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The distribution of *MLL* breakpoints correlates with outcome in infant acute leukaemia

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Summary

Acute leukaemia in early childhood - and mainly infant leukaemia (IL) – is characterized by acquired genetic alterations, most commonly by the presence of distinct *MLL* rearrangements (*MLL*-r). The aim of this study was to investigate possible correlations between clinical features and molecular analyses of a series of 545 childhood leukaemia ($\leq$24 months of age) cases: 385 acute lymphoblastic leukaemia (ALL) and 160 acute myeloid leukaemia (AML). The location of the genomic breakpoints was determined in a subset of 30 *MLL-r* cases. The overall survival of the investigated cohort was 60·5%, as determined by the Kaplan-Meier method. Worse outcomes were associated with age at diagnosis $\leq$6 months ($P < 0{\cdot}001$), high white blood cell count ($P = 0{\cdot}001$), and *MLL*-r ($P = 0{\cdot}002$) in ALL, while children with AML displayed a poorer outcome ($P = 0{\cdot}009$) regardless of their age strata. Moreover, we present first evidence that *MLL*-r patients with poor outcome preferentially displayed chromosomal breakpoints within *MLL* intron 11. Based on the literature, most *MLL*-r IL display a breakpoint localization towards intron 11, which in turn may explain their worse clinical course. In summary, the *MLL* breakpoint localization is of clinical importance and should be considered as a novel outcome predictor for *MLL*-r patients.

Keywords: age at diagnosis, breakpoint, infant, *MLL*, prognostic.

Acute leukaemia (AL) is one of the most common malignancies of early childhood, and is associated with a high incidence of early death during the perinatal period. In some cases, AL is present at birth and neonates die shortly thereafter, while other newborns develop normally for a few months and clinical and haematological problems appear later (Ross *et al*, 1994; Biondi *et al*, 2000). It is possible to correlate these differences in AL progression with variable genetic markers that are associated with the age of individual AL patients. *MLL* gene rearrangements (*MLL*-r) are a remarkable example: they constitute the most frequently identified genetic factor correlated with the development of acute lymphoblastic leukaemia (ALL) or acute myeloid leukaemia (AML) in early infancy ($\leq$12 months of age). Despite advances in most other age groups, the prognosis of infants remains poor (Pieters *et al*, 2007; Balgobind *et al*, 2009). Therefore, *MLL*-r is also a hallmark for epidemiological studies in infant leukaemias (ILs). Molecular studies have demonstrated that these critical molecular lesions occur *in utero* in early haematopoietic precursors (Ford *et al*, 1993; Greaves & Wiemels, 2003), and that maternal exposures during pregnancy may be associated with infant and childhood leukaemias (Alexander *et al*, 2001; Pombo-de-Oliveira & Koifman, 2006).

The extraordinary diversity and distribution of *MLL*-r in ALL versus AML, and in infants versus paediatric or adult patients, suggest different associated biological mechanisms that may also reflect diverging prognostic responses. Extensive analyses at the molecular level have allowed the identification of 71 different *MLL* fusion partners to date (Meyer *et al*, 2009; Marschalek, 2011). Efforts have been made to associate immunobiological patterns with different fusion partner genes and age at diagnosis (Jansen *et al*, 2007; Mann *et al*, 2007); however, it remains unclear which molecular mechanisms or which exposures to external poisons contribute to the biological differences between *MLL*-r in children of different age strata. Some questions are less explored, such as (i) the predictive value of *MLL*-r in children aged between

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13 and 24 months (ii) whether interethnic differences and/or differences in environmental exposure would influence the frequency of *MLL*-r-associated leukaemias; and (iii) whether differences in genetic susceptibility have pharmacogenomic implications. In this study, we analysed a series of patients enrolled in the Brazilian Collaborative Study Group of Infant Acute Leukaemia (BCSGIAL) in order to evaluate the frequencies of different *MLL* translocations and their association with demography, clinical and laboratory findings, and overall survival (OS).

Materials and methods

Subjects

Patients were gathered from 24 cities in the South, Southeast, Northeast, and Middle West regions of Brazil, covering both urban and rural areas, except the Amazon region. The BCSGIAL has addressed questions related to maternal environmental substance exposures during pregnancy, immunological factors, and genetic susceptibility associated with *MLL*-r leukaemia in Brazilian children (Pombo-de-Oliveira & Koifman, 2006; Zanrosso *et al*, 2010; Gonçalves *et al*, 2012). Additionally, the BCSGIAL studies include an accurate diagnosis of biomarkers to adjust treatment strategies and follow-up for children (Emerenciano *et al*, 2006; Pombo-de-Oliveira *et al*, 2009).

Data collection and laboratory procedures were evaluated and approved by the Ethics Committee of all participating hospitals. Data analysis was approved by the Research Ethics Committee, Instituto Nacional de Câncer (studies CEP #005/06 and #024/10; CONEP # 707/2010).

Leukaemia diagnosis

Diagnostic specimens of bone marrow aspirates and/or peripheral blood cells from children in early childhood ($\leq$24 months of age) were assigned to this study from January 2000 to January 2011. Samples from participants with ALL or AML were diagnosed prior to any treatment. The diagnosis was first established through morphological examinations of lymphoid and myeloid cells according to standard criteria. Depending on the immunophenotype, AL was categorized as precursor B-lineage ALL (B-I, B-II, B-III), T-ALL (T-I, T-II, T-III, T-IV), or AML (as defined by the World Health Organization classification and revised in Bain (2006). The immunophenotype was assessed by flow cytometry as outlined in detail previously (Emerenciano *et al*, 2011) using a FACS Calibur (Becton, Dickinson and Company, San Diego, CA, USA) and the CellQuest software package (Becton, Dickinson and Company). Subsequently, specimens were screened for somatic genetic abnormalities commonly observed in childhood ALL (*ETV6-RUNX1, TCF3-PBX1, BCR-ABL1, STIL-TAL1, TLX3, MLL*-r) and in AML (*RUNX1-RUNX1T1, CBFB-MYH11, PML-RARA, MLL*-r) (van Dongen *et al*, 1999). Childhood leukaemia associated with Down syndrome, monosomy 7, Fanconi anaemia, Bloom syndrome, Ataxia telangiectasia, neurofibromatosis, myelodysplasia, and children older than 24 months were excluded.

Detection of MLL rearrangements

Detection of an *MLL*-r was performed by conventional cytogenetics, reverse transcription polymerase chain reaction (PCR), and/or by fluorescence *in situ* hybridization (FISH; LSI MLL Dual Color Break Apart Rearrangement Probe, Vysis Inc., Downers Grove, IL, USA) as previously described (Emerenciano *et al*, 2006). Long distance inverse PCR (LDI-PCR) was used to identify the *MLL* fusion partners and the respective breakpoints. Briefly, 1 μg of genomic DNA was digested and the resulting DNA fragments were self-ligated. This re-ligated DNA was used for the subsequent LDI-PCR analysis. PCR amplimers were purified from the gel and subsequently sequenced to obtain chromosomal breakpoint information (Meyer *et al*, 2005).

Statistical analysis

The children recruited in the study received different chemotherapy treatments in accordance with the Brazilian ALL treatment protocol group (GBTLI-ALL93, GBTLI-ALL99), Berlin-Frankfurt-Munster (BFM), or the INTERFANT-based protocols (Schrappe *et al*, 2000; Pieters *et al*, 2007; Brandalise *et al*, 2010). Therefore, it was not possible to evaluate each biomarker with respect to the different treatment protocol strategies. To compare the frequency of *MLL*-r between distinct groups (for example, age strata, ALL versus AML patients, Whites versus Non-whites) the χ^2-test (two-sided) was used (or Fisher's Exact Test when expected values were less than five). *P*-values $\leq$0·05 were considered statistically significant. The disease risk associated with *MLL*-r occurrence in ALL or AML patients was determined according to demographic and laboratory findings by calculating odds ratios (ORs) with 95% confidence interval (CI) and unconditional logistic regression to adjust for potential confounding variables. A multivariate logistic regression model (method enter) was used to analyse associations between *MLL* genomic breakpoint status (intron 11 *vs.* others) and established demographic-clinical parameters [i.e. age at diagnosis and white blood cell (WBC) counts]. OS was determined in months from the diagnosis to outcome (death, alive, or last follow-up) using the Kaplan-Meier method. Patients lost to follow-up were censored at their date of last known contact. The differences between AL survival distributions were compared using the log-rank test. The multivariate Cox proportional hazard regression method was used to determine the independent prognostic factors influencing OS. All statistical analyses were performed using the Statistical Product and Services Solutions statistical package, version 18·0 (SPSS Inc, Chicago, IL, USA).

Results

Demography and immunophenotyping

A total of 545 children (385 ALL and 160 AML) were included in this study. They were first analysed by correlating age at diagnosis with leukaemia subtypes and *MLL* status (Table I). IL patients ($\leq$12 months old) and AL patients aged 13–24 months were matched regarding demography. The majority of samples (55%) displayed high WBC counts ranging from 1·1 −965·0 × 10^9/l.

Leukaemia subtype was successfully classified in 537 out of 545 (98·5%) samples; the immunophenotype of 8 ALL patients could not be analysed. B precursor-ALL (n = 354) and T-ALL (n = 23) comprised 91·9% and 5·9% of ALL cases, respectively. The B precursor-ALL series included 167 cases displaying a CD10 negative pro-B phenotype (B-I), while the remaining cases with CD10 positive had common ALL (B-II, n = 138) or pre-B ALL (B-III, n = 9) phenotypes. T-ALL cases included T-I (n = 13), T-II (n = 5), and T-III (n = 5) subtypes (Table I).

AML cases (n = 160) represented 29·4% of the total series; the M4-M5 subgroups accounted for the majority of these cases (61·3%), while 19·4% were M6-M7, 13·1% were M1-M2, 3·1% were M0, and 3·1% were M3.

MLL rearrangements and fusion partner genes

In 429 out of 545 children with AL (78·7%), the presence or absence of a rearranged *MLL* allele was successfully analysed.

Table I. Demographic and clinical characteristics of patients according to age at diagnosis.

	n (%)				
	All cases n = 545	$\leq$6 months n = 123	7–12 months n = 154	13–24 months n = 268	P
Gender					
Male	302 (55·4)	65 (52·8)	89 (57·8)	148 (55·2)	0·710
Female	243 (44·6)	58 (47·2)	65 (42·2)	120 (44·8)	
Race/skin colour*					
White	347 (63·7)	87 (70·7)	94 (61·0)	166 (61·9)	0·036
Non-White	188 (34·5)	31 (25·2)	57 (37·0)	100 (37·3)	
Other†	10 (1·8)	5 (4·1)	3 (1·9)	2 (0·7)	
Region of Brazil‡					
South	56 (10·3)	11 (8·9)	21 (13·6)	24 (9·0)	0·688
Southeast	232 (42·6)	58 (47·2)	62 (40·3)	112 (41·8)	
Northeast	171 (31·4)	36 (29·3)	49 (31·8)	88 (32·8)	
Middle-West	80 (14·7)	18 (14·6)	22 (14·3)	40 (14·9)	
Leukaemia subtype					
ALL	385 (70·6)	87 (70·7)	100 (64·9)	198 (73·9)	0·152
AML	160 (29·4)	36 (29·3)	54 (35·1)	70 (26·1)	
ALL subtype§					
Pro-B	167 (44·3)	71 (82·5)	54 (55·1)	42 (21·8)	<0·001
c-ALL	178 (47·2)	14 (16·3)	35 (35·7)	129 (66·8)	
Pre-B	9 (2·4)	0 (0·0)	1 (1·0)	8 (4·1)	
Pro-T	23 (6·1)	1 (1·2)	8 (8·2)	14 (7·3)	
AML subtype					
M0	5 (3·1)	1 (2·8)	2 (3·7)	2 (2·9)	0·279
M1-M2	21 (13·1)	6 (16·7)	4 (7·4)	11 (15·7)	
M3	5 (3·1)	0 (0·0)	0 (0·0)	5 (7·1)	
M4-M5	98 (61·3)	23 (63·8)	37 (68·5)	38 (54·3)	
M6-M7	31 (19·4)	6 (16·7)	11 (20·4)	14 (20·0)	
MLL gene status¶					
MLL rearranged	176 (41·0)	59 (59·0)	63 (48·1)	54 (27·3)	<0·001
MLL wild-type	253 (59·0)	41 (41·0)	68 51·9)	144 (72·7)	

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia.

*As defined by the mother of the index.

†Includes two Japanese descendants, two Indians and six without information.

‡Corresponds to the place of birth of the index.

§In 8 ALL patients (2·1%), it was not possible perform the immunophenotype sub-classification, given that 3 patients were aged $\leq$12 months and 5 were 13–24 months of age.

¶*MLL* gene status was indeterminate in 116 cases (21·3%): 46 in the age group $\leq$12 months and 70 in the 13–24 months group.

Table I shows that 41% of the patients investigated displayed a rearranged *MLL* allele ($n = 176$). In addition, *MLL*-r were significantly more frequent among patients aged ≤ 6 months than among patients aged >12 months at diagnosis ($P < 0{\cdot}001$). Most of the *MLL*-r cases displayed a pro-B ALL phenotype (56·2% of the overall samples and 75·6% of the ALL samples). *MLL*-r were detected in 27·3% ($n = 54$) of patients older than 12 months. Of these 54 patients, 37 had ALL (68·5%) and 17 had AML (31·5%). Of patients younger than 12 months with *MLL*-r, 77% had ALL and 23% had AML. In 80 cases, other recurrent chromosomal alterations frequently found in childhood leukaemia were detected. These aberrations were considered mutually exclusive to *MLL*-r. Thirty-six cases had inappropriate biological material to perform the analysis.

As summarized in Supplementary Table I, IL were more prone to harbouring an *MLL*-r, while children older than 12 months displayed less rearrangements (OR 0·26; 95% CI 0·15–0·43). This association was statistically significant when infant ALL cases were compared to childhood ALL cases (OR 0·13; 95% CI 0·06–0·24). *Vice-versa*, no such association was evident among children of the same age strata and diagnosed with AML (OR 1·15; 95% CI 0·43–3·03). Females were more likely than males to present with *MLL*-r (OR 1·55; 95% CI 1·05–2·29); this increased risk for females was higher in AML patients (OR 2·20; 95% CI 1·04–4·64) and not statistically significant in ALL patients. When analysed by place of birth, only ALL patients from the Northeast region exhibited a three-fold higher of *MLL*-r (OR 3·25; 95% CI 1·36–7·73) compared to ALL patients from the South or AML patients. Among patients with *MLL*-r, a higher WBC was more strongly associated with ALL (OR 3·20; 95% CI 1·94–5·29) than with AML (OR 2·19; 95% CI 1·05–4·57).

The precise translocation partner gene (TPG) of the rearranged allele was elucidated at the molecular level for 148 patients (84·1%). For 28 patients, no sufficient amount of genomic DNA material was left for further molecular analysis. Three TPG types accounted for 77·8% ($n = 137$) of all *MLL*-r leukaemia samples: 85 patients (48·3%) were diagnosed with an *MLL-AFF1*, 27 (15·3%) with an *MLL-MLLT3*, and 25 (14·2%) with an *MLL-MLLT1* fusion gene. The remaining 11 cases (6·2%) were diagnosed with the following *MLL* TPGs: *MLLT10* ($n = 4$), *ELL* ($n = 3$), *MLLT4* ($n = 1$), *EPS15* (1p32; $n = 1$), *NEBL* (3q21; $n = 1$), and *SEPT6* (17q23; $n = 1$) (Table II). The *MLLT3* partner gene was more frequently observed in males (63·0%, vs. 37·0% in females); the other TPGs presented with equal gender distributions. The presence of the *MLL-AFF1* fusion gene was strongly associated with a pro-B ALL immunphenotype. The co-existence of *MLL-AFF1* and *AFF1-MLL* fusion transcripts was observed in 71·1% of t (4;11) cases and, in 80·7% of these cases, a high WBC was observed ($P = 0{\cdot}029$). Patients diagnosed with both an *MLL*-r and T-ALL subtype were exclusively associated with the *MLL-MLLT1* fusion gene. In AML, *MLLT3* was the most common TPG, accounting for 44·5% of all AML-positive cases. All *MLL*-r patients were associated with a high WBC count. In case of *MLL-MLLT1* fusions, a greater frequency of hyperleucocytosis was observed.

MLL breakpoint distribution

The analysis of genomic breakpoints within the *MLL* breakpoint cluster region was performed in a subset of 55 *MLL*-r with available biological material and successfully determined in 30 cases. The correlation with age at diagnosis, gender, leukaemia subtype, and WBC count is shown in Fig 1. The breakpoint distribution according to age displays a preference for recombination events affecting *MLL* intron 11 in children ≤ 6 months at diagnosis (83·0%). Conversely, children aged 7–12 months presented with a preference for recombination events occurring in *MLL* intron 9 (63·6%). These differences were highly significant ($P = 0{\cdot}005$). Male patients presented with a similar distribution between *MLL* intron 9 (35·0%) and intron 11 (40·0%), while female patients displayed recombination events remarkably more frequently in *MLL* intron 11 (70·0%). ALL patients had chromosomal breakpoints within intron 11 in 11 of 20 (55·0%) cases, while AML cases had breakpoints in *MLL* intron 9 and intron 11 at similar frequencies (40%). A breakpoint within intron 11 was associated with a higher WBC count at diagnosis ($P = 0{\cdot}047$). The multivariate analysis showed that the risk of having the *MLL* breakpoint affecting intron 11 is increased for patients ≤ 6 months [(OR 22·9; 95% CI 2·2–237·4), $P = 0{\cdot}009$] and for patients with high WBC [(OR 13·2; 95% CI 1·1–152·9), $P = 0{\cdot}039$].

Survival analyses

OS was 73·3% for ALL patients and 45·4% for AML patients. Mean follow-up time from diagnosis was 75·1 months (95% CI 67·2–83·1). Follow up data was available for 469 patients of which 270 were alive (57·6%) and 199 died (42·3%). Of the 199 deceased patients, 150 deaths (73·4%) occurred in the first 12 months of treatment, and 115 deaths (57·8%) occurred in the first 6 months after diagnosis. Of the 270 living patients, 75% have been followed for at least 36 months. Variables associated with significant poorer outcomes were age at diagnosis ≤ 6 months ($P < 0{\cdot}001$), high WBC count ($P = 0{\cdot}048$), AML subtype ($P = 0{\cdot}009$), *MLL*-r ($P = 0{\cdot}002$), and *MLL* breakpoint region ($P = 0{\cdot}018$) (Table III; Fig 2). However, when leukaemia subtypes were analysed separately, a high WBC count and *MLL*-r conferred a significantly worse prognosis only for children with ALL, and not for those with AML. OS has also been associated with *MLL* TPGs. Although not statistically significant, *AFF1* and *MLLT3* presented with a poor prognosis in the analysis including all patients ($P = 0{\cdot}986$). The univariate analyses of OS according to TPG were also performed, separating *MLL-AFF1* versus other TPG in ALL cases and *MLL-MLLT3* versus other TPG in AML cases, but no statistically significant difference was observed.

Table II. Demographical and laboratorial characteristics of patients according to *MLL* rearrangements and specific fusion partners

	MLL rearranged (%)						
	All (n = 176)	*AFF1* (n = 85)			*MLLT3* (n = 25)	*MLLT3* (n = 27)	Other* (n = 39)
		Total†	Transcripts verified‡ (n = 45)				
			MLL-AFF1	*MLL-AFF1/AFF1-MLL*			
Age at diagnosis (months)							
≤6 months	59 (33·5)	33 (38·8)	7 (53·8)	13 (40·6)	10 (40·0)	6 (22·2)	10 (25·7)
7–12 months	63 (35·8)	28 (71·8)	3 (23·1)	12 (37·5)	10 (40·0)	9 (33·4)	16 (41·0)
13–24 months	54 (30·7)	24 (28·2)	3 (23·1)	7 (21·9)	5 (20·0)	12 (44·4)	13 (33·3)
			P = 0·623				
Gender							
Male	88 (50·0)	39 (45·9)	6 (46·1)	12 (38·7)	12 (48·0)	17 (63·0)	20 (51·3)
Female	88 (50·0)	46 (54·1)	7 (53·9)	19 (61·3)	13 (52·0)	10 (37·0)	19 (48·7)
			P = 0·048				
Race/skin colour							
White	104 (59·1)	48 (56·5)	8 (61·5)	19 (61·3)	18 (72·0)	15 (55·6)	23 (59·0)
Non-White	68 (38·6)	35 (41·2)	4 (30·8)	12 (38·7)	7 (28·0)	12 (44·4)	14 (35·9)
Other	4 (2·3)	2 (2·4)	1 (7·7)	-	0 (0·0)	0 (0·0)	2 (5·1)
			P = 0·280				
Region of Brazil							
South	13 (7·4)	4 (4·7)	1 (7·7)	1 (3·3)	1 (4·0)	3 (11·1)	5 (12·8)
Southeast	65 (36·9)	28 (32·9)	2 (15·4)	8 (25·8)	11 (44·0)	8 (29·6)	18 (46·2)
Northeast	75 (42·6)	38 (44·7)	8 (61·5)	13 (41·9)	13 (52·0)	11 (40·7)	13 (33·3)
Middle-West	23 (13·1)	15 (17·6)	2 (15·4)	9(29·0)	0 (0·0)	5 (18·5)	3 (7·7)
			P = 0·520				
Leukaemia subtype							
ALL	131 (74·4)	72 (84·7)	11 (84·6)	27 (87·1)	23 (92·0)	15 (55·5)	21 (53·8)
AML	45 (25·6)	13 (15·3)	2 (15·4)	4 (12·9)	2 (8·0)	12 (44·5)	18 (46·2)
			P = 0·048				
WBC count ($\times 10^9$/l)							
<50	51 (29·0)	24 (28·2)	7 (22·6)	6 (19·3)	4 (16·0)	12 (44·4)	11 (28·2)
≥50	125 (71·0)	61 (71·8)	6 (19·4)	25 (80·7)	21 (84·0)	15 (55·6)	28 (71·8)
			P = 0·029				

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; WBC, white blood cell.

*Includes *MLLT10* (n = 4), *ELL* (n = 3), *MLLT4* (n = 1), *EPS15* (1p32; n = 1), *NEBL* (n = 1), *SEPT6* (n = 1) and unidentified *MLL* translocation partner gene (n = 28).

†Total of all patients with *AFF1* as *MLL* translocation partner gene.

‡Patients with enough biological material were further investigated for the presence of both reciprocal *MLL-AFF1* and *AFF1-MLL* transcripts and the differences were statistically tested.

The univariate analyses of OS according to age group in AL are shown in Table IV. Of the patients aged ≤12 months, variables with significant poorer outcomes were high WBC count (P = 0·026), *MLL*-r (P = 0·047), and *MLL* breakpoint region (P = 0·052). When patients older than 12 months were analysed, only *MLL*-r conferred a significantly worse prognosis. Because an increased risk to harbour an *MLL*-r was observed among female patients, we analysed whether the combined status of gender and *MLL* affected the OS. There was a statistically significant difference between the groups (P < 0·001), with the worst survival observed in females with *MLL*-r (Fig 1F). This difference was also significant when analysing the different subgroups: ALL, AML, younger than 12 months, and aged 13–24 months (data not shown).

In the investigated cohort (ALL and AML), multivariate analysis revealed that age at diagnosis (P = 0·003; HR 0·74, 95% CI 0·60-0·90), WBC count (P = 0·049; HR 1·40, 95% CI 1·06–1·97), AL subtype (ALL vs. AML, P = 0·007; HR 1·59, 95% CI 1·14-2·23), *MLL* status (P = 0·034; HR 1·61, 95% CI 1·05–2·51), and *MLL* breakpoint region (P = 0·019; HR 4·95, 95% CI 1·30–18·85) conferred adverse risk. Likewise, Cox regression analysis in ALL patients demonstrated that age at diagnosis (P = 0·036; HR 0·76, 95% CI 0·59–0·97), WBC

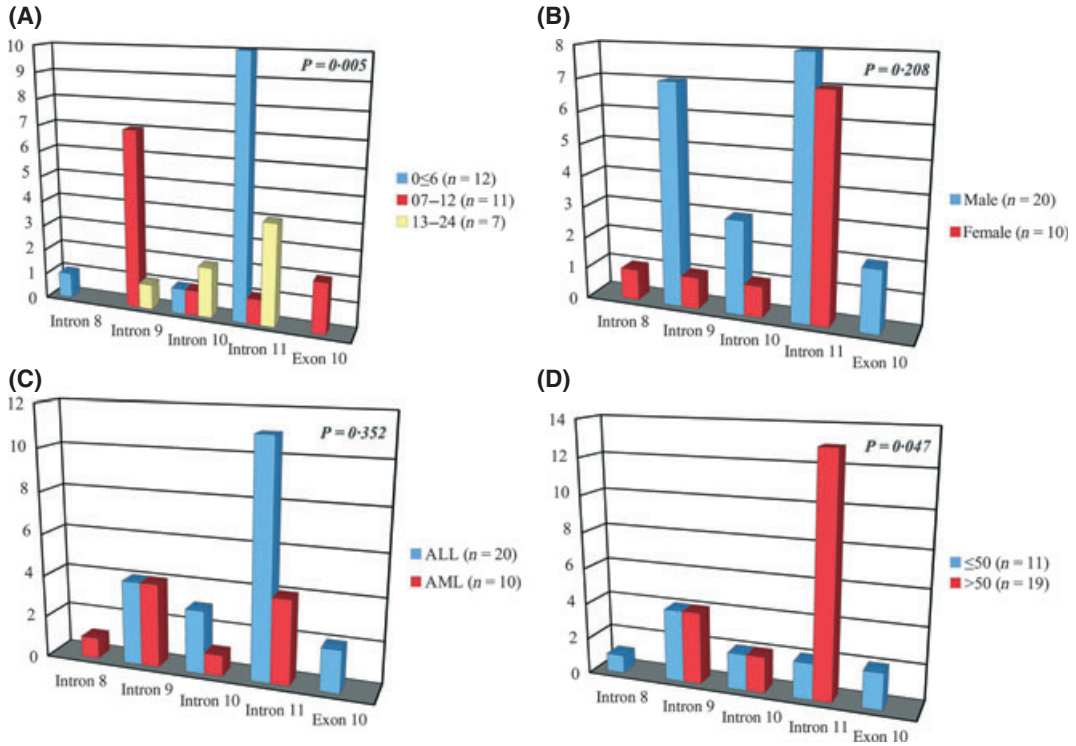

Fig 1. *MLL* breakpoints distribution according to (A) age at diagnosis (months) (B) gender (C) leukaemia subtype (D) white blood cell count ($\times 10^9$/l). ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia.

count (P = 0·002; HR 1·99, 95% CI 1·28–3·10), and *MLL* status (P = 0·020; HR 1·93, 95% CI 1·12–3·37) were identified as independent prognostic factors. No independent risk factors could be identified for the investigated AML cases.

Discussion

In this series of early childhood leukaemia, the frequency of *MLL*-r separated the cohort into 74·4% ALL and 25·6% AML, in agreement with several previous reports (Borkhardt *et al*, 2002; Greaves, 1999; Harrison *et al*, 2010; Jansen *et al*, 2007; Pieters *et al*, 2007; Pui *et al*, 1995; Sam *et al*, 2011; von Neuhoff *et al*, 2010). It also corroborates the findings of a recent report (Sam *et al*, 2011), in which younger age was predominantly associated with ALL rather than with AML. The observation that *MLL*-r IL patients are diagnosed earlier than *MLL* germline patients has been demonstrated by others (Jansen *et al*, 2007; Pieters *et al*, 2007; Pui *et al*, 1995; Sam *et al*, 2011; Sorensen *et al*, 1994). The molecular epidemiological approach in several genetic studies has raised the concept that most, if not all, childhood leukaemia cases originate *in utero* (Greaves & Wiemels, 2003). Despite these findings, the underlying mechanism that determines the latency of leukaemia development remains to be resolved. Increased apoptotic rates due to the presence of certain MLL fusion proteins in non-permissive cell types may be a potential mechanism that explains the likelihood of developing a leukaemia (reviewed in Marschalek, 2011); however, the knowledge concerning the tolerance, lineage restriction, and other potential mechanisms deriving from different *MLL*-r alleles remains limited. For example, children with T-ALL are usually diagnosed at older ages. However, in this same series of IL cases, the existence of T-ALL was identified. The availability of such a rare epidemiological set of cases prompted us to conduct a molecular screening of leukaemia- and clone-specific biological markers, which could be helpful in understanding the critical events leading to T-ALL in such a short latency time (Mansur *et al*, 2010).

In agreement with earlier studies (Ross & Robison, 1997; Sam *et al*, 2012), the increased likelihood of harbouring an *MLL*-r in females was observed here. This risk was increased in female patients with AML. It has been postulated that an elevated risk of IL in females would suggest that there is a hormonal trigger associated with female and higher risk (Ross *et al*, 1997). Indeed, while testing the potential role of intrauterine and perinatal factors associated with IL risk, we have demonstrated a greater association between maternal use of oestrogen during pregnancy and IL risk (OR 8·76; 95% CI 2·85–26·93) (Pombo-de-Oliveira & Koifman, 2006). Motivated by this epidemiological data, Schnyder *et al* (2009) performed an *in vitro* model of translocation susceptibility and determined that higher concentrations of oestrogens (estradiol-E2 and 4-OH-E2) might indeed be implicated in the occurrence of *MLL*-r. In addition, we describe here that

Table III. Univariate analysis of overall survival (OS) according to AL subtypes

	All cases		ALL		AML	
	OS* (95% CI)	*P*†	OS* (95% CI)	*P*†	OS* (95% CI)	*P*†
Age at diagnosis (months)						
≤6	10·7 (7·1–14·2)	<0·001	12·2 (8·6–15·7)	<0·001	6·5 (1·0–12·0)	0·016
7–12	37·3 (16·0–58·6)		42·1 (4·1–80·2)		29·0 (8·6–49·3)	
13–24	63·0 (52·9–73·2)		67·1 (55·6–78·6)		30·9 (21·3–40·5)	
WBC ($x10^9$/l)						
<50	72·1 (64·1–86·2)	0·048	85·3 (72·1–98·5)	0·001	13·1 (2·6–23·6)	0·337
≥50	24·5 (8·0–41·0)		23·1 (4·5–41·7)		29·0 (22·3–45·3)	
MLL status						
Rearranged	14·2 (10·1–18·2)	0·002	16·1 (7·9–24·2)	0·002	7·3 (1·8–12·7)	0·087
Germline	57·3 (51·3–68·4)		72·1 (63·9–74·7)		21·5 (5·1–37·9)	
MLL TPGs						
AFF1	13·8 (7·3–20·3)	0·986	14·1 (9·9–18·3)	0·920	7·3 (2·7–11·8)	0·857
MLLT1	34·2 (0·0–70·6)		34·2 (0·0–85·9)		2·2 (0·0–11·7)	
MLLT3	17·3 (10·4–24·1)		21·1 (13·6–28·5)		3·6 (1·4–5·8)	
Other[c]	12·2 (8·8–15·5)		12·2 (7·9–24·2)		4·8 (0·0–18·5)	
MLL breakpoint region						
Intron 11	6·8 (0·0–14·5)	0·018	6·8 (0·0–14·9)	0·085	6·6 (0·0–16·5)	0·091
Other	42·1 (15·2–69·1)		21·1 (13·4–28·8)		61·1 (22·3–99·9)	
t(4;11) transcripts (ALL cohort)						
MLL-AFF1	2·3 (0·5–11·8)	0·206	1·0 (0·6–3·2)	0·161	–	
MLL-AFF1/AFF1-MLL	8·2 (0·7–17·3)		9·0 (0·8–21·6)		–	

OS, overall survival; 95% CI, 95% confidence interval; WBC, white blood cell count; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; TPGs, translocation partner genes; ND, not determined; cIncludes *MLLT10* ($n = 4$), *ELL* ($n = 3$), *MLLT4* ($n = 1$), *EPS15* (1p32; $n = 1$), *NEBL* ($n = 1$), *SEPT6* ($n = 1$) and unidentified *MLL* TPG ($n = 31$).

*Median survival.

†Mantel-Cox (log-rank) test.

66·7% of female patients had the breakpoints located in intron 11. Thus, the reported higher incidence of *MLL*-r in females may account for the higher probability of recombinant events in intron 11, which also correlated with younger age at diagnosis, ALL subtype, and higher WBC count.

We also tested whether ethnicity was associated with the likelihood of acquiring an *MLL*-r. We were able to demonstrate that children defined as non-White had an increased likelihood of AL with an *MLL*-r. Aware of the pitfalls of separating the Brazilian mixed population by skin colour (Pena *et al*, 2011), we analysed the patients according to Brazilian regions. Non-Whites are more prevalent in the Northeast of Brazil, which presented a greater likelihood of *MLL*-r. This result partly contradicts the findings described by Sam *et al* (2012), which claimed that Blacks had a lower probability of *MLL*-r. However, because of the differences in ethnicity definition among Brazilians, it is difficult to draw conclusions from this comparison. Altogether, both our and the former studies suggest that differences in genetic susceptibility and the variability of environmental exposures may explain these findings. Furthermore, infants can also be indirectly exposed to toxins through maternal exposure and the physiological immaturity of the xenobiotic system. We have previously investigated the effect of genotype of enzymes that act in the detoxification phase of the xenobiotic system. We were able to identify a four-fold increased risk for developing IL when *NAT2* displays a slow acetylating profile. The combination of maternal and fetal slow acetylating profiles was one of the most important risk factors for IL and early childhood leukaemia (Zanrosso *et al*, 2010). We also demonstrated an association between maternal pesticide exposure (postulated to cause DNA alterations) during the periconceptional period or during early pregnancy and the development of childhood leukaemia (Pombo-de-Oliveira & Koifman, 2006; Ferreira *et al*, 2012).

In our study, patients born in the Northeast presented with a higher risk for *MLL*-r; however, the frequency of *MLL* TPGs *MLL-AFF1* (46%), *MLL-MLLT1* (18%) and *MLL-MLLT3* (16%) among these patients was similar to those of patients from other areas. Therefore, we conclude that the frequency of specific *MLL* TPGs in Brazilian IL resembles the findings that were obtained worldwide. Our data shows an expressive percentage of *MLL*-r cases in children older than 12 months, 30·6% (54 out of 176 rearranged cases). Regarding the prognosis value of *MLL* TPGs, a major observational study of infant ALL did not identify any association between type of *MLL*-r and outcome (Pieters *et al*, 2007). This is consistent with our findings in

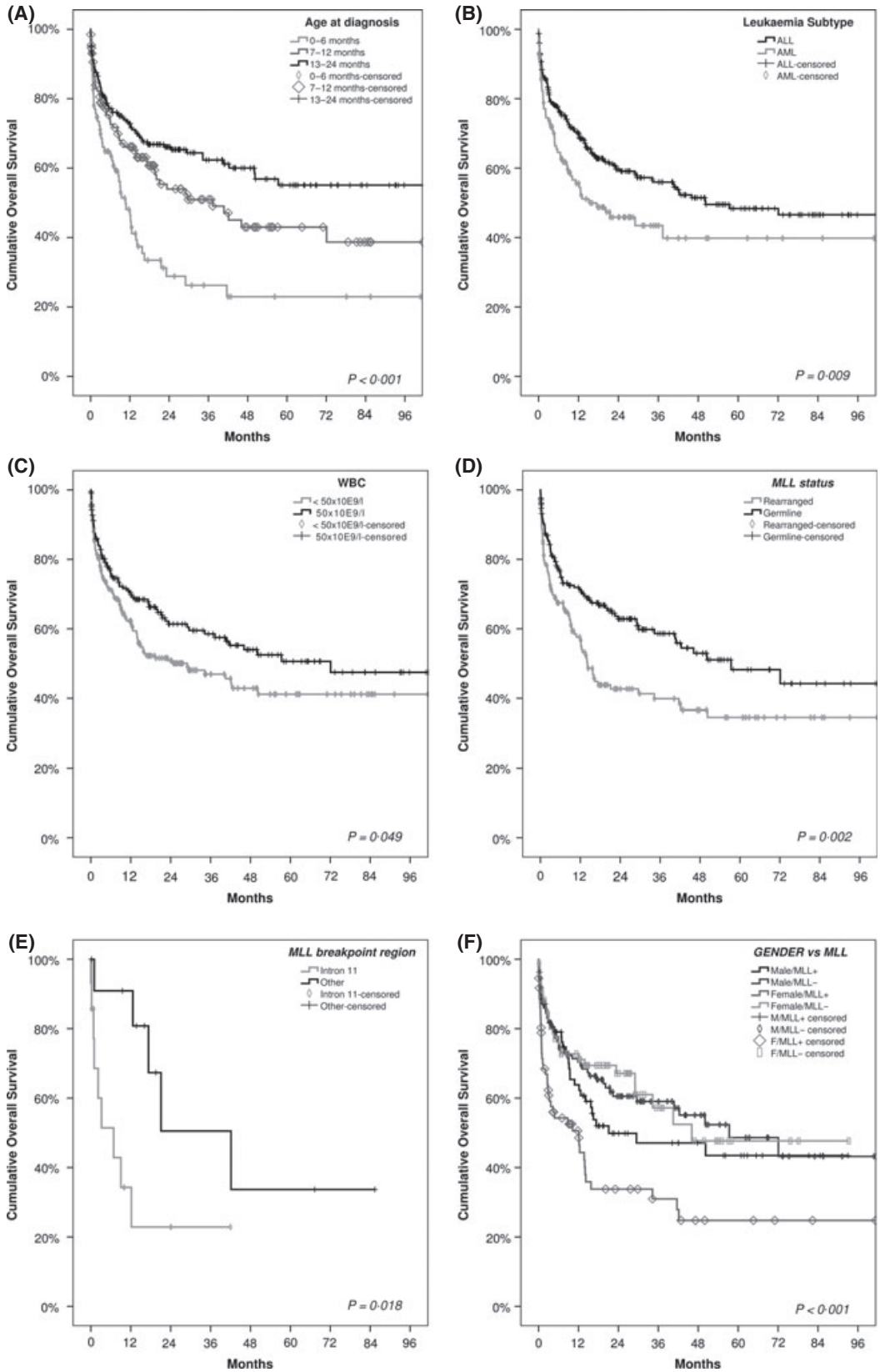

Fig 2. Kaplan-Meier overall survival (OS) curves for infant patients according to (A) age at diagnosis (months) (B) acute leukaemia subtype (ALL [acute lymphoblastic leukaemia] vs. AML [acute myeloid leukaemia]) (C) white blood cell (WBC) count ($\times 10^9$/l) (D) *MLL* status (E) *MLL* breakpoint region, and (F) gender (M, male; F, female) and *MLL* status. Symbols represent censored cases.

Table IV. Univariate analysis of overall survival according to age groups in AL patients.

	≤ 12 months		13–24 months	
	OS* (95% CI)	*P*†	OS[a]* (95% CI)	*P*†
WBC (x10^9/l)				
<50	37·3 (16·9–57·7)	0·026	85·4 (71·6–99·3)	0·815
≥ 50	12·3 (8·5–16·1)		64·7 (53·5–75·9)	
Leukaemia				
ALL	19·7 (8·6–30·8)	0·128	87·1 (75·6–98·6)	0·073
AML	12·5 (1·0–24·0)		40·9 (31·3–50·5)	
MLL status				
Rearranged	12·3 (7·7–16·9)	0·047	46·8 (32·8–60·8)	0·020
Germline	29·0 (9·4–48·6)		67·4 (56·6–78·3)	
MLL TPGs				
AFF1	12·3 (7·5–17·1)	0·791	48·2 (27·4–69·1)	0·840
MLLT1	14·2 (8·7–25·2)		26·2 (5·8–46·5)	
MLLT3	16·4 (0·9–39·8)		49·2 (24·5–73·9)	
Other[c]	10·8 (1·3–20·3)		77·9 (37·6–118·2)	
MLL breakpoint region				
Intron 11	6·8 (0·0–14·9)	0·052	0·6 (0·0–2·3)	0·222
Other	21·1 (0·0–44·6)		12·6 (5·6–15·6)	
t(4;11) transcripts (ALL cohort)				
MLL-AFF1	6·9 (1·4–18·7)	0·830	0·5 (0·2–1·9)	0·010
MLL-AFF1/AFF1-MLL	8·2 (3·4–16·4)		13·7 (0·2–27·1)	

WBC, white blood cell count; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; TPGs, translocation partner genes; ND, not determined; cIncludes *MLLT10* (*n* = 4), *ELL* (*n* = 3), *MLLT4* (*n* = 1), *EPS15* (1p32; *n* = 1), *NEBL* (*n* = 1), *SEPT6* (*n* = 1) and unidentified *MLL* TPG (*n* = 31).

*Median survival.

†Mantel-Cox (log-rank) test.

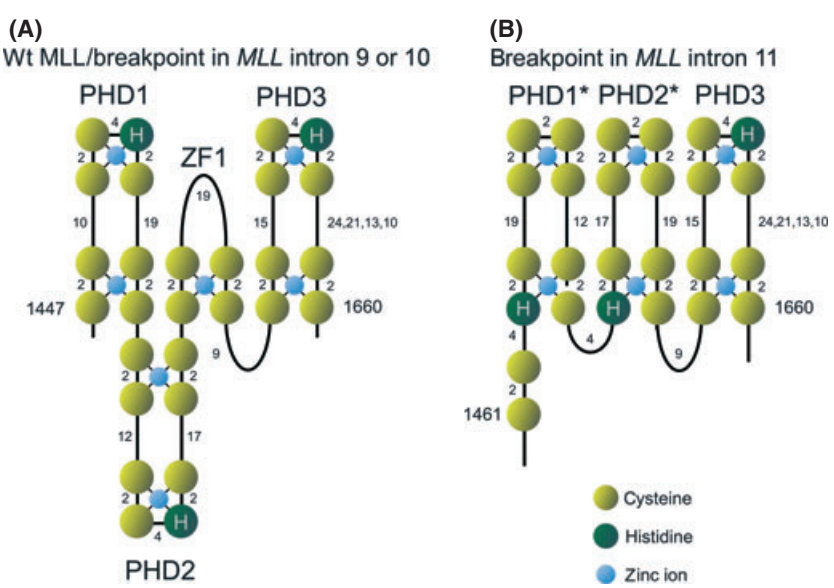

Fig 3. Schematic structure of the MLL PHD domain. PHD Fingers are composed of amino acid sequences Cys(2)Cys(X)Cys(2)Cys(4)His (2)Cys(Y)Cys(2)C (numbers represent spacing amino acids). Cysteine residues are displayed as light green spheres, while histidine residues are displayed as dark green spheres. Smaller light blue spheres indicate zinc ions. (A) The position of the MLL wild type PHD domain is displayed. Between PHD2 and 3, a normal zinc finger (ZF) is present. (B) The situation after deleting *MLL* exon 11 encoded amino acids is displayed. Assuming that the consecutive cysteine and histidine residues are still able to fold into distinct structures, PHD1 and PHD2 are changed into non-PHD fingers and ZF1, a portion of extended PHD3, is missing. This may cause an inability to bind to CYP33.

ALL cases. In paediatric AML, in which the frequency of *MLL*-r varies between 15% and 20% the outcome was not significantly different when comparing the different *MLL* TPGs, as we observed a dismal OS for all of them. The prognostic significance of *MLL* TPGs in AML remains uncertain. Nevertheless, recent studies have contributed to future risk stratification. A study evaluating incidence and risk of chromosomal abnormalities in 720 children (0 to 16 years) registered on Medical Research Council AML trials did not show a significantly improved outcome of

patients with t(9;11) when compared with other *MLL* subgroups (Harrison *et al*, 2010). Another study analysed the cytogenetic data of a large cohort treated nearly uniformly in AML-Berlin-Frankfurt-Münster (BFM) 98 and observed that children with t(9;11) and additional aberrations and children with *MLL*-r other than *t*(9;11) and *t*(11;19) had an unfavourable outcome (von Neuhoff *et al*, 2010). A large retrospective analysis observed that patients with a *t*(1;11) showed independent favourable outcome and several other rearrangements were identified as predictors of poor clinical outcome, including *t*(6;11)(q27;q23), *t*(10;11)(p11·2;q23), *t*(4;11)(q21;q23), and *t*(10;11)(p12;q23) (Balgobind *et al*, 2009). In both AL subtypes, few investigations had examined the aetiology and prognosis of children aged between 13 and 24 months, taking into account that a prenatal origin for these *MLL*-r is also plausible (Alexander *et al*, 2001; Pombo-de-Oliveira & Koifman, 2006). We could verify that *MLL*-r is an independent prognostic factor for ALLs conferring poor outcome even for the patients aged 13–24 months.

Finally, we identified a strong association between breakpoint distributions within *MLL*-r cases and clinical course. Breakpoints in *MLL* intron 11 were described as associated with infant-ALL and therapy-related leukaemia (Reichel *et al*, 2001). There is also a known higher frequency of breakpoints within intron 11 in paediatric ALL (Meyer *et al*, 2009). To our knowledge, this is the first time that a breakpoint-dependent impact of *MLL*-r on clinical progression – synonymously reflected by initial high WBC count and young age at diagnosis – has been identified in a clinical study. Although the survival analyses according to *MLL* breakpoints was jeopardized due to the number of available cases, the OS for patients with intron 11 breakpoints was significantly poorer compared to other *MLL*-r introns. This might be explained by the fact that *MLL* exons 11–16 encode the PHD domain (PHD1, PHD2, and PHD3). Chromosomal breakpoints within *MLL* intron 11 will disrupt the structure of the first PHD finger, and thus, similarly cause mis-folding of the remaining domain due to alternative Cys/His-pairings (see Fig 3). Only breakpoints within *MLL* introns 9 and 10 will not affect the three PHD finger domains. Mis-folding of these important domains is related to two different mechanisms: PHD2 is able to confer dimerization of MLL, while PHD3 is able to bind to cyclophylin 33 (CYP33) (Fair *et al*, 2001). Binding of CYP33 to PHD3 causes a regulatory switch of the MLL protein, because CYP33 binds to HDAC1/2, CtBP, and the Polycomb-group proteins HCP2 and BMI1. This causes a switch of a promoter-activating MLL complex to a promoter-repressing MLL complex, as explored in detail elsewhere (Rössler & Marschalek, 2012). It is therefore a plausible hypothesis that breakpoints in *MLL* intron 11 result in fusion proteins that exert different biological functions on the affected cells, most likely eliminating a negative regulatory function normally exerted by the wild type MLL complex. By contrast, breakpoints that occur in other introns of the *MLL* breakpoint cluster region should not have such an impact. This difference may explain our observed clinical variations in *MLL*-r patients with a breakpoint in *MLL* intron 11.

In summary, we have shown for the first time that *MLL* intron 11 recombination events are associated with early disease onset and high WBC count, indicating that a disruption of the PHD domains may be associated with a worse clinical course. This new knowledge should be validated by additional studies. Finally, if this information can be verified, both the presence of an *MLL*-r itself and the location of the breakpoint should be taken into account in clinical practice.

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Authors' contributions

ME, RM, and MSPO contributed to study design. ME, CM, MBM, RM, and MSPO contributed to drafting of the manuscript. ME and CM performed the molecular studies. ME and MBM contributed to the statistical analysis. MSPO contributed to the conception of the study, data collection, and critical analysis of the data. All co-authors of the Brazilian Collaborative Study Group of Infant Acute Leukaemia contributed with clinical and demographical data. LCST contributed to the statistical analysis.

Conflict of interest

The authors have no competing interests.

Appendix I

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. Comparisons regarding *MLL* final status and demographical and laboratorial characteristics.

References

Alexander, F. E., Patheal, S. L., Biondi, A., Brandalise, S., Cabrera, M. E., Chan, L. C., Chen, Z., Cimino, G., Cordoba, J. C., Gu, L. J., Hussein, H., Ishii, E., Kamel, A. M., Labra, S., Magalhaes, I. Q., Mizutani, S., Petridou, E., de Oliveira, M. P., Yuen, P., Wiemels, J. L. & Greaves, M. F. (2001) Transplacental chemical exposure and risk of infant leukemia with MLL gene fusion. *Cancer Research*, **61**, 2542–2546.

Bain, B. J. (2006) Blood Cells: a practical guide, 4th edn. Blackwell Publishing, Oxford.

Balgobind, B. V., Raimondi, S. C., Harbott, J., Zimmermann, M., Alonzo, T. A., Auvrignon, A., Beverloo, H. B., Chang, M., Creutzig, U., Dworzak, M. N., Forestier, E., Gibson, B., Hasle, H., Harrison, C. J., Heerema, N. A., Kaspers, G. J., Leszl, A., Litvinko, N., Nigro, L. L., Morimoto, A., Perot, C., Pieters, R., Reinhardt, D., Rubnitz, J. E., Smith, F. O., Stary, J., Stasevich, I., Strehl, S., Taga, T., Tomizawa, D., Webb, D., Zemanova, Z., Zwaan, C. M. & Van den Heuvel-Eibrink, M. M. (2009) Novel prognostic subgroups in childhood 11q23/MLL-rearranged acute myeloid leukemia: results of an international retrospective study. *Blood*, **114**, 2489–2496.

Biondi, A., Cimino, G., Pieters, R. & Pui, C. H. (2000) Biological and therapeutic aspects of infant leukemia. *Blood*, **96**, 24–33.

Borkhardt, A., Wuchter, C., Viehmann, S., Pils, S., Teigler-Schlegel, A., Stanulla, M., Zimmermann, M., Ludwig, W. D., Janka-Schaub, G., Schrappe, M. & Harbott, J. (2002) Infant acute lymphoblastic leukemia - combined cytogenetic, immunophenotypical and molecular analysis of 77 cases. *Leukemia*, **16**, 1685–1690.

Brandalise, S. R., Pinheiro, V. R., Aguiar, S. S., Matsuda, E. I., Otubo, R., Yunes, J. A., Pereira, W. V., Carvalho, E. G., Cristofani, L. M., Souza, M. S., Lee, M. L., Dobbin, J. A., Pombo-de-Oliveira, M. S., Lopes, L. F., Melnikoff, K. N., Brunetto, A. L., Tone, L. G., Scrideli, C. A., Morais, V. L. & Viana, M. B. (2010) Benefits of the intermittent use of 6-mercaptopurine and methotrexate in maintenance treatment for low-risk acute lymphoblastic leukemia in children: randomized trial from the Brazilian Childhood Cooperative Group-protocol ALL-99. *Journal of Clinical Oncology*, **28**, 1911–1918.

van Dongen, J. J., Macintyre, E. A., Gabert, J. A., Delabesse, E., Rossi, V., Saglio, G., Gottardi, E., Rambaldi, A., Dotti, G., Griesinger, F., Parreira, A., Gameiro, P., Diaz, M. G., Malec, M., Langerak, A. W., San Miguel, J. F. & Biondi, A. (1999) Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. *Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. Leukemia*, **13**, 1901–1928.

Emerenciano, M., Agudelo Arias, D. P., Coser, V. M., de Brito, G. D., Macedo Silva, M. L. & Pombo-de-Oliveira, M. S. (2006) Molecular cytogenetic findings of acute leukemia included in the Brazilian Collaborative Study Group of Infant acute leukemia. *Pediatric Blood & Cancer*, **47**, 549–554.

Emerenciano, M., Renaud, G., Sant'Ana, M., Barbieri, C., Passetti, F. & Pombo-de-Oliveira, M. S. (2011) Challenges in the use of NG2 antigen as a marker to predict MLL rearrangements in multi-center studies. *Leukemia Research*, **35**, 1001–1007.

Fair, K., Anderson, M., Bulanova, E., Mi, H., Tropschug, M. & Diaz, M. O. (2001) Protein interactions of the MLL PHD fingers modulate MLL target gene regulation in human cells. *Molecular Cell Biology*, **21**, 3589–3597.

Ferreira, J. D., Couto, A. C., Pombo-de-Oliveira, M. S. & Koifman, S. & Brazilian Collaborative Study Group of Infant Acute Leukemia. (2012) *In utero* pesticide exposure and leukemia in Brazilian children < 2 years of age. *Environmental Health Perspectives*. [Epub ahead of print] doi: 10·1289/ehp.1103942.

Ford, A. M., Ridge, S. A., Cabrera, M. E., Mahmoud, H., Steel, C. M., Chan, L. C. & Greaves, M. (1993) *In utero* rearrangements in the trithorax-related oncogene in infant leukaemias. *Nature*, **363**, 358–360.

Gonçalves, B. A. A., Vasconcelos, G. M., Thuler, L. C., Andrade, C., Faro, A. & Pombo-de-Oliveira, M. S. & Brazilian Collaborative Study Group of Infant Acute Leukemia. (2012) NQO1 rs1800566 (C609T), PON1 rs662 (Q192R), and PON1 rs854560 (L55M) polymorphisms segregate the risk of childhood acute leukemias according to age range distribution. *Cancer Causes and Control*, **23**, 1811–1819.

Greaves, M. (1999) Molecular genetics, natural history and the demise of childhood leukaemia. *European Journal of Cancer*, **35**, 1941–1953.

Greaves, M. F. & Wiemels, J. (2003) Origins of chromosome translocations in childhood leukaemia. *Nature Reviews Cancer*, **3**, 639–649.

Harrison, C. J., Hills, R. K., Moorman, A. V., Grimwade, D. J., Hann, I., Webb, D. K., Wheatley, K., de Graaf, S. S., van den, B. E., Burnett, A. K. & Gibson, B. E. (2010) Cytogenetics of childhood acute myeloid leukemia: United Kingdom Medical Research Council Treatment trials AML 10 and 12. *Journal of Clinical Oncology*, **28**, 2674–2681.

Jansen, M. W., Corral, L., van, d. V., Panzer-Grumayer, R., Schrappe, M., Schrauder, A., Marschalek, R., Meyer, C., den den Boer, M. L., Hop, W. J., Valsecchi, M. G., Basso, G., Biondi, A., Pieters, R. & van Dongen, J. J. (2007) Immunobiological diversity in infant acute lymphoblastic leukemia is related to the occurrence and type of MLL gene rearrangement. *Leukemia*, **21**, 633–641.

Mann, G., Cazzaniga, G., van, d. V., Flohr, T., Csinady, E., Paganin, M., Schrauder, A., Dohnal, A. M., Schrappe, M., Biondi, A., Gadner, H., van Dongen, J. J. & Panzer-Grumayer, E. R. (2007) Acute lymphoblastic leukemia with t(4;11) in children 1 year and older: the 'big sister' of the infant disease? *Leukemia*, **21**, 642–646.

Mansur, M. B., Emerenciano, M., Splendore, A., Brewer, L., Hassan, R. & Pombo-de-Oliveira, M. S., & Brazilian Collaborative Study Group of Infant Acute Leukemia. (2010) T-cell lymphoblastic leukemia in early childhood presents NOTCH1 mutations and MLL rearrangements. *Leukemia Research*, **34**, 483–486.

Marschalek, R. (2011) Mechanisms of leukemogenesis by MLL fusion proteins. *British Journal of Haematology*, **152**, 141–154.

Meyer, C., Schneider, B., Reichel, M., Angermueller, S., Strehl, S., Schnittger, S., Schoch, C., Jansen, M. W., van Dongen, J. J., Pieters, R., Haas, O. A., Dingermann, T., Klingebiel, T. & Marschalek, R. (2005) Diagnostic tool for the identification of MLL rearrangements including unknown partner genes. *Proceedings of the National Academy of Sciences*, **102**, 449–454.

Meyer, C., Kowarz, E., Hofmann, J., Renneville, A., Zuna, J., Trka, J., Ben Abdelali, R., Macintyre, E., De Braekeleer, E., De Braekeleer, M., Delabesse, E., Pombo-de-Oliveira, M. S., Cave, H. & Marschalek, R. (2009) New insights to the MLL recombinome of acute leukemias. *Leukemia*, **23**, 1490–1499.

von Neuhoff, C., Reinhardt, D., Sander, A., Zimmermann, M., Bradtke, J., Betts, D. R., Zemanova, Z., Stary, J., Bourquin, J. P., Haas, O. A., Dworzak, M. N. & Creutzig, U. (2010) Prognostic impact of specific chromosomal aberrations in a large group of pediatric patients with acute myeloid leukemia treated uniformly according to trial AML-BFM 98. *Journal of Clinical Oncology*, **28**, 2682–2689.

Pena, S. D., Di, P. G., Fuchshuber-Moraes, M., Genro, J. P., Hutz, M. H., Kehdy, F. S., Kohlrausch, F., Magno, L. A., Montenegro, R. C., Moraes, M. O., de Moraes, M. E., de Moraes, M. R., Ojopi, E. B., Perini, J. A., Racciopi, C.,

Ribeiro-Dos-Santos, A. K., Rios-Santos, F., Romano-Silva, M. A., Sortica, V. A. & Suarez-Kurtz, G. (2011) The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected. *PLoS ONE*, **6**, e17063.

Pieters, R., Schrappe, M., de, L. P., Hann, I., De, R. G., Felice, M., Hovi, L., LeBlanc, T., Szczepanski, T., Ferster, A., Janka, G., Rubnitz, J., Silverman, L., Stary, J., Campbell, M., Li, C. K., Mann, G., Suppiah, R., Biondi, A., Vora, A. & Valsecchi, M. G. (2007) A treatment protocol for infants younger than 1 year with acute lymphoblastic leukaemia (Interfant-99): an observational study and a multicentre randomised trial. *Lancet*, **370**, 240–250.

Pombo-de-Oliveira, M. S. & Koifman, S. (2006) Infant acute leukemia and maternal exposures during pregnancy. *Cancer Epidemiology Biomarkers & Prevention*, **15**, 2336–2341.

Pombo-de-Oliveira, M. S., Koifman, S., Vasconcelos, G. M., Emerenciano, M. & de Oliveira, N. C. & Brazilian Collaborative Study Group of Infant Acute Leukemia. (2009) Development and perspective of current Brazilian studies on the epidemiology of childhood leukemia. *Blood Cells, Molecules and Disease*, **42**, 121–125.

Pui, C. H., Kane, J. R. & Crist, W. M. (1995) Biology and treatment of infant leukemias. *Leukemia*, **9**, 762–769.

Reichel, M., Gillert, E., Angermuller, S., Hensel, J. P., Heidel, F., Lode, M., Leis, T., Biondi, A., Haas, O. A., Strehl, S., Panzer-Grumayer, E. R., Griesinger, F., Beck, J. D., Greil, J., Fey, G. H., Uckun, F. M. & Marschalek, R. (2001) Biased distribution of chromosomal breakpoints involving the MLL gene in infants versus children and adults with t (4;11) ALL. *Oncogene*, **20**, 2900–2907.

Ross, J. A. & Robison, L. L. (1997) MLL rearrangements in infant leukemia: is there a higher frequency in females? *Leukemia Research*, **21**, 793–795.

Ross, J. A., Davies, S. M., Potter, J. D. & Robison, L. L. (1994) Epidemiology of childhood leukemia, with a focus on infants. *Epidemiologic Reviews*, **16**, 243–272.

Ross, J. A., Potter, J. D., Shu, X. O., Reaman, G. H., Lampkin, B. & Robison, L. L. (1997) Evaluating the relationships among maternal reproductive history, birth characteristics, and infant leukemia: a report from the Children's Cancer Group. *Annals of Epidemiology*, **7**, 172–179.

Rössler, T. & Marschalek, R. (2012) An alternative splice process renders the MLL protein either into a transcriptional activator or repressor. *Die Pharmazie*, [in press]. doi: 10·1691/ph.2013·6514.

Sam, T. N., Kersey, J. H., Linabery, A. M., Johnson, K. J., Heerema, N. A., Hilden, J. M., Davies, S. M., Reaman, G. H. & Ross, J. A. (2012) MLL gene rearrangements in infant leukemia vary with age at diagnosis and selected demographic factors: a Children's Oncology Group (COG) study. *Pediatric Blood & Cancer*, **58**, 836–839.

Schnyder, S., Du, N. T., Le, H. B., Singh, S., Loredo, G. A. & Vaughan, A. T. (2009) Estrogen treatment induces MLL aberrations in human lymphoblastoid cells. *Leukemia and Research*, **33**, 1400–1404.

Schrappe, M., Reiter, A., Ludwig, W. D., Harbott, J., Zimmermann, M., Hiddemann, W., Niemeyer, C., Henze, G., Feldges, A., Zintl, F., Kornhuber, B., Ritter, J., Welte, K., Gadner, H. & Riehm, H., German-Austrian-Swiss ALL-BFM Study Group. (2000) Improved outcome in childhood acute lymphoblastic leukemia despite reduced use of anthracyclines and cranial radiotherapy: results of trial ALL-BFM 90. *Blood*, **95**, 3310–3322.

Sorensen, P. H., Chen, C. S., Smith, F. O., Arthur, D. C., Domer, P. H., Bernstein, I. D., Korsmeyer, S. J., Hammond, G. D. & Kersey, J. H. (1994) Molecular rearrangements of the MLL gene are present in most cases of infant acute myeloid leukemia and are strongly correlated with monocytic or myelomonocytic phenotypes. *Journal of Clinical Investigation*, **93**, 429–437.

Zanrosso, C. W., Emerenciano, M., Goncalves, B. A., Faro, A., Koifman, S. & Pombo-de-Oliveira, M. S. (2010) N-acetyltransferase 2 polymorphisms and susceptibility to infant leukemia with maternal exposure to dipyrone during pregnancy. *Cancer Epidemiology Biomarkers & Prevention*, **19**, 3037–3043.