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Prognostic impact of *IKZF1* deletions in association with vincristine–dexamethasone pulses during maintenance treatment of childhood acute lymphoblastic leukemia on trial ALL-BFM 95

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Acute lymphoblastic leukemia (ALL) is the most common malignancy of childhood and is cured by application of modern treatment protocols in more than 80% of cases.[1,2] However, the ~20% of children with ALL suffering from relapse still exemplify the ongoing need for improved risk stratification and adapted treatment strategies.[1,2] In several studies, deletions of *IKZF1*—encoding IKAROS, a transcription factor important for lymphoid development and differentiation—have been associated with a poor treatment outcome in precursor B cell ALL.[3–5] Deletions of *IKZF1* are observed in 10–15% of pediatric ALLs and affect either the entire *IKZF1* gene or appear as focal deletions.[3–5] The most common of the latter ones (Δ4–7) includes the DNA-binding region and results in a dominant-negative isoform (Ik6), impairing cell differentiation in CD34+ lymphoid progenitor cells.[3–5]

Recently, Clappier *et al.*[6] from the EORTC Study Group suggested that patients with *IKZF1*-deleted ALL and intermediate-risk features—so-called average-risk (AR) patients—treated on the BFM-based EORTC protocol 58951 may benefit from intensification of conventional maintenance therapy by application of vincristine–glucocorticoid pulses. We previously reported that on ALL-BFM protocols *IKZF1* status exerts additive value as a prognostic factor, especially in the intermediate-risk group, in which still the majority of relapses occur.[4] Therefore, we investigated the impact of additional pulses during maintenance therapy for *IKZF1*-deleted ALL in a large cohort of intermediate-risk patients treated according to the ALL-BFM 95 protocol.[7] These patients were included in the I-BFM study on pulses of vincristine and dexamethasone in BFM protocols for children with ALL.[8]

Between April 1995 and December 2000, patients diagnosed with *de novo* ALL were enrolled in ALL-BFM 95 from Austrian, German and Swiss study centers.[7] Treatment was stratified in three risk groups as follows: standard (SR), intermediate (IR) and high risk (HR). Risk group definition was as follows: HR, prednisone poor response and/or no complete remission on day 33, and/or evidence of t(9;22) (or BCR/ABL), and/or evidence of t(4;11) (or MLL/AF4); IR, no HR criteria and initial white blood cell (WBC) count 20×10^9/l or more and/or age at diagnosis less than 1 or 6 years, or older, and/or T-ALL; SR, no HR criteria and initial WBC less than 20×10^9/l, and age at diagnosis between 1 and 6 years, and no T-ALL. CNS status was no stratification criterion. Before start of maintenance treatment, patients of the IR group who were in complete remission were randomly assigned to receive either conventional mercaptopurine and methothrexate chemotherapy or mercaptopurine and methothrexate supplemented with additional pulses of vincristine (1.5 mg/m^2 weekly for 2 weeks) and dexamethasone (6 mg/m^2 daily for 7 days) every 10 weeks for six cycles. *IKZF1* deletion status in leukemic DNA isolated from initial bone marrow smears was assessed by a multiplex PCR assay[9] (Supplementary Figure 1).

We were able to isolate leukemic DNA from bone marrow smears of 655 German precursor B cell ALL patients—corresponding to 95% of the entire randomized German cohort of the patients who were treated as randomized–who were subsequently subjected to PCR screening for *IKZF1* deletion status.[9] Analyzed patients did not differ from those not included due to lack of biological specimens (Supplementary Table 1). Characteristics of IR patients were comparable in the two randomized arms (Supplementary Table 2). In accordance with previously reported results of the I-BFM study on pulses,[8] no differences in outcome were observed between the experimental and standard treatment arms (Supplementary Figure 2). Out of the 655 patients included in our study, 80 tested positive for an *IKZF1* deletion (12.2%). In analyses of the entire group, *IKZF1* deletion was associated with a worse treatment outcome compared to *IKZF1* wild-type status (5-year event-free survival (EFS): 0.66 ± 0.05 vs 0.82 ± 0.02, $P = 0.001$; Supplementary Figure 3). When outcome was analyzed restricted to *IKZF1*-deleted patients, 5-year EFS tended to be lower in patients treated on the experimental arm incorporating pulses compared to those on standard therapy (0.57 ± 0.08 vs 0.74 ± 0.07, $P = 0.083$) (Figure 1a). Non-relapse events were more frequent in the experimental arm and contributed to the differences observed in EFS analyses (Supplementary Table 3). Thirteen (35.1%) patients exposed to experimental treatment suffered from relapse compared to 11 (25.6%) patients on the standard arm; respective cumulative relapse incidences (CIR) were not significantly different (0.35 ± 0.08 vs 0.26 ± 0.07; $P = 0.370$; Figure 1b). In multivariate analysis within the IR group, *IKZF1* deletion was significantly associated with risk of an event only in patients receiving pulsed maintenance treatment, while this effect was not observed for *IKZF1*-deleted patients on the standard arm (Table 1). However, as mentioned already above, the significant hazard ratio for *IKFZ1* deletion on the experimental arm is likely to be explained by the higher number of non-relapse events observed in this group (Supplementary Table 3). Thus, application of pulsed maintenance therapy did not specifically improve outcome for *IKZF1*-deleted IR patients on ALL-BFM 95.

For 34 (15.5%) *IKZF1*-deleted out of 219 randomized AR patients on EORTC trial 58951, Clappier and colleagues described an outcome for *IKZF1*-deleted patients who received pulses, which was identical to that of non-deleted patients (8-year DFS: 93.3 versus 89.5%; $P = 0.600$), whereas the outcome for deleted patients who did not receive pulses was significantly worse compared to *IKZF1* wild-type patients (8-year DFS 42.1 versus 88.8%; HR = 6.65; $P < 0.001$).[6] Consequently, the authors suggested that intensification of maintenance therapy with vincristine–glucocorticoid pulses had contributed to prevent relapses in patients with deletions of *IKZF1*. Treatment on both trials ALL-BFM 95 and EORTC trial 58951 was BFM-based and comparable, as was overall

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IR and AR outcome with 6-year DFS of 83.2 and 82.2%, respectively. However, potential explanations for the observed differences in treatment results for *IKZF1*-deleted patients receiving pulsed maintenance therapy on these two trials include, for example, differences in risk group definition. IR patients on ALL-BFM 95 were aged less than 1 or 6 years or older, or had initial WBC counts of 20×10^9/l or more, had a favorable cytomorphologically assessed treatment response and were negative for a t(4;11) or its molecular equivalent *MLL-AF4*. Minimal residual disease (MRD) analyses were not performed on ALL-BFM 95. On EORTC 58951, AR patients were not restricted to certain age groups, had initial WBC counts of 10×10^9/l or more, had a favorable cytomorphologically assessed treatment response, were not only negative for all *MLL* rearrangements, but also for high hyperdiploidy (51 or more chromosomes or DNA index >1.16 and <1.50) as well as low hypodiploidy or near haploidy. In addition, the EORTC trial excluded patients with high MRD levels (10^{-2} or more) after induction treatment from the AR group. Therefore, the stratification strategy on ALL-BFM 95 may have selected for a different spectrum of *IKZF1*-deleted ALL patients in our study in comparison to the EORTC study. To get an estimate on the percentage of patients, which would have been excluded from the IR group when MRD-PCR analyses would have been conducted, we applied the ALL-BFM 95 stratification criteria to patients from trial ALL-BFM 2000, which used DNA-PCR-based MRD analyses for stratification.[4,10] Of 2386 ALL-BFM 2000 patients fulfilling the ALL-BFM 95 IR stratification criteria and having the MRD data available, only 159 (6.7%) would have been stratified into the HR group according to the level of $\geqslant 10^{-2}$ as applied in the EORTC 58951 trial (Supplementary Table 4), which could have added to the differences observed between the two studies. One additional indicator for differences in patient populations could be the fact that the relapse cascade on ALL-BFM 95 started already during maintenance treatment, while on EORTC 58951 the majority of relapses was observed after cessation of treatment. If we look at the relapse pattern of patients from ALL-BFM 2000 with the available MRD data but stratified according to the ALL-BFM 95 criteria, we observe a similar relapse cascade for these "ALL-BFM 95 IR patients" who would have been stratified to HR on ALL-BFM 2000 according to their MRD levels.[10] As treatment approaches between the ALL-BFM 95 IR and EORTC 58951 AR groups were comparable, this suggests that the stratification strategy on trial EORTC 58951 was more effective in preventing early relapses—probably through exclusion of patients with MRD levels of $\geqslant 10^{-2}$ after induction to the HR group.

Lastly, our diagnostic approach itself could have influenced results through selective exclusion of patients affected by whole *IKZF1* gene deletions.[9] We are not aware of the distribution of these patients in our study cohort, which could have led to bias in our assessment. However, assuming a balanced distribution in the two randomized groups—as observed for focal deletions detected by our multiplex PCR approach—the probability of a severe influence on our results seems negligible.

In conclusion, our data demonstrate that intensification of maintenance therapy with vincristine–glucocorticoid pulses in IR patients with *IKZF1*-deleted ALL is not associated with improved outcome on trial ALL-BFM 95.

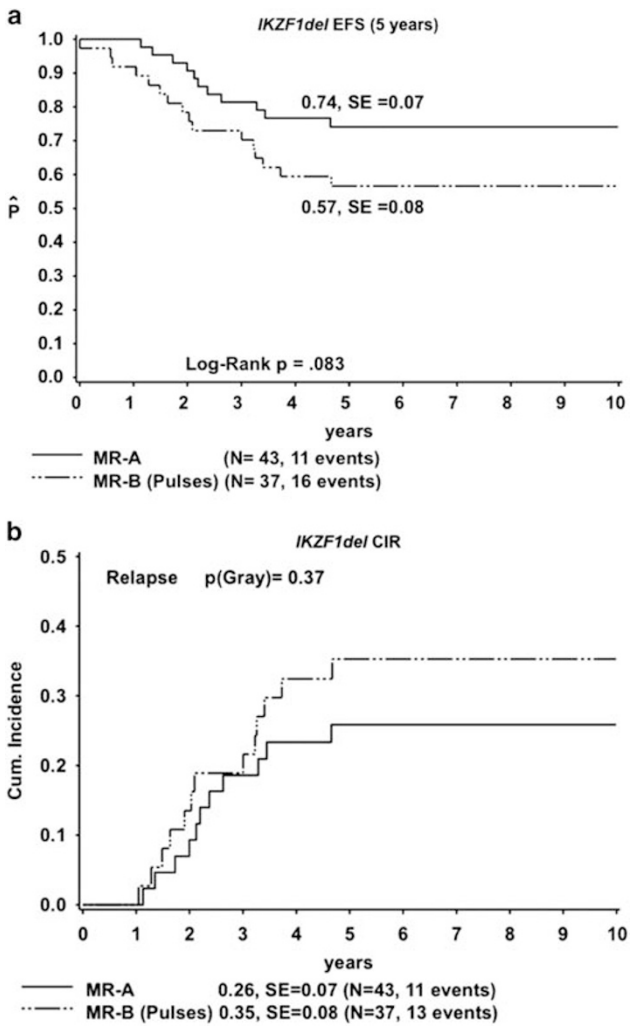

Figure 1. Treatment outcome of *IKZF1* deletion-positive patients by conventional and pulsed maintenance therapy. Kaplan–Meier estimates of 5-year event survival (**a**) and cumulative incidences of relapse (**b**) are shown.

Table 1. Multivariate hazard ratios for an event: comparison of conventional with pulsed maintenance therapy

	Conventional maintenance therapy			*pulsed maintenance therapy*		
	Hazard ratio	*95% confidence interval*	P-*value*	*Hazard ratio*	*95% confidence interval*	P-*value*
IKZF1 deletion[a]	1.32	0.69–2.52	0.403	2.97	1.67–5.29	<0.001
WBC count at diagnosis $\geqslant$100 000/μl[b]	3.12	1.54–6.31	0.002	1.41	0.60–3.33	0.431
Sex[c]	0.65	0.41–1.05	0.078	0.80	0.48–1.34	0.420
Age at diagnosis $\geqslant$10 years[d]	1.35	0.82–2.22	0.243	1.59	0.94–2.69	0.087

Abbreviations: ALL, acute lymphoblastic leukemia; WBC, white blood cell. [a]In comparison to negative patients. [b]In comparison to WBC count at diagnosis $<$100 000/μl. [c]Females compared to male patients. [d]In comparison to patients $<$10 years.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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