (4%)		15 (13%)	6 (6%)
Deletion	1 (4%)		1 (4%)		11 (10%)	11 (11%)
MME (3q25.2)						
Deletion	1 (4%)		1 (4%)		2 (2%)	2 (2%)
EVI1 (3q26)						
Deletion	1 (4%)		1 (4%)		3 (3%)	3 (3%)
	T-ALL patients (n=3)				–	
STIL and PTEN‡						
Deletion	1 (33%)		NA		NA	NA
PICALM (11q14.2)						
Deletion	1 (33%)		NA		NA	NA

**As numerical and structural aberrations can occur combined in one patient, numbers mentioned do not necessarily add up to 100%. †B-ALL patients including patients with unknown immunophenotype. ‡Fluorescent* in situ *hybridization (FISH) confirmed a SIL-TAL1 fusion caused by sub-deletion. HD: high hyperdiploid karyotype (51-65 chromosomes). ^Fisher's exact tests for structural aberrations were all non-significant. #Pediatric non-infant B-ALL cohort consisting of one (1%) TCF3-PBX1 translocated patient, 12 (10%) high hyperdiploid patients, 3 (3%) MLL-rearranged patients, 51 (44%) ETV6-RUNX1 translocated patients, and 48 (42%) B-other patients of whom 8 are positive for iAMP21 (17%).*

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