## IKZF1 deletion is an independent predictor of outcome in pediatric acute lymphoblastic leukemia treated according to the ALL-BFM 2000 protocol

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SNP 6.0								MLPA P335								
		3	IKZF1	-Exon				IKZF1-Exon								
1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	
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		In3 <sup>(2)</sup>														
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**Online Supplementary Figure** S1. Comparison of results from Affymetrix SNP 6.0 arrays and MLPA kit P335, regarding IKZF1 deletions, in 25 selected patients. 1Each row represents one patient (the first 2 patients were chosen as negative controls), each colored lane represents a deletion in the respective exon. Light red deletions are heterozygous, dark red deletions are homozygous and light pink deletions are sub-clonal. <sup>2</sup>Deletion on SNP 6.0 was found in Intron 3.

		MLPA P335 MLPA P202 IKZF1- Exon IKZF1-Exon																							
_	_					_					_	_		_					_			-	_	_	
1	2	3	4	5	6	7	8	1 a	1 b	1 c	2 a	2 b	3 a	3 b	3 In <sup>2</sup>	4 a	4 b	5 a	5 b	6 a	6 b	7 a	7 b	8 a	8 b
																									9
									4																
5 3														1											

Online Supplementary Figure S2. Results for all 84 *IKZF1* deleted patients as analyzed with MLPA kit P335 and validation with MLPA kit P202.  $^4$ MLPA kit P202-A1 contains additional markers, compared to kit P335-A3, identical markers are highlighted in bold. Each row represents one patient, each colored lane represents a deletion in the respective exon. Light red deletions are heterozygous, dark red deletions are homozygous and light pink deletions are subclonal. In one subclonal sample, results were discordant between P335 and P202, as indicated. Of the 6 un-validated samples 4 had a  $\Delta$ 1-8, one a  $\Delta$ 4-7 and one a  $\Delta$ 5-7 deletion in P335.  $^2$ In: Intron.

Online Supplementary Table 1. Patients' characteristics and response to treatment in the study cohort of 694 pediatric patients with ALL, in comparison to the remaining ALL-BFM 2000 patient cohort that was not studied.

Feature Number of patients	Cohort not studied (%) 2133 (100)	Study cohort (%) 694 (100)	<b>P</b> <sup>1</sup>
Sex		202 (202)	0.20
Male	1170 (54.9)	400 (57.6)	
Female	963 (45.1)	294 (42.4)	
Age at diagnosis (years)	4000 (77 0)	<b></b>	0.90
1-9 ± 10	1603 (75.2) 530 (24.8)	520 (74.9) 174 (25.1)	
Presenting WBC count (x	. ,	111 (20.1)	< 0.0001
<1 <1	1091 (51.1)	257 (37.0)	<b>10.0001</b>
1-4.99	666 (31.2)	256 (36.9)	
5-9.99	175 (8.2)	97 (14.0)	
≥ 10	201 (9.4)	84 (12.1)	
Prednisone response <sup>2</sup>			0.005
Good	1920 (90.0)	601 (86.7)	
Poor No information	189 (8.9) 24 (1.1)	87 (12.5) 6 (0.8)	
MRD <sup>3</sup>	24 (1.1)	0 (0.0)	0.57
MRD-SR	687 (32.2)	249 (35.9)	0.51
MRD-IR	847 (39.7)	276 (39.8)	
MRD-HR	132 (6.2)	46 (6.6)	
No information	467 (21.9)	123 (17.7)	
Immunology			0.03
Non-T-ALL	1787 (83.8)	572 (82.5)	
T-ALL No information	280 (13.1) 66 (3.1)	116 (16.6) 6 (0.9)	
ETV6/RUNX1	00 (5.1)	0 (0.3)	0.80
Negative	1488 (69.8)	498 (71.7)	0.00
Positive	449 (21.0)	146 (21.1)	
No information	196 (9.2)	50 (7.2)	
BCR/ABL		** (**=)	0.58
Negative	2053 (96.2)	668 (96.2)	
Positive No information	39 (1.8) 41 (2.0)	15 (2.2) 11 (1.6)	
MLL/AF4	11 (2.0)	11 (1.0)	0.23
Negative	1979 (92.8)	651 (93.8)	0.20
Positive	10 (0.5)	1 (0.1)	
No information	144 (6.7)	42 (6.1)	
Final risk group			0.003
SR	667 (31.3)	240 (34.6)	
IR HR	1167 (54.7) 299 (14.0)	331 (47.7) 123 (17.7)	
IIIX	400 (14.0)	120 (11.1)	

 $<sup>^1\</sup>gamma_c^2$  test comparing study cohort and patients not studied, patients with no information excluded from test;  $^2$ good: less than 1000 leukemic blood blasts/ $\mu$ L on treatment Day 8, poor: more than 1000/ $\mu$ L.  $^3$ MRD risk groups  $^{i7}$ : MRD-SR: TP1+2 negative, MRD-IR: TP1 and/or TP2 <10 $^3$ , MRD-HR: TP2  $\geq$ 10 $^3$ .

Online Supplementary Table 2. Multivariate Cox's regression analysis for event-free survival, including all 694 patients.

Feature	Hazard ratio	95% CI	P
IKZF1	2.28	1.44-3.60	< 0.0001
Sex	0.91	0.62-1.33	0.62
SR	0.56	0.34 - 0.93	0.03
HR	1.91	1.20-3.03	0.006
ETV6/RUNX1	1.00	0.58 - 1.73	0.99
Immunology	0.99	0.59-1.67	0.97
WBC $\geq x10^9/L$	1.46	0.89-2.39	0.14

Online Supplementary Table 3. Multivariate Cox's regression analysis for event-free survival, excluding 27 *P2RY8-CRLF2* positive patients from the cohort.

Feature	Hazard ratio	95% CI	P
IKZF1	1.94	1.19-3.16	0.008
Gender	0.91	0.61-1.34	0.62
SR	0.58	0.34-0.99	0.05
HR	1.98	1.24-3.17	0.004
ETV6/RUNX1	1.03	0.59-1.79	0.92
WBC $\geq x10^9/L$	1.53	0.93-2.51	0.09

Online Supplementary Table 4. Multivariate Cox's regression analysis for event-free survival, including *P2RY8-CRLF2* status in the model.

Feature	Hazard ratio	95% CI	P
IKZF1	2.08	1.32-3.28	0.002
P2RY8-CRLF2	2.04	0.94-4.41	0.07
Gender	0.90	0.6s2 - 1.32	0.591
SR	0.54	0.33-0.91	0.02
HR	2.07	1.31-3.28	0.002
ETV6/RUNX1	1.07	0.60-1.85	0.81
WBC $\geq x10^9/L$	1.44	0.89 - 2.32	0.14