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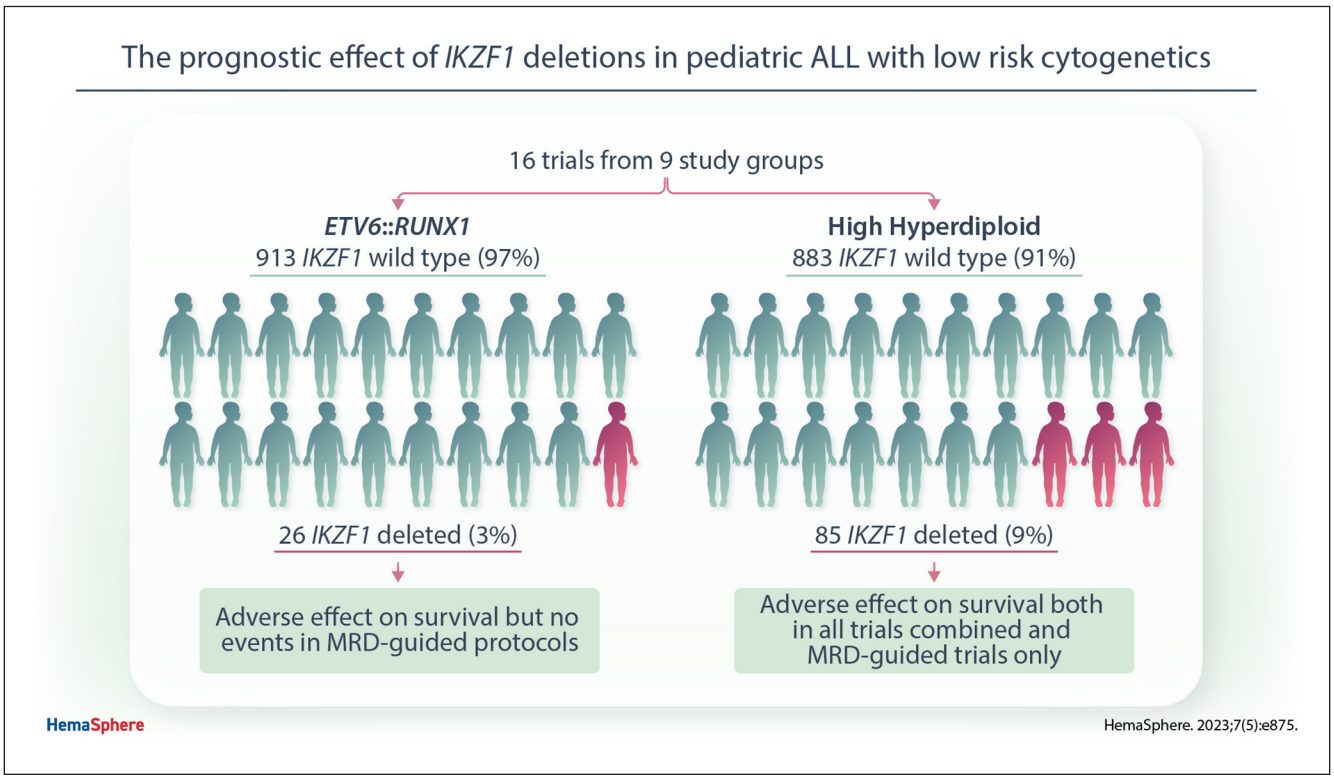
Anna Østergaard[1,2,*], Amir Enshaei[3,*], Rob Pieters[1], Ajay Vora[4], Martin A. Horstmann[5,6], Gabriele Escherich[5], Bertil Johansson[7,8], Mats Heyman[9,10], Kjeld Schmiegelow[11], Peter M. Hoogerbrugge[1], Monique L. den Boer[1,2], Roland P. Kuiper[1,12], Anthony V. Moorman[3,**], Judith M. Boer[1,2,**], Frank N. van Leeuwen[1,**]

GRAPHICAL ABSTRACT



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The Prognostic Effect of *IKZF1* Deletions in *ETV6*::*RUNX1* and High Hyperdiploid Childhood Acute Lymphoblastic Leukemia

Anna Østergaard[1,2,*], Amir Enshaei[3,*], Rob Pieters[1], Ajay Vora[4], Martin A. Horstmann[5,6], Gabriele Escherich[5], Bertil Johansson[7,8], Mats Heyman[9,10], Kjeld Schmiegelow[11], Peter M. Hoogerbrugge[1], Monique L. den Boer[1,2], Roland P. Kuiper[1,12], Anthony V. Moorman[3,**], Judith M. Boer[1,2,**], Frank N. van Leeuwen[1,**]

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ABSTRACT

IKZF1 deletions are an established prognostic factor in childhood acute lymphoblastic leukemia (ALL). However, their relevance in patients with good risk genetics, namely *ETV6*::*RUNX1* and high hyperdiploid (HeH), ALL remains unclear. We assessed the prognostic impact of *IKZF1* deletions in 939 *ETV6*::*RUNX1* and 968 HeH ALL patients by evaluating data from 16 trials from 9 study groups. Only 3% of *ETV6*::*RUNX1* cases (n = 26) were *IKZF1*-deleted; this adversely affected survival combining all trials (5-year event-free survival [EFS], 79% versus 92%; *P* = 0.02). No relapses occurred among the 14 patients with an *IKZF1* deletion treated on a minimal residual disease (MRD)-guided protocols. Nine percent of HeH cases (n = 85) had an *IKZF1* deletion; this adversely affected survival in all trials (5-year EFS, 76% versus 89%; *P* = 0.006) and in MRD-guided protocols (73% versus 88%; *P* = 0.004). HeH cases with an *IKZF1* deletion had significantly higher end of induction MRD values (*P* = 0.03). Multivariate Cox regression showed that *IKZF1* deletions negatively affected survival independent of sex, age, and white blood cell count at diagnosis in HeH ALL (hazard ratio of relapse rate [95% confidence interval]: 2.48 [1.32-4.66]). There was no evidence to suggest that IKZF1 deletions affected outcome in the small number of *ETV6*::*RUNX1* cases in MRD-guided protocols but that they are related to higher MRD values, higher relapse, and lower survival rates in HeH ALL. Future trials are needed to study whether stratifying by MRD is adequate for HeH patients or additional risk stratification is necessary.

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most prevalent childhood malignancy and recent cure rates approach 90% on first-line therapy.[1,2] This is mostly due to trials using increasingly precise risk stratification, but changes in drug doses and schedule as well as improved supportive care also play a role.[1,2] Risk stratification is based on clinical and genetic parameters present at diagnosis[2] and on early response to treatment as ascertained by minimal residual disease (MRD) analyses.[3] Some study groups additionally use copy number alterations to adjust risk stratification.[4–6] *IKZF1* deletions have been reported as an unfavorable prognostic factor by various study groups,[7–16] and some have consequently incorporated *IKZF1* status in risk stratification.[5,6] *IKZF1* deletion is associated with older age,[17] higher white blood cell count (WCC) at diagnosis,[10] and higher MRD[10,18] and is thus overrepresented in high-risk patients. In contrast, *IKZF1* deletions are rare in

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Ethical statement: The study was conducted in accordance with the Declaration of Helsinki. All research protocols were approved by the local Ethics Committees of the included trials. Informed consent was obtained from all participants and/or their parents or caretakers.
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ETV6::*RUNX1* and high hyperdiploid (HeH) ALL,[11,12] the 2 cytogenetic subgroups with the most favorable prognosis.[19,20] Because of the rarity of *IKZF1* deletions within the subset of patients with favorable cytogenetics, its prognostic effect remains unclear.[10–12] Therefore, we assessed the prognostic impact of *IKZF1* deletions in *ETV6*::*RUNX1*- and HeH-positive ALL by evaluating previously published data from 16 international trials.[19,21–32]

MATERIALS AND METHODS

Patients

We performed this retrospective analysis on data of children and adolescents of 1–18 years with B-cell precursor ALL diagnosed in 1991–2016 and treated on 1 of the 16 trials from 9 study groups, of which 6 were MRD guided (Table 1; Suppl. Table S1).[33,34] Cytogenetic, fluorescence in-situ hybridization, and reverse transcription polymerase chain reaction (RT-PCR) analyses of pretreatment bone marrow samples to determine ploidy and fusion gene status were performed locally. Trials using MRD for risk stratification applied either PCR[19,21,23,25] or flow cytometry analyses.[22,24] The *IKZF1* status was determined by multiplex ligation-dependent probe amplification (MLPA) by each individual study group (Suppl. Table S1). In addition, we classified cases according to the *IKZF1*^{plus} profile described by Stanulla et al.[16] Because we did not have data on *ERG* deletions, we used a modified definition of *IKZF1*^{plus}, namely an *IKZF1* deletion plus a deletion involving *CDKN2A/B*, *PAX5*, or PAR1. Intragenic *ERG* deletions are exclusively observed in cases with *IGH*::*DUX4*, which is widely considered mutually exclusive with *ETV6*::*RUNX1* and high hyperdiploidy.[35] All trials were approved by the local ethics committees and patients, parents, or guardians gave written consent.

Procedures

We used several previously curated datasets to define 4 datasets for our analysis based on genetics and the availability of MRD data (Table 1; Suppl. Table S1). Overlap in patients between datasets might be present and, therefore, datasets were analyzed separately. Because the largest datasets (A and B) did not contain MRD data, datasets C and D were acquired. Dataset C contains categorized MRD data, meaning MRD values have been divided into 5 categories between MRD positive but not quantifiable and 5%. Dataset D contains exact quantitative MRD values without categorization. ALL with both HeH and *ETV6*::*RUNX1* was classified as the latter on the assumption that the fusion gene was the primary genetic abnormality.

Statistics

The survival analyses considered 3 end points: event-free survival (EFS), relapse rate (RR), and overall survival (OS). An event was defined as either relapse, second malignant neoplasm, or death. All end points were censored at last contact. Survival rates were calculated and compared using Kaplan-Meier curves, log-rank tests, and univariate and multivariate Cox regression models. Variables included in the models were sex, age < or ≥10 years, and WCC <50 or ≥50 × 10^9/L. All variables were linear and conformed to the proportional hazard assumption. Hazard ratios are reported with 95% confidence interval. Survival analyses presented in results were not stratified per trial because of small sample sizes (Suppl. Table S1) and resulting low number of events. However, where number of included patients permitted (>20 cases within a cytogenetic subgroup), survival analysis was performed per trial to assess differences in outcome among trials and these differences did not affect further analyses (Suppl. Table S2). Forest plots were drawn to depict variation in effect size of *IKZF1* deletions on outcome among studies.

Table 1
Datasets Used for Assessing the Prognostic Value of *IKZF1* Deletions in *ETV6*::*RUNX1* and High Hyperdiploid Acute Lymphoblastic Leukemia

Dataset	Genetic Subtype	*IKZF1* Status: Deleted n	*IKZF1* Status: Wild-type n	Inclusion Criteria From Original Study	Time Period	Trials/Protocols
A	*ETV6*::*RUNX1*	26	913	Availability of MLPA data	1991–2015	BFM-ALL 2000, IC-BFM ALL 2002/2009, ANZCHOG ALL8, DCOG ALL8/9/10, NOPHO ALL-92/2000/2008, UKALL2003, MB-ALL-2002/2008, GBTLI LLA-2009, JACLS-ALL02, Other
B	High hyperdiploid	85	883	Availability of MLPA data		
C	High hyperdiploid	34	299	EOI MRD >0% and <5%	2003–2014	CoALL07-03, DCOG ALL10, NOPHO ALL2008, UKALL2003
D	High hyperdiploid	29	276	Participation in clinical trial	2003–2013	DCOG ALL10, UKALL2003

EOI MRD = end of induction minimal residual disease; MLPA = multiplex ligation-dependent probe amplification.

Heterogeneity was tested using Higgins I^2 test.[36] An I^2 statistic ≥50% was considered representing statistically significant heterogeneity.

Categorical variables were compared between groups with Fishers exact test continuous variables with Wilcoxon-rank sum test. To examine MRD as a continuous variable in dataset D, we log-transformed quantitative MRD values, assigned patient-cases with undetectable MRD a value of 1×10^{-6} (one log below the minimum detection level of 1×10^{-5}) and assumed a maximum value of 0.99999.[37] Normality was assessed by using the skewness, kurtosis, and Shapiro-Wilk test. Log normal distributions were compared by *t* test.

RESULTS

ETV6::RUNX1

Among the 939 *ETV6*::*RUNX1* positive cases in dataset A (Table 1), 3% had an *IKZF1* deletion (Table 2). There was no significant difference between *IKZF1*-deleted and wild-type cases in terms of age, sex and WCC ($P > 0.168$ for all, Table 2). When assessing all protocols, *ETV6*::*RUNX1* positive cases with an *IKZF1* deletion had a significantly worse outcome (5-year EFS, 79% versus 92%; RR, 18% versus 6%; OS, 87% versus 97%, respectively; $P > 0.03$ for all, Table 2; Suppl. Figure S1A). However, among the 14 patients with an *IKZF1* deletion treated on and MRD-guided protocol (n = 646), no adverse events occurred; suggesting that MRD stratification negated the adverse prognostic impact of an *IKZF1* deletion (5-year EFS, 100% versus 93%; RR, 0% versus 5%; OS, 100% versus 98%; $P > 0.34$ for all; Table 2 and Suppl. Figure S1B).

Summarizing, dataset A shows that *IKZF1* deletions do not affect survival in *ETV6*::*RUNX1* ALL for patients treated on MRD-guided protocols.

High hyperdiploidy

Among the 968 HeH ALL cases in dataset B (Table 1), 9% had an *IKZF1* deletion (Table 3). *IKZF1*-deleted cases were significantly older (median of 5 versus 4 years at diagnosis, $P = 0.005$; Table 3), but sex and WCC were not significantly different between *IKZF1*-deleted and wild-type patients ($P > 0.557$; Table 3). There was no difference in the survival of HeH cases between non-MRD-guided and MRD-guided protocols (hazard ratio of EFS non-MRD-guided trials versus MRD-guided: 0.83 [0.55-1.30], $P = 0.372$; Table 3). However, the outcome of HeH patients was not equivalent across all the trials. In ALL-IC BFM 2002, ANZCHOG ALL8, and DCOG ALL10, patients with HeH showed a significantly higher hazard ratio for RR, EFS, and OS compared with patients treated on UKALL2003 (Suppl. Table S2). *IKZF1*-deleted HeH ALL had a significantly worse outcome than HeH with *IKZF1* wild-type (5-year EFS, 76% versus 89%; RR, 20% versus 8%; OS, 88% versus 94%, respectively; $P < 0.01$ for all; Table 3 and Figure 1A) when examining the total cohort (dataset B). When we examined MRD-guided and non-MRD-guided protocols separately, we observed a lower and nonsignificant hazard ratio among the non-MRD-guided protocols but the test for heterogeneity was not significant (Figure 1C). *IKZF1* deletions had a negative effect in MRD-guided protocols (n = 696): 5-year EFS, 73% versus 88%; RR, 23% versus 9%; OS, 88% versus 94%; $P < 0.01$ for all; Table 3 and Figure 1B. When adjusting for clinical parameters by including sex, age, and WCC in a multivariate Cox regression model, *IKZF1* status still affected survival significantly in HeH cases treated on MRD-guided protocols (hazard ratio *IKZF1*-deleted versus wild-type EFS, 2.09 [1.19-3.65]; RR, 2.48 [1.32-4.66]; OS, 2.37 [1.17-4.79]; $P < 0.03$ for all). The individual MRD-guided protocols had percentages of 0%–4% of *IKZF1*-deleted cases (Suppl. Table S1). However, among the 5 MRD-guided protocols, there was an evidence of heterogeneity albeit with a marginal *P*-value ($P = 0.0495$) indicating that the prognostic impact of *IKZF1* deletions may be protocol specific (Figure 1D). Applying the modified *IKZF1*^{plus} profile in HeH cases did not show any additional effect on survival over *IKZF1* deletions only ($P > 0.18$ for all, Suppl. Table S3). In summary, dataset B shows that *IKZF1* deletions can lead to lower survival and higher RRs in HeH ALL cases treated on MRD-guided protocols and that *IKZF1*^{plus} does not have additional prognostic effect over *IKZF1* deletion alone.

High hyperdiploidy in ALLTogether

The new European collaborative treatment protocol ALLTogether uses the UKALL-CNA profile[5,34] along with other genetic abnormalities and MRD for risk stratification (AVM, personal communication, March 2, 2022). Patients are assigned at the end of induction to standard-, intermediate-, or high-risk groups based on MRD and the presence of selected high-risk features.

HeH patients allocated to the initial intermediate-risk group (detectable MRD <5% at the end of induction) are further stratified into the intermediate low-risk group if they fulfill either of the following criteria: (a) MRD <0.03% or (b) a good risk UKALL-CNA profile and MRD <0.05%. The presence of an *IKZF1* deletion would not influence the first criterion but would prevent the patient qualifying on the basis of the second criterion, because the *IKZF1* deletion would automatically assign them to a UKALL-CNA poor risk profile and would lead to assignment to the intermediate-risk-high group if their MRD was ≥0.03%.

Therefore, we examined the prognostic effect of *IKZF1* deletion in this specific subgroup in more detail by looking at the 333 HeH patients with detectable MRD <5% at the end of induction in dataset C[33] (Table 1 and Suppl. Table S4). Among these cases, 10% carried an *IKZF1* deletion and while the age difference between *IKZF1*-deleted and wild-type cases was not significant in this subgroup, a significant difference in sex distribution was seen (68% versus 44% males, respectively; $P = 0.011$; Table 4). Within dataset C, *IKZF1*-deleted cases also had a worse outcome compared with *IKZF1* wild-type cases (5-year EFS, 78% versus 92%; RR, 18% versus 6%; OS, 88% versus 97%; $P < 0.05$ for all; Table 4 and Figure 2A). When adjusting for sex, age, and WCC in a multivariate Cox regression model, *IKZF1* deletion still affected survival significantly (hazard ratio of *IKZF1*-deleted versus wild-type EFS, 2.96 [1.23-7.14]; RR, 3.03 [1.08-8.54]; OS, 4.20 [1.41-12.53]; $P < 0.05$ for all). The prognostic effect of *IKZF1* deletion was again largest in the DCOG ALL10 trial (n = 95), while no events were observed in *IKZF1*-deleted cases treated on CoALL 07-03 (n = 2) or NOPHO ALL2008 (n = 5) (Figure 2B, Suppl. Table S5). There was strong evidence for statistical heterogeneity among studies ($P = 0.004$). When assessing the quantitative MRD data of 305 HeH patients from the UKALL2003 and DCOG ALL10 cohorts in dataset D (Table 1), cases with an *IKZF1* deletion had significantly higher MRD values ($P = 0.03$; Figure 3) and 80% had an MRD ≥0.03%, which would already exclude them from the intermediate low-risk group, based on MRD levels only.

Together, dataset B, C, and D show that *IKZF1* deletions can lead to lower survival and higher RRs in HeH ALL with intermediate MRD at the end of induction and to higher MRD levels in general.

DISCUSSION

IKZF1 deletions are an established poor prognostic factor in childhood ALL, but their value in the favorable cytogenetic subgroups *ETV6*::*RUNX1* and HeH is unclear. The present comprehensive analysis of 16 trials assesses the prognostic effect of *IKZF1* deletions in these 2 subgroups. Our results show that *IKZF1* deletions can predict significantly lower survival in HeH ALL, even when treated on MRD-adapted protocols and when adjusting for other clinical parameters. However, *IKZF1*

Table 2

Features and Treatment Outcome of *ETV6::RUNX1* Acute Lymphoblastic Leukemia

	All Protocols			MRD-guided Protocols Only			Non-MRD-guided Protocols Only		
	IKZF1			*IKZF1*			*IKZF1*		
ETV6::RUNX1	**Deleted**	**Wild-type**		**Deleted**	**Wild-type**		**Deleted**	**Wild-type**	
n = 939	**n = 26**	**n = 913**	***P*-value**	**n = 14**	**n = 632**	***P*-value**	**n = 12**	**n = 281**	***P*-value**
Age									
<10	22 (85%)	837 (92%)	0.27	13 (93%)	585 (93%)	1.00	9 (75%)	252 (90%)	0.132
≥10	4 (15%)	76 (8%)		1 (7%)	47 (7%)		3 (25%)	29 (10%)	
Median (IQR)	5.00 (3.03–6.21)	4.00 (3.00–6.00)	0.434	4.50 (3.19–5.96)	4.00 (3.00–6.00)	0.741	5.35 (2.67–8.75)	4.00 (3.00–6.00)	0.486
Sex			0.844			1.00			1.00
Male	13 (50%)	478 (52%)		7 (50%)	335 (53%)		6 (50%)	143 (51%)	
Female	13 (50%)	435 (48%)		7 (50%)	297 (47%)		6 (50%)	138 (49%)	
WCC									
$<50 \times 10^9$/L	19 (73%)	769 (84%)	0.168	11 (79%)	546 (86%)	0.421	8 (67%)	223 (79%)	0.288
$\geq 50 \times 10^9$/L	7 (27%)	143 (16%)		3 (21%)	85 (13%)		4 (33%)	58 (21%)	
Median (IQR)	16.35 (6.02–47.53)	10.34 (5.20–30.30)	0.268	16.35 (6.03–31.95)	9.80 (5.02–25.65)	0.572	16.00 (9.47–130.40)	11.40 (5.60–40.80)	0.34
CNS disease			1.00			1.00	Unknown	Unknown	
Yes	0	1 (<0.5%)		0 (<0.5%)	1 (<0.5%)				
No	5 (19%)	226 (25%)		5 (36%)	226 (36%)				
Clinical remission			1.00			1.00			1.00
Yes	26 (100%)	908 (99%)		14 (100%)	629 (100%)		12 (100%)	279 (99%)	
No	0	4 (<0.5%)		0	2 (<0.5%)		0	2 (1%)	
Induction death	0	1 (<0.5%)		0	1 (<0.5%)		0	0	
Outcome									
5-y EFS	79% (63%-97%)	92% (90%-94%)	0.021	100%	93% (91%-95%)	0.341	58% (36%-94%)	91% (87%-94%)	<0.001
5-y RR	18% (0%-33%)	6% (4%-8%)	0.026	0%	5% (3%-7%)	0.41	37% (0%-59%)	7% (4%-10%)	<0.001
5-y OS	87% (74%-100%)	97% (96%-98%)	0.013	100%	98% (67%-99%)	0.626	74% (53%-100%)	95% (92%-98%)	0.009
Hazard ratio EFS	2.80 (1.10–6.90)	Reference	0.027	No event	Reference		5.10 (1.90–13.00)	Reference	<0.001
Follow-up in years, median (IQR)	6.72 (4.65–8.71)	7.40 (5.45–9.22%)		6.28 (2.25–8.57)	7.30 (5.71–9.13)		7.28 (5.84–9.13)	7.67 (5.08–9.73)	

Data are n (%), rates at 5 y (95% CI) or median (IQR).

WCC = white blood cell count; CNS = central nervous system; CI = confidence interval; EFS = event-free survival; IQR = inter-quartile range; OS = overall survival; RR = relapse rate.

Table 3

Features and Treatment Outcome of High Hyperdiploid Acute Lymphoblastic Leukemia

	All Protocols			MRD-guided Protocols Only			Non-MRD-guided Protocols Only		
	IKZF1			*IKZF1*			*IKZF1*		
High Hyperdiploid	**Deleted**	**Wild-type**		**Deleted**	**Wild-type**		**Deleted**	**Wild-type**	
n = 968	**n = 85**	**n = 883**	***P*-value**	**n= 65**	**n = 631**	***P*-value**	**n = 20**	**n = 252**	***P*-value**
Age									
<10	68 (80%)	780 (88%)	0.037	51 (78%)	556 (88%)	0.032	17 (85%)	224 (89%)	0.485
≥10	17 (20%)	103 (12%)		14 (22%)	75 (12%)		3 (15%)	28 (11%)	
Median (IQR)	5.00 (3.09–7.83)	4.00 (2.52–6.45)	0.005	5.36 (3.28–7.92)	3.94 (2.60–6.41)	0.003	4.00 (2.91–6.55)	4.00 (2.00–6.86)	0.676
Sex			0.734			1.00			0.493
Male	42 (49%)	457 (52%)		33 (51%)	322 (51%)		9 (45%)	135 (54%)	
Female	43 (51%)	426 (48%)		32 (49%)	309 (49%)		11 (55%)	117 (46%)	
WCC									
$<50 \times 10^9$/L	76 (89%)	803 (91%)	0.557	60 (92%)	577 (91%)	1.00	16 (80%)	226 (90%)	0.247
$\geq 50 \times 10^9$/L	9 (11%)	79 (9%)		5 (8%)	54 (9%)		4 (20%)	25 (10%)	
Median (IQR)	7.20 (3.40–17.80)	7.30 (3.50–18.35)	0.922	7.10 (3.40–16.50)	7.00 (3.40–17.30)	0.781	15.95 (3.32–32.59)	8.10 (3.89–21.80)	0.599
CNS disease			1.00			1.00			
Yes	0	2 (<0.5%)		0	2 (0.3%)		Unknown	Unknown	
No	17 (20%)	204 (23%)		17 (26%)	204 (32%)				
Clinical remission			1.00			1.00			
Yes	84 (99%)	870 (99%)		65 (100%)	623 (99%)		19 (95%)	247 (98%)	0.37
No	1 (1%)	8 (1%)		0	5 (1%)		1 (5%)	3 (1%)	
Induction death	0	5 (1%)		0	3 (<0.5%)		0	2 (1%)	
Outcome									
5-y EFS	76% (67%-87%)	89% (87%-91%)	0.006	73% (63%-86%)	88% (86%-91%)	0.004	85% (70%-100%)	90% (87-94%)	0.62
5-y RR	20% (10%-28%)	8% (6%-10%)	0.003	23% (10%-33%)	9% (6%-11%)	0.002	11% (0%-24%)	6% (3-9%)	0.576
5-y OS	88% (81%-96%)	94% (93%-96%)	0.005	88% (79%-97%)	94% (92%-96%)	0.004	90% (78%-100%)	94% (91-97%)	0.541
Hazard ratio EFS	2.00 (1.20–3.30)	Reference	0.007	2.20 (1.30%–3.90)	Reference	0.005	1.40 (0.41%–4.50)	Reference	0.621
Follow-up in years, median (IQR)	6.93 (4.48–9.83)	7.19 (4.89–9.10)		6.90 (4.48–8.96)	6.95 (4.70–8.86)		8.64 (4.68%–10.52)	7.62 (5.68–10.43)	

Data are n (%), rates at 5 y (95% CI) or median (IQR).

WCC = white blood cell count; CNS = central nervous system; CI = confidence interval; EFS = event-free survival; IQR = inter-quartile range; OS = overall survival; RR = relapse rate.

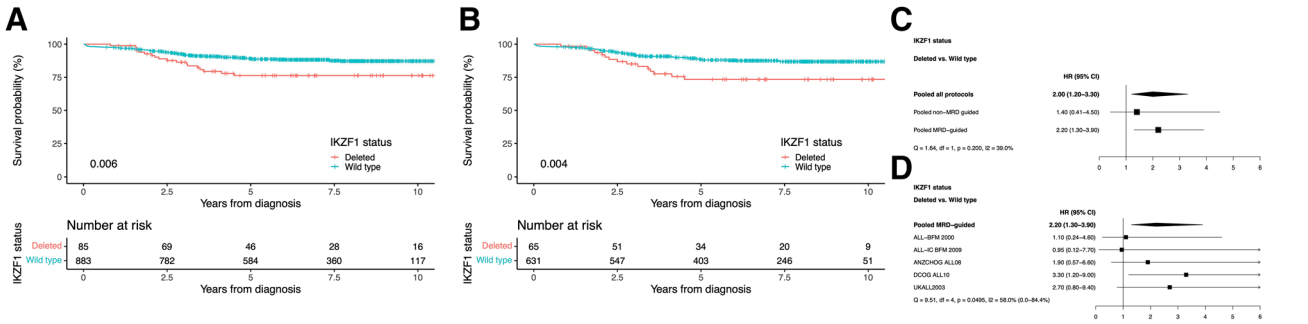

Figure 1. Outcome of patients with high hyperdiploid acute lymphoblastic leukemia. (A) Kaplan-Meier of event-free survival including both MRD-guided and non-MRD-guided trials. (B) Kaplan-Meier of event-free survival of MRD-guided trials. (C) Effect of *IKZF1* deletions on hazard ratio of event-free survival: pooled data from MRD-guided and non-MRD-guided trials. (D) Effect of *IKZF1* deletions on hazard ratio of event-free survival: data from each MRD-guided trial. *P*-values from log-rank test for comparing survival function estimates. MRD = minimal residual disease.

Table 4

Features and Treatment Outcome of High Hyperdiploid Acute Lymphoblastic Leukemia and Minimal Residual Disease at the End of Induction >0% and <5%

	IKZF1		
High Hyperdiploid	**Deleted**	**Wild-type**	***P*-value**
n = 333	**n = 34**	**n = 299**	
Age			
<10	30 (88%)	261 (87%)	1.00
≥10	4 (12%)	38 (13%)	
Median (IQR)	5.13 (3.72–6.84)	4.00 (2.66–6.12)	0.072
Sex			0.011
Male	23 (68%)	133 (44%)	
Female	11 (32%)	166 (56%)	
WCC			
$<50 \times 10^9$/L	31 (91%)	247 (83%)	0.334
$\geq 50 \times 10^9$/L	1 (3%)	25 (8%)	
Median (IQR)	6.65 (3.40–13.72)	7.70 (4.10–21.50)	0.167
CNS disease			1.00
Yes	0	1 (<0.5%)	
No	15 (44%)	128 (43%)	
Clinical remission			1.00
Yes	34 (100%)	299 (100%)	
No	0	0	
Induction death	0	0	
Ouctome			
5-y EFS	78% (64%-94%)	92% (89%-95%)	0.01
5-y RR	18% (2%-31%)	6% (3%-9%)	0.041
5-y OS	88% (77%-100%)	97% (95%-99%)	0.041
Hazard ratio EFS	2.90 (1.20–6.80)	Reference	0.014
Follow-up in years, median (IQR)	6.74 (4.50–8.88)	6.69 (5.31–8.29)	

Data are n (%), rates at 5 y (95% CI) or median (IQR).
WCC = white blood cell count; CNS = central nervous system; CI = confidence interval; EFS = event-free survival; IQR = inter-quartile range; OS = overall survival; RR = relapse rate.

deletions do not have a prognostic effect in *ETV6*::*RUNX1* ALL when treated on MRD-adapted protocols.

Previous DCOG trials showed that *IKZF1* deletions do not have prognostic value in patients stratified as standard risk[18] or in *ETV6*::*RUNX1* as a subgroup.[11] However, it did have prognostic value in HeH patients[6,11] and in patients stratified as medium risk, independent of the MRD level within the medium risk arm.[6,18] These findings led to the design of the DCOG ALL11 trial in which having an *IKZF1* deletion resulted in longer treatment (3 years) in the medium risk arm.[6] However, in the current trial used by many European countries, ALLTogether, *IKZF1* status alone is not used for treatment stratification of *ETV6*::*RUNX1* and HeH cases. For these 2 subgroups, specific optimal MRD thresholds at the end of induction have been established and are the main parameters used for stratification to intermediate-risk-low or intermediate-risk-high treatment arms.[37]

MRD was previously shown to be a very accurate prognostic parameter for treatment outcome in childhood ALL,[19,38,39] also in combination with *IKZF1* status.[11,18] Unfortunately, we could not take MRD values into account for the largest datasets A and B. Therefore, we only compared quantitative values of the first time point (end of induction) of 2 recent trials (dataset D). Our analysis of quantitative MRD data of HeH patients showed that *IKZF1*-deleted cases showed higher MRD levels than wild-type cases at the end of induction. To determine how HeH cases with *IKZF1* deletions would be stratified in the new ALLTogether trial, we examined how cases assigned to the initial intermediate-risk

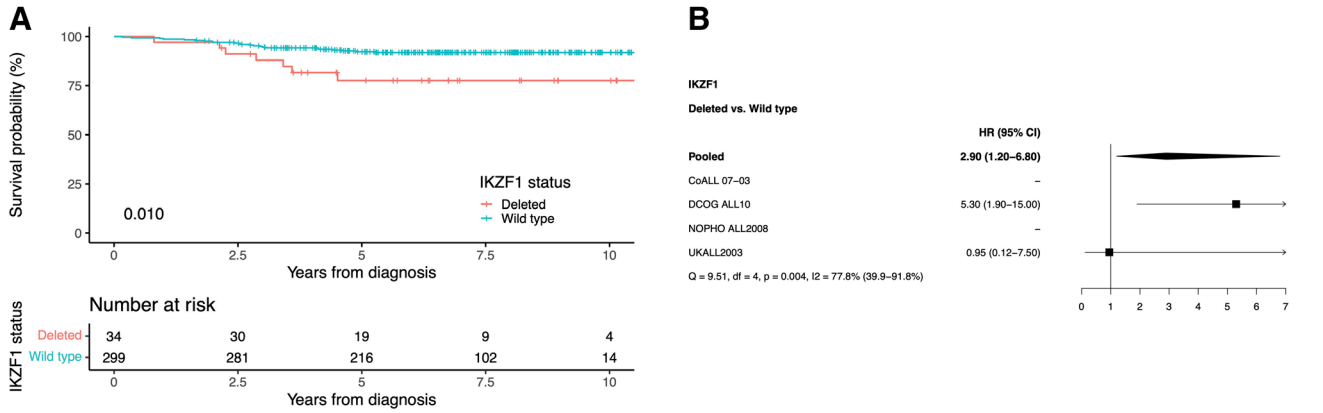

Figure 2. Outcome of patients with high hyperdiploid acute lymphoblastic leukemia and minimal residual disease at the end of induction between >0% and <5%. (A) Kaplan-Meier of event-free survival. (B) Effect of *IKZF1* deletion on hazard ratio of event-free survival. *P*-values from log-rank test for comparing survival function estimates. No hazard ratio for NOPHO and CoALL due to no events.

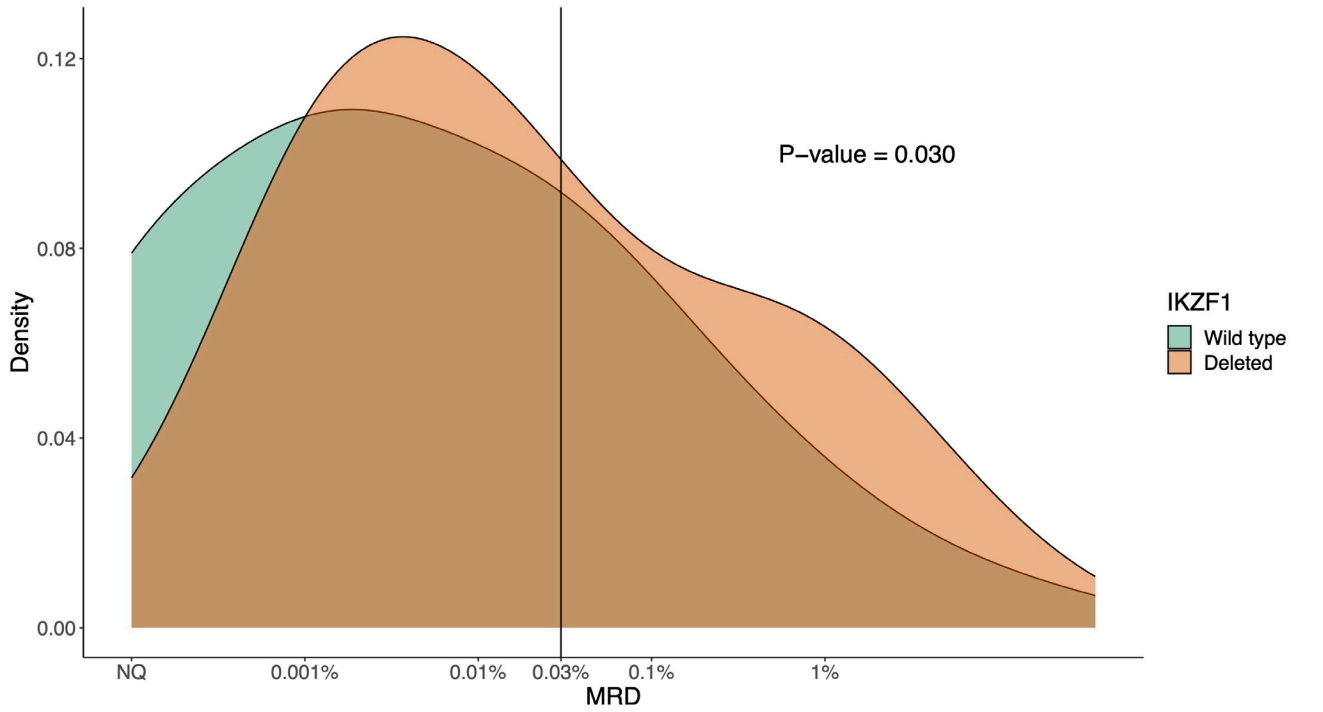

Figure 3. Distribution of the log-transformed MRD value, τ(MRD), at the end of induction of 305 patients with HeH ALL treated on UKALL2003 or DCOGALL10 trials. Smoothed density plots of the log-transformed minimal τ(MRD) by *IKZF1* status: not deleted (green) and deleted (orange); 0.03% is MRD cutoff at the end of induction for HeH ALL for placement in intermediate-risk-low or intermediate-risk-high in ALLTogether trial. HeH = high hyperdiploid; MRD = minimal residual disease.

group would distribute across the intermediate-risk-low and intermediate-risk-high arms based on quantitative end of induction MRD. The vast majority of these HeH cases (80% in dataset D) would be allocated to intermediate-risk-high group of the current ALLTogether trial and would not be eligible for any treatment reduction. This is the same treatment arm non-HeH cases with *IKZF1* deletions would be placed in, based on the copy number alteration profile. In this scenario, stratification by primary genetic subtype (HeH) and MRD appears to have the same effect as stratifying by primary and secondary genetic abnormality. The pattern of chromosomal gain in HeH has been linked to both MRD and outcome. A recent study identified that the pattern of gain of 4 chromosomes (5, 17, 18, and 20) could defined low- and high-risk subtypes of HeH ALL.[40] Interestingly, the proportion of cases with an *IKZF1* deletion was higher in the UKALL-HeH high-risk group compared with the low-risk group: 11% versus 6%, $P = 0.66$.

One of the limitations of this study is that it is based on data from 16 trials spanning 25 years and during this period OS for ALL has increased. However, *IKZF1* deletions are rare in *ETV6*::*RUNX1* and HeH ALL; hence, analysis of large retrospective multitrial datasets are the only practical source of information. All retrospective studies spanning long periods are limited by the fact that patients are treated on different, often improving protocols. To address this issue, we present data by trial and also by 2 major eras—pre and post the advent of MRD

risk stratification. Survival analysis of patients with HeH ALL treated on different protocols showed several differences in RR, EFS, and OS, although the trials were recent and MRD-guided. It is not clear why this difference in survival occurs. All trials use similar drugs but in different doses and regimens. In our analysis, the prognostic effect of *IKZF1* deletion was largest in the DCOG ALL10 trial and even more pronounced when we examined intermediate-risk patients as defined by the ALLTogether trial (Dataset C). This difference cannot be explained by difference in methodology or classification. MLPA was used to detect all IKZF1 deletions, and we use the same definition of HeH, that is, cytogenetic presence of an abnormal clone with 51–67 chromosomes. Furthermore, we re-examined the DCOG cases to ensure that there was no misclassification or inclusion of masked near-haploidy. Because ALL with *IKZF1* deletions has been shown to be more resistant to therapeutic drugs, this mechanism of drug resistance might underlie the difference in prognostic effect of *IKZF1* deletions among trials.[6]

Stanulla et al[16] described the prognostic effect of the *IKZF1*^{plus} profile characterized by a co-occurrence of *IKZF1* deletions with deletions of *CDKN2A*, *CDKN2B*, or *PAX5* or the PAR1 region in the absence of *ERG* deletion. We did not have data on *ERG* status and could, therefore, not assess the *IKZF1*^{plus} profile, although the numbers for this co-occurrence are expected to be small in the *ETV6*::*RUNX1* and HeH subtypes. Our analysis using the modified profile without *ERG* status did not show an additional prognostic effect of *IKZF1*^{plus} over assessing *IKZF1* status only. In addition, due to slight differences in *IKZF1* status calling between trials and in concordance with previous reports on the prognostic value of copy number alterations, single-exon deletions have not been categorized as *IKZF1*$^{-}$deleted in this dataset. Although single-exon deletions can have a prognostic effect,[41] they only comprise <10% of *IKZF1* deletions[11] and are, therefore, unlikely to influence our conclusions.

In conclusion, our analysis of a large composite cohort consisting of 16 trials shows no evidence to suggest that IKZF1 deletions affect outcome in the small number of *ETV6*::*RUNX1* cases in MRD-guided protocols. In contrast, our data show that in HeH ALL, *IKZF1* deletions are associated with lower survival rates, higher RRs, and higher MRD values. Future results of current trials such as the ALLTogether will likely reveal whether risk stratification predominantly reliant on MRD is adequate for HeH patients or whether stratification by copy number alteration profile, including *IKZF1* status, or by other methods, would be more suitable.

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AUTHOR CONTRIBUTIONS

FvL, RK, and RP conceived and designed the study. AV, MAH, GE, BJ, MH, MLdB, and KS supplied data. AE and AVM collected and assembled the data. AØ and AE analyzed the data. AØ, AE, PH, MLdB, RP, AVM, FNvL, and JB interpreted the data. AØ wrote the original draft. All authors reviewed and approved the final article.

DATA AVAILABILITY

The data used in this study are available from the corresponding author.

DISCLOSURES

The authors have no conflicts of interest to disclose.

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