

IKZF1 alterations are not associated with outcome in 498 adults with B-precursor ALL enrolled on the UKALL14 trial

Supplementary methods and data

Methods

PCR reaction

Each reaction included 2µl 10x reaction buffer (Qiagen), 0.5µl 10mM dNTPs (Promega), 0.125µl HotStarTaq (Qiagen), 2.5µl each of the 10mM primers (table 3), made up to a final volume of 20µl with 1.375µl dH₂O.

Cycling conditions

Step	Cycling condition
1	95°C for 10 minutes
2	95°C for 50 seconds
3	58°C for 50 seconds
4	72°C for 50 seconds
5	Repeat steps 2-4 for 44 more times
6	72°C for 10 minutes
7	4°C forever

Primers for IKZF1 PCR

	Location	Sequence
Forward Primers	Δ2a	5'-CAACAAGTGACCCATCCTTTG-3'
	Δ2b	5'-CACACACTTCAAGATTATGCATTT-3'
	Δ4	5'-TGTGAAGGTCACACCCTCTG-3'
	Albumin	5'-TGAAACATACGTTCCCAAAGAGTTT-3'
Reverse Primers	Δ7	5'-AAAGAACCCTCAGGCATTCA-3'
	Δ8	5'-GGGGACTGGAAGTCACAGAA-3'
	Albumin	5'-CTCTCCTTCTCAGAAAGTGTGCATAT-3'

Well 1		Well 2	
Forward	Reverse	Forward	Reverse
Δ2a	Δ7	Δ2a	Δ8
Δ2b	-	Δ2b	-
Δ4	-	Δ4	-
Alb	Alb	Alb	Alb

Supplementary table 1

Patient characteristics of the 'post amendment' cohort for survival analyses

	PCR Sample	No PCR Sample	p-value ¹	MLPA Sample	No MLPA Sample	p-value ¹	All UKALL14 B-cell patients
	N=437	N=140		N=193	N=384		N=577
Rituximab given, N (%)	227 (51.9)	62 (44.3)		198 (51.6)	91 (47.2)		289 (50.1)
Median Age (years)	45.0(23 - 65)	46.5(22 - 65)	0.20	44.0(23 - 65)	48.0(22 - 65)	<0.001	46.0(22 - 65)
Sex ² , N (%)			0.47			0.79	
Male	237 (54.2)	81 (57.9)		210 (54.7)	108 (56.0)		318 (55.1)
ECOG, score 0 N (%)	264 (61.0)	87 (63.0)	0.66	233 (61.2)	118 (62.1)	0.80	351 (61.5)
Baseline WBC	10.7(.11 - 889.6)	4.9(.4 - 372)	<0.001	10.7(.11 - 889.6)	4.9(.4 - 372)	<0.001	8.0(.11 - 889.6)
Genetic Subgroup, N (%)							
BCR-ABL1	144 (33.0)	28 (20.0)	0.004	134 (34.9)	38 (19.7)	<0.001	172 (29.8)
Complex karyotype	14 (3.2)	3 (2.1)	NA	12 (3.1)	5 (2.6)	NA	17 (2.9)
High hyperdiploid	9 (2.1)	3 (2.1)	NA	8 (2.1)	4 (2.1)	NA	12 (2.1)
JAK-STAT	25 (5.7)	5 (3.6)	NA	23 (6.0)	7 (3.6)	NA	30 (5.2)
KMT2A other	5 (1.1)	1 (0.7)	NA	5 (1.3)	1 (0.5)	NA	6 (1.0)
KMT2A-AFF1	36 (8.2)	6 (4.3)	NA	34 (8.9)	8 (4.1)	NA	42 (7.3)
Low hypodiploid/Near-triploid	33 (7.6)	16 (11.4)	NA	3 (0.8)	46 (23.8)	NA	49 (8.5)
Other	112 (25.6)	46 (32.9)	NA	113 (29.4)	45 (23.3)	NA	158 (27.4)
TCF3-PBX1	14 (3.2)	0	NA	14 (3.6)	0	NA	14 (2.4)
Failed/no data	45 (10.3)	32 (22.9)		38 (9.9)	39 (20.2)		77 (13.3)
Baseline UKALL 14 risk ³ , N (%)			0.76			0.018	
High risk	364 (83.3)	111 (79.3)		308 (80.2)	167 (86.5)		475 (82.3)
IKZF1 by PCR, N (%)	98 (22.4)			92 (24.9)			98 (22.4)
IKZF1 by MLPA, N (%)	146 (39.5)			149 (38.8)			149 (38.8)

¹ p-values are chi-squared or for binary variables and Wilcoxon-Mann-Whitney for continuous (missing values excluded). ²One intersex patient (genetically male but identified as female) has been excluded from all comparisons by sex. ³For the purposes of the trial, patients missing risk factor data (but no high risk factors in the data available) were assumed to be standard risk. For any comparisons these patients were excluded (~5% of all B-cell patients) NA – P values not provided where numbers too low for meaningful comparisons (p-values for BCR-ABL status compare positive vs negative)

Supplementary Table 2: Univariable and multivariable analysis of EFS and OS in relation to MLPA-determined *IKZF1* status

	EFS			OS		
	Events/N	HR (95% CI)	p	Events/N	HR (95% CI)	p
All patients						
WT	133/235	1.00	0.64	116/235	1.00	0.52
<i>ΔIKZF1</i>	84/149	1.07 (0.81 – 1.40)		66/149	0.91 (0.67 – 1.23)*	
WT	133/235	1.00	0.63	116/235	1.00	0.79
<i>ΔIKZF1</i> DN (4-7)	28/46	1.21 (0.81 – 1.83)		22/46	1.01 (0.64 – 1.59)	
<i>ΔIKZF1</i> LOF	54/100	1.00 (0.73 – 1.38)		44/100	0.89 (0.63 – 1.26)	
WT	133/235	1.00	0.062	116/235	1.00	0.051
<i>ΔIKZF1</i> , not IKZF1 plus	31/66	0.80 (0.54 – 1.18)		23/66	0.64 (0.41 – 1.00)	
IKZF1 plus	53/83	1.33 (0.97 – 1.83)		43/83	1.16 (0.82 – 1.65)	
BCR-ABL1- ALL						
WT	105/184	1.00	0.26	94/184	1.00	0.80
<i>ΔIKZF1</i> (all BCR-ABL1-)	40/66	1.24 (0.86 – 1.78)		33/66	1.05 (0.71 – 1.56)	
MVA: WT	86/149	1.00	0.23	77/149	1.00	0.33
MVA: <i>ΔIKZF1</i>	37/60	1.28 (0.85 – 1.92)		32/60	1.24 (0.80 – 1.91)	
WT	105/184	1.00	0.51	94/184	1.00	0.94
<i>ΔIKZF1</i> DN (4-7)	11/18	1.37 (0.74 – 2.56)		8/18	1.11 (0.56 – 2.20)	
<i>ΔIKZF1</i> LOF	28/47	1.17 (0.77 – 1.78)		24/47	1.05 (0.67 – 1.64)	
MVA: WT	86/149	1.00	0.37	77/149	1.00	0.48
MVA: <i>ΔIKZF1</i> DN (4-7)	10/16	1.65 (0.83 – 3.28)		9/16	1.54 (0.75 – 3.16)	
MVA: <i>ΔIKZF1</i> LOF	26/43	1.15 (0.73 – 1.82)		23/43	1.17 (0.72 – 1.91)	
WT	105/184	1.00	0.26	94/184	1.00	0.18
<i>ΔIKZF1</i> , not IKZF1 plus	12/24	0.97 (0.53, 1.76)		9/24	0.67 (0.34 – 1.33)	
IKZF1 plus	28/42	1.40 (0.92, 2.13)		24/42	1.34 (0.85 – 2.10)	
MVA: WT	86/149	1.00	0.47	77/149	1.00	0.43
MVA: <i>ΔIKZF1</i> , not IKZF1 plus	11/22	1.17 (0.61, 2.24)		9/22	0.98 (0.49 – 1.99)	
MVA: IKZF1 plus	26/38	1.33 (0.84, 2.12)		23/38	1.39 (0.85 – 2.25)	

BCR-ABL1 + ALL						
WT	28/51	1.00	0.91	22/51	1.00	0.70
<i>ΔIKZF1 (all BCR-ABL1+)</i>	44/83	0.97 (0.60 – 1.56)		33/83	0.90 (0.52 – 1.55)	
p190 breakpoint						
<i>MVA: WT</i>	22/31	1.00	0.047	18/31	1.00	0.022
<i>MVA : ΔIKZF1 deleted (p190 breakpoint)</i>	26/49	0.55 (0.31 – 0.99)		18/49	0.45 (0.22 – 0.99)	
<i>MVA: WT</i>	22/31	1.00		18/31	1.00	
<i>MVA: ΔIKZF1 DN (4-7)</i>	8/15	0.46 (0.18 – 1.15)	0.097	5/15	0.29 (0.09 – 0.87)	0.027
<i>MVA: ΔIKZF1 LOF</i>	17/32	0.59 (0.31 – 1.13)	0.11	13/32	0.58 (0.27 – 1.24)	0.16
<i>MVA: WT</i>	22/31	1.00		18/31	1.00	
<i>MVA: ΔIKZF1, not IKZF1 plus</i>	13/29	0.45 (0.23 – 0.91)	0.025	9/29	0.40 (0.18 – 0.91)	0.03
<i>MVA: IKZF1 plus</i>	13/20	0.74 (0.35 – 1.55)	0.43	9/20	0.51 (0.22 – 1.22)	0.13
p210 breakpoint						
<i>MVA: WT</i>	4/15	1.00	0.26	2/15	1.00	0.14
<i>MVA : ΔIKZF1 deleted (p210 breakpoint)</i>	14/29	1.90 (0.62 – 5.87)		12/29	3.18 (0.69 – 14.59)	
<i>MVA: WT</i>	4/15	1.00		2/15	1.00	
<i>MVA: ΔIKZF1 DN (4-7)</i>	7/11	2.19 (0.87 – 5.51)	0.097	6/11	5.26 (1.00 – 27.58)	0.049
<i>MVA: ΔIKZF1 LOF</i>	7/18	1.29 (0.49 – 3.41)	0.61	6/18	2.24 (0.44 – 11.36)	0.33
<i>MVA: WT</i>	4/15	1.00		2/15	1.00	
<i>MVA: ΔIKZF1, not IKZF1 plus</i>	6/13	1.88 (0.53 – 6.74)	0.33	5/13	3.36 (0.66 – 18.07)	0.14
<i>MVA: IKZF1 plus</i>	8/16	1.92 (0.57 – 6.52)	0.30	7/16	2.97 (0.59 – 14.96)	0.19

Univariable analyses are shown in bold and multivariable (MVA) in italics. All MVA analyses include age, sex, ECOG, log WBC and rituximab randomisation. BCR-ABL1- models include genetic risk group (good, standard or high) and BCR-ABL1+ models also included breakpoint (p190 or p210) and an interaction term between IKZF1 and breakpoint (p-values for interaction deleted/WT: EFS p = 0.045 and OS p = 0.011). *Fails the assumption of proportional hazards.

Supplementary Table 3: Univariable and multivariable analysis of time to relapse in relation to PCR and MLPA-determined *IKZF1* status

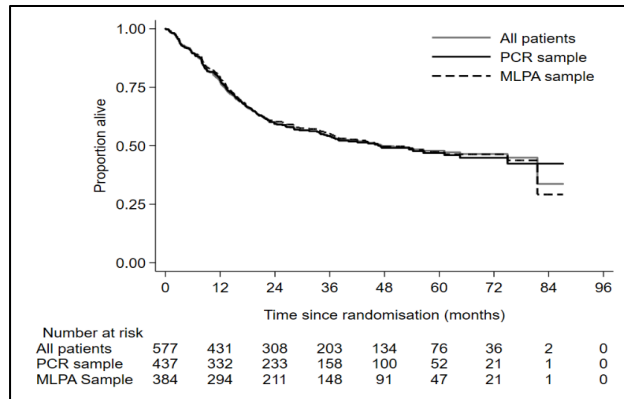
All patients	Time to relapse PCR-determined <i>IKZF1</i>			Time to relapse MLPA-determined <i>IKZF1</i>		
	Events/N	HR (95% CI)	p	Events/N	HR (95% CI)	p
WT	110/339	1.00	0.65	79/235	1.00	0.50
<i>ΔIKZF1</i>	31/98	0.91 (0.60-1.37)		44/149	0.88 (0.60 – 1.28)	
WT	110/339	1.00	0.77	79/235	1.00	0.77
<i>ΔIKZF1</i> DN (4-7)	18/58	0.83 (0.49 – 1.40)		14/46	0.88 (0.49 – 1.58)	
<i>ΔIKZF1</i> LOF	13/40	1.03 (0.58 – 1.84)		28/100	0.87 (0.56 – 1.33)	
WT	-	-	-	79/235	1.00	0.57
<i>ΔIKZF1</i> , not <i>IKZF1</i> plus	-	-		18/66	0.75 (0.44 – 1.28)	
<i>IKZF1</i> plus	-	-		26/83	0.98 (0.63 – 1.53)	
BCR-ABL1- ALL						
WT	82/251	1.00	0.80	61/184	1.00	0.84
<i>ΔIKZF1</i>	15/42	1.08 (0.60 – 1.94)		21/66	1.05 (0.63 – 1.76)	
<i>MVA: WT</i>	68/206	1.00	0.92	51/149	1.00	0.63
<i>MVA: ΔIKZF1</i>	13/38	1.03 (0.54 – 1.98)		18/60	0.87 (0.48 – 1.55)	
WT	82/251	1.00	0.87	61/184	1.00	0.78
<i>ΔIKZF1</i> DN (4-7)	9/24	0.97 (0.45 – 2.10)		7/26	0.79 (0.29 – 2.17)	
<i>ΔIKZF1</i> LOF	6/18	1.24 (0.54 – 2.85)		13/39	1.15 (0.65 – 2.03)	
<i>MVA: WT</i>	68/206	1.00	0.99	51/149	1.00	0.80
<i>MVA: ΔIKZF1</i> DN (4-7)	8/22	1.05 (0.45 – 2.48)		4/16	0.68 (0.21 – 2.25)	
<i>MVA: ΔIKZF1</i> LOF	5/16	1.01 (0.40 – 2.56)		13/43	0.92 (0.49 – 1.74)	
WT	-	-	-	61/184	1.00	0.95
<i>ΔIKZF1</i> , not <i>IKZF1</i> plus	-	-		7/24	0.96 (0.38 - 2.39)	
<i>IKZF1</i> plus	-	-		14/42	1.09 (0.61 – 1.94)	
<i>MVA: WT</i>	-	-	-	51/149	1.00	0.87
<i>MVA: ΔIKZF1</i> , not <i>IKZF1</i> plus	-	-		6/22	0.81 (0.29 – 2.27)	
<i>MVA: IKZF1</i> plus	-	-		12/38	0.89 (0.46 – 1.69)	

BCR-ABL1 + ALL						
WT	28/88	1.00	0.59	18/51	1.00	0.31
<i>ΔIKZF1 (all BCR-ABL1+)</i>	16/56	0.84 (0.46 – 1.56)		23/83	0.73 (0.39 – 1.35)	
WT	28/88	1.00	0.83	18/51	1.00	0.43
<i>ΔIKZF1 DN (4-7)</i>	9/34	0.79 (0.37 – 1.68)*		9/28	0.88 (0.40 – 1.97)	
<i>ΔIKZF1 LOF</i>	7/22			13/53	0.63 (0.31 – 1.29)	
WT	-	-	-	18/51	1.00	0.50
<i>ΔIKZF1, not IKZF1 plus</i>	-	-		11/42	0.64 (0.30 – 1.35)	
<i>IKZF1 plus</i>	-	-		12/41	0.84 (0.40 – 1.74)	
p190 breakpoint						
<i>MVA: WT</i>	19/53	1.00	0.56	13/31	1.00	0.16
<i>MVA :ΔIKZF1</i>	11/32	0.79 (0.35 – 1.76)		15/49	0.57 (0.26 – 1.24)	
<i>MVA: WT</i>	19/53	1.00		13/31	1.00	
<i>MVA: ΔIKZF1 DN (4-7)</i>	6/19	0.62 (0.20 – 1.90)	0.41	6/15	0.69 (0.22 – 2.19)	0.53
<i>MVA: ΔIKZF1 LOF</i>	5/13	0.97 (0.36 – 2.66)	0.96	8/32	0.50 (0.20 – 1.23)	0.13
<i>MVA: WT</i>	-	-	-	13/31	1.00	
<i>MVA: ΔIKZF1, not IKZF1 plus</i>	-	-		8/29	0.51 (0.21 – 1.24)	0.14
<i>MVA: IKZF1 plus</i>	-	-		7/20	0.69 (0.26 – 1.88)	0.47
p210 breakpoint						
<i>MVA: WT</i>	6/27	1.00	0.74	3/15	1.00	0.88
<i>MVA :ΔIKZF1 (p210 breakpoint)</i>	4/20	0.81 (0.23 – 2.89)		6/29	0.90 (0.22 – 3.64)	
<i>MVA: WT</i>	6/27	1.00		3/15	1.00	
<i>MVA: ΔIKZF1 DN (4-7)</i>	2/13	0.65 (0.13 – 3.26)	0.60	2/11	0.78 (0.13 – 4.82)	0.79
<i>MVA: ΔIKZF1 LOF</i>	2/7	1.07 (0.21 – 5.46)	0.94	4/18	0.98 (0.22 – 4.42)	0.98
<i>MVA: WT</i>	-	-	-	3/15	1.00	
<i>MVA: ΔIKZF1, not IKZF1 plus</i>	-	-		3/13	1.05 (0.21 – 5.30)	0.96
<i>MVA: IKZF1 plus</i>	-	-		3/16	0.80 (0.16 – 4.01)	0.79

Univariable analyses are shown in bold and multivariable (MVA) in italics. All MVA analyses include age, sex, ECOG, log WBC and rituximab randomisation. *BCR-ABL1*- models include genetic risk group (good, standard or high) and *BCR-ABL1*+ models also included breakpoint (p190 or p210) and an interaction term between *IKZF1* status and breakpoint (p-values for interaction deleted/WT in MVA: p= 0.98 (PCR) and p=0.57(MLPA)). Models were also run censoring at SCT in first CR I and including post induction MRD as a covariate with no change in conclusions. *Fails the assumption of proportional hazards.

Supplementary figure 1

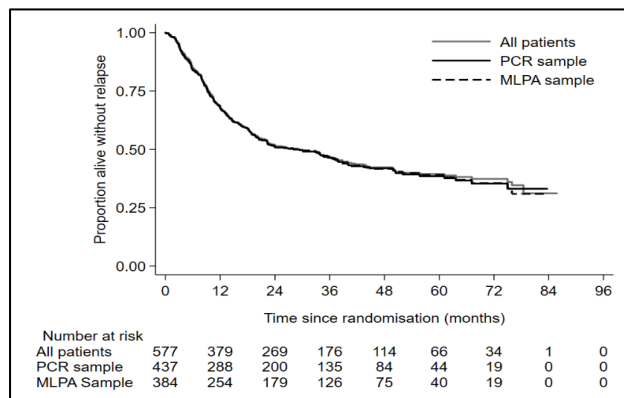
Kaplan Meier Curves of EFS, OS and CIR by availability of sample for PCR or MLPA compared to the whole trial population



EVENTS (DEATH)

182/384 in the MLPA population

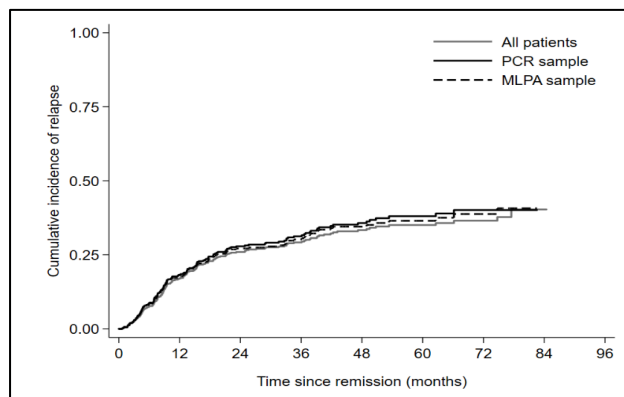
208/437 in the PCR population



EVENTS (RELAPSE and DEATH)

217/384 in the MLPA population

245/437 in the PCR population



RELAPSE

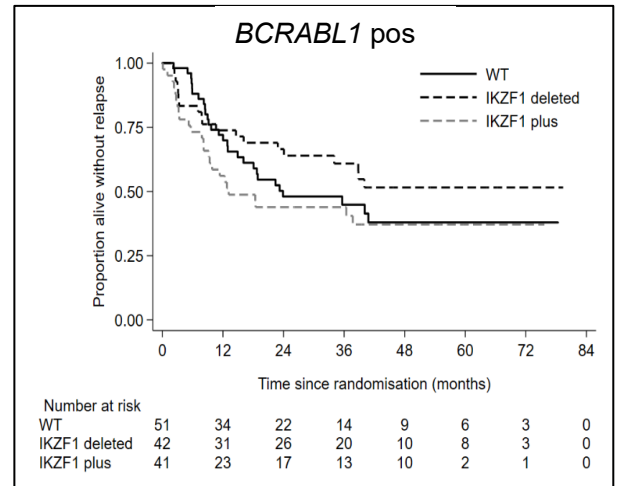
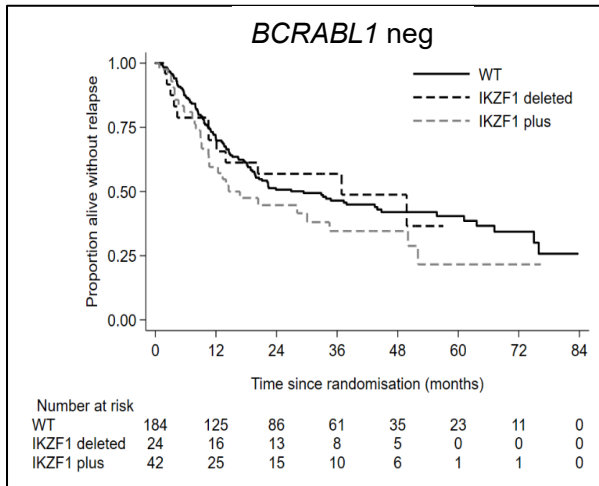
123/384 in the MLPA, population

141/437 in the PCR population

Supplementary figure 2

Kaplan Meier curves of EFS and OS by *IKZF1* deletion status, shown separately by MLPA-determined *BCRABL1* status

EFS



OS

