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Original Research

Minimal residual disease and outcome characteristics in infant KMT2A-germline acute lymphoblastic leukaemia treated on the Interfant-06 protocol

J. Stutterheim [a,*], P. de Lorenzo [b,c], I.M. van der Sluis [a], J. Alten [d], P. Ancliffe [e], A. Attarbaschi [f], L. Aversa [g], J.M. Boer [a,m], A. Biondi [c], B. Brethon [h], P. Diaz [i], G. Cazzaniga [j], G. Escherich [k], A. Ferster [l], R.S. Kotecha [n,o], B. Lausen [p], Alex WK. Leung [q], F. Locatelli [r], L. Silverman [s], J. Stary [t], T. Szczepanski [u], V.H.J. van der Velden [v], A. Vora [e], J. Zuna [w], M. Schrappe [x], M.G. Valsecchi [b], R. Pieters [a,y]

[a] *Pediatric Oncology, Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands*
[b] *Center of Bioinformatics, Biostatistics and Bioimaging, University of Milano-Bicocca, Monza, Italy*
[c] *Pediatrics, School of Medicine and Surgery, University of Milano- Bicocca, Fondazione MBBM/San Gerardo Hospital, Monza, Italy*
[d] *Pediatrics, University Medical Center Schleswig-Holstein, Christian-Albrechts-University of Kiel, Germany*
[e] *United Kingdom Children Cancer Study Group, London, United Kingdom*
[f] *St Anna Children's Hospital, Pediatric Hematology and Oncology, Austria*
[g] *GATLA, Buenos Aires, Argentina*
[h] *Department of Pediatric Hematology, University Robert Debre Hospital, APHP, Paris, France*
[i] *Chilean National Pediatric Oncology Group, Santiago, Chile*
[j] *Tettamanti Research Center, Pediatrics, School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy*
[k] *German Cooperative Study Group for Childhood Acute Lymphoblastic Leukemia, Hamburg, Germany*
[l] *European Organisation for Research and Treatment of Cancer Children Leukemia Group, Brussels, Belgium*
[m] *Oncode Institute, Utrecht, the Netherlands*
[n] *Australian and New Zealand Children's Haematology/Oncology Group, Perth Children's Hospital, Perth, Australia*
[o] *Telethon Kids Cancer Centre, Telethon Kids Institute, University of Western Australia, Perth, Australia*
[p] *Rigshospitalet, University Hospital, Department of Pediatrics, Copenhagen, Denmark*
[q] *The Chinese University of Hong Kong, Shatin, Hong Kong, Special Administrative Region, People's Republic of China*
[r] *Department of Pediatric Haematology and Oncology, IRCCS Ospedale Pediatrico Bambino Gesù, Sapienza University of Rome, Rome, Italy*
[s] *Dana-Farber Cancer Institute, Pediatric Oncology, Boston, MA, USA*
[t] *Czech Working Group for Pediatric Hematology, Prague, Czech Republic*
[u] *Polish Pediatric Leukemia/Lymphoma Study Group, Department of Pediatric Hematology and Oncology, Medical University of Silesia, Zabrze, Katowice, Poland*
[v] *Department of Immunology, Erasmus University Medical Center, the Netherlands*

* *Corresponding author*: Princess Máxima Center, Heidelberglaan 25, 3435 CS, Utrecht, the Netherlands.
E-mail address: j.stutterheim@prinsesmaximacentrum.nl (J. Stutterheim).

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[w] CLIP, Department of Paediatric Haematology and Oncology, Second Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic
[x] Berlin-Frankfurt-Münster Group Germany, Kiel, Germany
[y] Dutch Childhood Oncology Group, Utrecht, the Netherlands

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Abstract ***Background:*** The outcome of infants with *KMT2A*-germline acute lymphoblastic leukaemia (ALL) is superior to that of infants with *KMT2A*-rearranged ALL but has been inferior to non-infant ALL patients. Here, we describe the outcome and prognostic factors for 167 infants with *KMT2A*-germline ALL enrolled in the Interfant-06 study.
Methods: Univariate analysis on prognostic factors (age, white blood cell count at diagnosis, prednisolone response and CD10 expression) was performed on *KMT2A*-germline infants in complete remission at the end of induction (EOI; n = 163). Bone marrow minimal residual disease (MRD) was measured in 73 patients by real-time quantitative polymerase chain reaction at various time points (EOI, n = 68; end of consolidation, n = 56; and before OCTADAD, n = 57). MRD results were classified as negative, intermediate ($<5^{*}10^{-4}$), and high ($\geq 5*10^{-4}$).
Results: The 6-year event-free and overall survival was 73.9% (standard error [SE] = 3.6) and 87.2% (SE = 2.7). Relapses occurred early, within 36 months from diagnosis in 28 of 31 (90%) infants. Treatment-related mortality was 3.6%. Age <6 months was a favourable prognostic factor with a 6-year disease-free survival (DFS) of 91% (SE = 9.0) compared with 71.7% (SE = 4.2) in infants >6 months of age ($P = 0.04$). Patients with high EOI MRD $\geq 5 \times 10^{-4}$ had a worse outcome (6-year DFS 61.4% [SE = 12.4], n = 16), compared with patients with undetectable EOI MRD (6-year DFS 87.9% [SE = 6.6], n = 28) or intermediate EOI MRD $<5 \times 10^{-4}$ (6-year DFS 76.4% [SE = 11.3], n = 24; $P = 0.02$).
Conclusion: We conclude that young age at diagnosis and low EOI MRD seem favourable prognostic factors in infants with *KMT2A*-germline ALL and should be considered for risk stratification in future clinical trials.

1. Introduction

Acute lymphoblastic leukaemia (ALL) occurring in infants, defined as children aged <365 days at diagnosis, is a rare and aggressive type of leukaemia. Rearrangement of the lysine methyltransferase 2A gene (*KMT2A*), formerly known as the mixed lineage leukaemia (*MLL*) gene, occurs in 75% of infants with ALL and is associated with a poor outcome [1,2]. Infants with *KMT2A*-germline (*KMT2A*-g) ALL have better survival rates, reaching approximately 75% [1–3]. Infants with *KMT2A-g* ALL are defined as low-risk patients in most recent treatment protocols for infants with ALL. Although the outcome for *KMT2A*-g infants has been inferior to older children with ALL (aged 1–18 years), recent Japanese studies with low patient numbers reported survival rates above 90% [4,5], highlighting the need for prognostic markers is needed to guide therapy for infants with *KMT2A*-g ALL.

In *KMT2A*-rearranged (*KMT2A*-r) infants, high-risk features are age <6 months at diagnosis, a high white blood cell (WBC) count at diagnosis ($\geq 300 \times 10^{9}$/L), poor prednisone response [1,2,6] and high level of minimal residual disease (MRD) after induction and consolidation [5,7]. In infants with *KMT2A*-g ALL, few studies have reported clinical and molecular genetic characteristics. These studies showed that infants with *KMT2A*-g ALL share cytogenetic abnormalities with older children with ALL, albeit with a different distribution; the proportion of patients with favourable risk genetics (hyperdiploidy, *ETV6-RUNX1*) is lower (12% versus 60% in older children) [8,9]. Furthermore, we showed in a small cohort of patients that an immature (CD10-negative) pro-B immunophenotype predicted a worse outcome in infants with *KMT2A*-g ALL [10]. More recently, although in a limited series of cases, a poor prognostic value was shown for *PAX5* fusions and a good outcome was associated with *NUTM1* gene fusions, both correlated with MRD response [11,12]. Overall, the relevance of MRD is still unknown in infants with *KMT2A*-g ALL.

The purpose of this study was to describe the outcome and to investigate prognostic factors, including MRD, in infants with *KMT2A*-g ALL that were homogeneously treated according to the Interfant-06 protocol.

2. Material and methods

2.1. Patients and treatment protocol

Patients were included in this study if they were registered on Interfant-06 and were *KMT2A*-germline. Patients were recruited from February 2006 to July 2016, as described previously [2]. The presence of KMT2A gene rearrangements was excluded using fluorescence in situ hybridisation (FISH), reverse transcription polymerase chain reaction and/or Southern blotting. Each national study group provided patient data, including cytogenetics, FISH and molecular results. NUTM1 status was known in 106 patients, as described previously [12]. The final follow-up was updated on 31st December 2017, and the median follow-up time was 5.3 years (range, 0.1–11.4 years). Presenting features, including age, WBC at diagnosis, CD10 expression and prednisone response, were prospectively collected for all patients.

For the analyses of MRD, *KMT2A*-g patients were included if they had reached morphological complete remission at the end of induction (EOI) (CR1) and had MRD data for at least one of the protocol specified follow-up time points (TPs) up to the start of OCTADAD (see Supplementary Figure 1 for treatment schema). As reporting of MRD was not mandatory for this protocol, MRD data were available in only 73 of 163 patients.

All patients were enrolled onto the Interfant-06 protocol, with parental written consent obtained according to the Declaration of Helsinki. The study was approved by the Ethics Committee of participating institutions and registered with the European Clinical Trials database (EudraCT 2005-004599-19) and at http://www/cancer.gov/clinicaltrials (NTC0550992).

2.2. Detection of MRD

Bone marrow (BM) samples were obtained at diagnosis, at the EOI (TP2; n = 68), at the end of consolidation (EOC; TP4; n = 56) and after MARMA (TP5; n = 57). BM samples were available at both EOI and EOC for 55 patients. Samples were shipped to and analysed by the national reference laboratories according to EuroMRD guidelines, all being part of EuroMRD and participating in the EuroMRD quality assurance programme, as described before [13,14]. MRD results were classified as negative (undetectable), intermediate (detectable but $<5 \times 10^{-4}$), and high ($\geq 5 \times 10^{-4}$).

2.3. Statistical analysis

Fisher's exact test was used to compare patients with and without MRD evaluations with respect to potential prognostic features. Disease-free survival (DFS) was defined as the time from complete remission to relapse, death in CR from any cause or second malignant neoplasm, whichever occurred first. Event-free survival (EFS) was defined as time from diagnosis to first failure, including death in induction, resistance to induction therapy (i.e. no CR at the EOI), relapse, death in CR from any cause, or second malignant neoplasm, and overall survival (OS) as time from diagnosis to death from any cause. DFS, EFS and OS curves were computed according to Kaplan–Meier estimator, their standard errors (SEs) according to Greenwood formula, and were compared with log-rank test. The multivariable analysis of prognostic relevance of patients' characteristics at diagnosis (age, WBC, and CD10 expression; MRD was not considered because data were available in only half of the patients) on DFS was performed with the Cox model (Wald test). Analyses were performed using SAS version 9.4.

3. Results

3.1. Patient characteristics

Of 651 infants enrolled in the Interfant-06 trial, 167 (25.7%) were *KMT2A*-g. Patients' characteristics are reported in Table 1. Compared with infants with *KMT2A-r* ALL, infants with *KMT2A*-g ALL were older (age ≥6 months 77.8% versus 39.9%, $P < 0.0001$), had a lower WBC at diagnosis ($\leq 100 \times 10^9$/L, 76.0% versus 36.5%, $P < 0.0001$), were more often CD10 positive (CD10 negative 15.0% versus 70.1%, $p < 0.0001$), and were more likely to have a good prednisone response (86.3% versus 74.9%, $P = 0.0027$). Cytogenetics data were available for 127 patients. Twenty-six patients had a NUTM1 rearrangement, ten had hyperdiploid ALL (HeH), one patients was BCR-ABL1 positive, and four patients carried a TCF3/PBX1 translocation (t(1;19)) (Table 1). In the registry, there were no patients with ETV6/RUNX1 fusions.

3.2. Outcome

In the Interfant-06 study, the 6-year EFS (SE) and OS (SE) for infants with *KMT2A*-g ALL was 73.9% (3.6) and 87.2 (2.7), respectively [2]. CR1 was attained in 97.6% (n = 163/167). One patient died during induction, and three patients had resistant disease. Relapse occurred in 19% (n = 31/163) of patients who achieved CR1, of whom 13 died. Notably, 90.3% (n = 28/31) relapses occurred early, within 36 months from diagnosis. Sites of relapse included 15 (48.4%) isolated BM, 6 (19.4%) isolated central nervous system (CNS), 5 (16.1%) combined BM and CNS, 1 (3.2%) combined BM and testis, and 4 (12.9%) others. Six patients died in CR1 (3.7%), of which five deaths were due to infection. Three out of these six patients died of infections during the MARMA course.

3.2.1. Post-induction outcome by patient characteristics

In 163 patients who achieved CR1 at the EOI, young age at diagnosis (<6 months) was associated with a significantly better outcome. The 34 patients aged <6

Table 1
Patient characteristics by *KMT2A* status.

	KMT2A-germline infant ALL (n = 167)	*KMT2A*-rearranged infant ALL (n = 476)	*P* value
Sex			0.0006
Male	95 (56.9%)	196 (41.2%)	
Female	72 (43.1%)	280 (58.8%)	
Age at diagnosis			<0.0001
<6 months	37 (22.2%)	286 (60.1%)	
≥6 months	130 (77.8%)	190 (39.9%)	
WBC count (cells/L)			<0.0001
$\leq 100 \times 10^9$	127 (76.0%)	173 (36.5%)	
$>100 \times 10^9$	40 (24.0%)	301 (63.5%)	
Not known	0	2	
Immunophenotype			<0.0001
B-ALL CD10 neg	25 (15.0%)	331 (70.1%)	
B-ALL CD10 pos	124 (74.2%)	80 (16.9%)	
B-ALL CD10 not known	7 (4.2%)	36 (7.7%)	
T-lineage	7 (4.2%)	3 (0.6%)	
Other[a]	4 (2.4%)	22 (4.7%)	
Not known	0	4	
Prednisone response			0.0027
Good response	138 (86.3%)	341 (74.9%)	
Poor response	22 (13.7%)	114 (25.1%)	
Not known	7	21	
Cytogenetics in B-ALL[b]	n = 160		
Data not available	33		
Normal karyotype	43		
Aberrant karyotype	43		
NUTM1	26		
HeH	10		
TCF3-PBX1	4		
BCR/ABL1	1		

ALL, acute lymphoblastic leukaemia; WBC, white blood cell.
[a] Includes acute undifferentiated and biphenotypic leukaemia.
[b] 'Data not available' includes patients with unknown karyotype/partial cytogenetics investigations; NUTM1 are described in Boer, J.M. *et al.*, Leukemia (2021).

months had a 6-year of DFS of 91% (5.0) compared with 71.7% (4.2) for 129 patients aged ≥6 months ($P = 0.04$, Table 2). Sex, WBC at diagnosis, CD10 expression and prednisone response had no significant impact on DFS (Table 2). To assess the impact of patients' characteristics at diagnosis on DFS, a multivariable analysis was performed with the Cox model on 147 patients (32 events) with data available on age, WBC and CD10 expression. In this model, there was a trend toward a favourable impact of young age, although this was not statistically significant (hazard ratio 0.34, 95% CI 0.10–1.14, $P = 0.08$, supplementary table 1).

Of patients with favourable genetics (e.g. HeH, t(1;19)), one patient with t(1:19) ALL experienced relapse. All 14 patients with HeH or t(1;19) ALL were older than 6 months. As described before, none of the 26 patients with a NUTM1 rearrangement experienced a relapse [12], and 14 (53.8%) of them were <6 months at diagnosis.

3.2.2. Outcome by MRD

Of 163 *KMT2A*-g infants enrolled in the Interfant-06 protocol and in CR1 at EOI, MRD data were available for 73. Of these, 12 (16.4%) relapsed, and 1 (1.4%) died in CR1. There was no significant difference in patients characteristics (supplementary table 2) and 6-year DFS (SE) between patients with and without MRD data available (79.5% [5.2] versus 72.7% [4.8], respectively; $P = 0.267$).

3.2.2.1. Prognostic significance of MRD at the EOI

At EOI, 76.5% (n = 52/68) of patients were either MRD negative (41.2%, n = 28) or intermediate MRD (35.3%, n = 24). 23.5% (n = 16) had high EOI MRD, which was associated with significantly lower 6-year DFS (SE), compared to patients with intermediate or negative EOI MRD (61.4% (12.4), 76.4% (11.3) and 87.9% (6.6), respectively; p = 0.02, Fig. 1a). Interestingly, isolated CNS relapse occurred in all three patients who relapsed after achieving negative EOI MRD. The four relapses in patients with intermediate EOI MRD levels were extramedullary in two cases (1 isolated CNS), 1 was

Table 2
Univariate analysis by prognostic factors of outcome of infants with KMT2A-germline ALL who achieved complete remission at the end of induction.

	N	N. events (relapses)	6-year DFS	SE	*P* value
Sex					0.7802
Male	94	21 (19)	75.6	4.8	
Female	69	16 (12)	75.7	5.3	
Age at diagnosis					0.0407
<6 months	34	3 (2)	91.0	5.0	
≥6 months	129	34 (29)	71.7	4.2	
WBC count (cells/L)					0.4499
$\leq 100 \times 10^9$	126	27 (23)	76.8	4.0	
$>100 \times 10^9$	37	10 (8)	72.0	7.5	
Immuno phenotype					0.8975*
B-ALL CD10 neg	24	5 (4)	78.7	8.5	
B-ALL CD10 pos	123	27 (22)	76.4	4.0	
B-ALL CD10 not known	7	1 (1)	–	–	
T-lineage	6	4 (4)	–	–	
Other	3	0 (0)	–	–	
Prednisone response					0.3124
Good response	136	33 (27)	74.1	3.9	
Poor response	20	3 (3)	84.4	8.3	

*P value for comparison of B-ALL CD10 neg versus B-ALL CD10 pos.
DFS, disease-free survival; WBC, white blood cell.
Note: n = 3 resistant patients and n = 1 death in induction are excluded from this analysis. Treatment of resistant patients was as follows: 1 received Interfant-06 treatment with Protocol IB (alive at 11 years after diagnosis), 1 received Interfant-06 treatment with Protocol IB and transplant (alive at 2 years after diagnosis), and one received Interfant-06 treatment with ADE + MAE (alive at 3.5).

combined BM and CNS and one isolated BM, while all five relapses in patients with high MRD EOI involved the bone marrow (three isolated BM and two combined BM and CNS).

3.2.2.2. End of induction MRD and age

Of the 68 patients with available EOI MRD data, 13 were <6 months of age at diagnosis, of whom five had negative, seven intermediate and one had high EOI MRD. Only one of these 13 patients relapsed (8%). In infants ≥6 months of age at diagnosis, 15 of 55 patients had high EOI MRD levels and five of them relapsed (33%), whereas six relapses occurred in 40 patients with low MRD levels (15%; Table 3).

3.2.2.3. Prognostic significance of MRD at the EOC

At EOC, 55.4% (n = 31/56) of patients were MRD negative. Outcome by MRD levels at EOC was not significantly different ($P = 0.24$); the 6-year DFS (SE) of negative and intermediate EOC MRD patients was 89.0% (6.0) and 72.7% (10.6), respectively, whereas only one of the five patients with high EOC MRD relapsed in BM and CNS (Fig. 1b).

MRD data for both EOI and EOC were available for 55 patients. Of these patients, 18 were MRD negative at EOI and EOC, with a 6-year DFS 93.3% (SE = 6.4). Five patients had negative EOI MRD but showed intermediate EOC MRD levels; none of these patients relapsed. There were 12 of 55 patients who were MRD positive at EOI and became MRD negative at EOC. These patients had a 6-year DFS of 82.5% (SE = 11.3). Patients with detectable disease at both time points had a 6-year DFS of 68.3% (SE = 10.8; n = 20, Fig. 1c). These data suggest that in the Interfant-06 protocol, MRD negativity at EOI is more important for outcome than MRD negativity at EOC.

3.2.2.4. Prognostic significance of MRD at the end of MARMA (TP5)

At the end of MARMA (TP5), 77.2% (n = 44/57) of patients were MRD negative. MRD at TP5 was significantly related to DFS (Fig. 1d); the 6-year DFS was 89.6% (SE = 5.0) for MRD negative patients, compared with 65.6% (SE = 14.0) for patients with intermediate MRD levels ($P = 0.039$). There was one patient with high MRD levels after MARMA, who is alive in complete remission at the last follow-up.

4. Discussion

In this study, we describe the outcome and prognostic factors in the largest cohort of uniformly treated infants with KMT2A-g ALL, treated according to the Interfant-06 protocol. The outcome of infants with *KMT2A*-g ALL is worse than non-infant ALL, and almost all relapses occurred early within 36 months of diagnosis. Furthermore, we have shown that MRD in infants with *KMT2A*-g ALL was predictive for outcome, and young age was surprisingly related to a favourable outcome.

In contrast to infants with *KMT2A*-r ALL, we identified young age at diagnosis (<6 months) as a favourable clinical prognostic factor in infants with *KMT2A*-g ALL. This has not been shown in previous studies. The COG P9407 study included only four very young infants who were <90 days of age at diagnosis with *KMT2A*-g ALL [3]. In our previous study in a smaller cohort (treated according to Interfant99 [n = 61] or Interfant-06 [n = 17]), there was a trend towards a lower relapse rate in younger infants aged <6 months at diagnosis with a 5-year cumulative incidence of relapse of 12% versus 25% in infants >6 months ($P = 0.32$) [10].

Previously, we have shown that infants with *KMT2A*-g ALL have a lower incidence of good risk abnormalities, such as high hyperdiploidy and *ETV6/RUNX1* fusions [8], which could explain the inferior outcome of infants with *KMT2A*-g ALL compared with non-infant ALL. The vast majority (80%) had intermediate cytogenetic profiles as defined by Moorman *et al.* [9], suggesting that other prognostic genetic aberrations could affect infants with *KMT2A*-g ALL. Recently, new *KMT2A* fusion partners such as MLL-USP2 have been identified using new sequencing approaches [15]. Standard FISH analysis and CGH array do not permit reliable detection of this fusion. Two patients in the present cohort appeared to harbour such new KMT2A fusion by sequencing, so the incidence of *KMT2A*-rearrangements may have been slightly underestimated as in other studies. Given the rarity of this, it is unlikely that this would have significantly impacted the survival rates of our *KMT2A*-germline cohort.

The favourable outcome in young *KMT2*A-g infants may partly be explained by their favourable genetics. In contrast to favourable HeH in older infants, the newly identified aberration *NUTM1*-rearrangement is a predominantly found in young *KMT2A*-g ALL. Recently, a collaborative international Ponte-di-Legno study characterised *NUTM1*-rearranged infant ALL as a good prognostic subtype and reported that the incidence of the *NUTM1* rearrangement was higher in infants <6 months of age at diagnosis [12]. Using a custom next-generation sequencing panel RNA sequencing, rearrangements that are associated with poor outcome have also recently been identified, including *PAX5* rearrangements [11].

This is the largest study that reports on MRD in *KMT2A*-g infant ALL. In the literature, only two relatively small studies have been published. In the Interfant-99 study, MRD data were available for 19 *KMT2A*-g infants. Only one patient had very high MRD levels ($>10^{-2}$) and relapsed; all other patients had lower MRD levels and remained in remission [16]. In the recent Japanese MLL-10 study, MRD was negative (defined as $<5 \times 10^{-4}$) in all 13 *KMT2A*-g patients, and none of these patients experienced a relapse [5]. In the

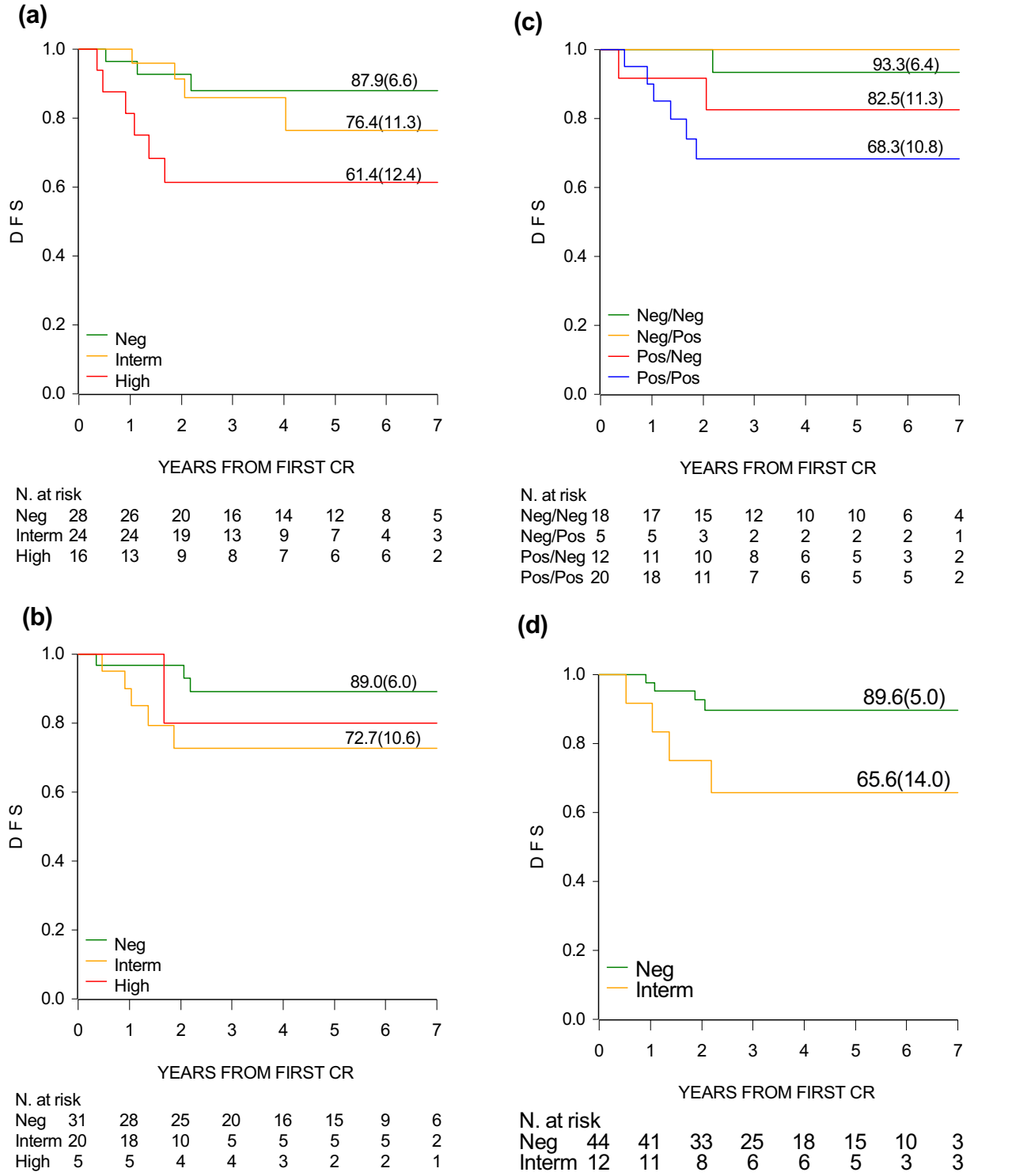

Fig. 1. (**a**) Prognostic impact of minimal residual disease (MRD) levels at the end of induction (EOI) as shown by Kaplan–Meier estimates of disease-free survival. (**b**) Prognostic impact of minimal residual disease (MRD) levels at end of consolidation (EOC) as shown by Kaplan–Meier estimates of disease-free survival. Note: Due to the small number of patients with high MRD (N = 5 with 1 relapse at 1.7 years), no DFS estimate can be given. (**c**) Prognostic impact of EOC MRD levels for all patients with MRD at EOI and EOC, as shown by Kaplan–Meier estimates of disease-free survival. Note: The subgroup Neg/Pos includes only N = 5 patients with no events. (**d**) Prognostic impact of MRD levels after MARMA as shown by Kaplan–Meier estimates of disease-free survival. Note: The figure does not report the outcome of the single patient with MRD High at TP5 (no events).

Table 3
End of induction MRD by age at diagnosis.

EOI MRD (TP2)	Age at diagnosis	
	<6 months	≥6 months
Neg	5/0 (–)	23/3 (3)
<5*10–4	7/1 (1)	17/3 (3)
≥5*10–4	1/0 (–)	15/6 (5)
Overall	13 (1)	55 (11)

N.pts/N.events (N. relapses).
$P = 0.20$ by Fisher exact test on association.

present study, MRD was identified as a significant prognostic factor. We showed that among the MRD timepoints investigated, EOI was the most indicative for outcome. Patients with high EOI MRD, defined as MRD $\geq 5 \times 10^{-4}$, had an inferior outcome compared with patients with negative or intermediate EOI MRD.

Notably, the three patients with relapse after negative EOI MRD, all experienced an isolated CNS relapse, whereas all five relapses in patients with high EOI MRD involved the BM. This highlights the need to use other markers to predict extramedullary relapse in these patients that may guide CNS directed therapy to prevent CNS relapse [17].

At the EOC, patients with negative MRD had a slightly, but not significantly, better outcome than patients with detectable EOC MRD. In infants with KMT2A-r ALL and in older children EOC MRD is of great prognostic significance [7,18], even if only intermediate genetics (non-ETV6/RUN1) was taken into account [18]. We hypothesise that the moderate prognostic value of MRD in *KMT2A*-g treated on Interfant-06 could be explained by (1) the relatively small number of patients studied and the low event rate, (2) subsequent therapy after consolidation being intensive enough to rescue patients with high MRD at EOI or EOC, (3) the relatively high incidence of relapses outside the BM mainly in patients with low EOI MRD, generally occurring earlier than isolated BM relapses [17], and (4) the differences in genetic makeup between patients with *KMT2A*-g infant ALL, for example, the relatively high incidence of the favourable NUTM1-rearrangement. In the present study, *NUTM1* status was known in 53 patients with MRD data. Of them, 13 harboured a *NUTM1* rearrangement. Seven of 11 (63.6%) were aged <6 months, and 6 of 42 (14.3%) were older. None of them relapsed, despite positive EOI MRD detected in eight cases (Supplementary Table 3).

We conclude that young age at diagnosis and low EOI MRD are favourable prognostic factors in infants with *KMT2A*-germline ALL. However, the prognostic value of MRD is not as strong as in infants with *KMT2A*-rearranged ALL or older children with ALL. This can partly be explained by the differences in genetic makeup of infants with *KMT2A*-germline ALL, thus supporting the hypothesis that in the future, a combined MRD- and genetic-based stratification of *KMT2A*-g infants might be considered.

Authors' contribution

J.S. contributed to conceptualisation, formal analysis, and writing the original article. P.d.L. contributed to methodology, formal analysis, writing the original article, and project administration. I.v.d.S. contributed to conceptualisation, investigation, formal analysis, and reviewing and editing the article. J.A., J.B., G.C., and V.v.d.V. contributed to investigation and reviewing and editing the article. P.A., A.A., L.A., A.B., B.B., P.D., G.E., A.F., R.K., B.L., A.L., F.L., L.S., J.S., T.S., and A.V. contributed to resources and reviewing and editing the article. J.Z. contributed to investigation and reviewing and editing the article (contributed vital new reagents or analytical tools). M.S. contributed to investigation, resources, and reviewing and editing the article. M.G.V. contributed to methodology, formal analysis, reviewing and editing the article, and project administration. R.P. contributed to conceptualisation, investigation, formal analysis, writing the original article, supervision, and project administration.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Disclaimers

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2021.10.004.

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